Dipeptidyl peptidase IV inhibitor sitagliptin reduces local inflammation in adipose tissue and in pancreatic islets of obese mice

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Obesity is frequently associated with type 2 diabetes, and both conditions are characterized by chronic low-grade inflammation (47). Increased inflammation in adipose tissue and in the pancreatic islets is proposed as a key contributor to insulin resistance and type 2 diabetes, yet the mechanisms are not completely elucidated. Dipeptidyl peptidase IV (DPP IV) inhibitors have recently become widely used for treatment of type 2 diabetes. Sitagliptin is a commonly used DPP IV inhibitor in type 2 diabetic patients, and numerous randomized control trials have proved efficient in increasing insulin secretion in a glucose-dependent manner (20). In vivo DPP IV inhibition improves glucose homeostasis via multiple mechanisms. One of the mechanisms explaining the insulinotropic actions of sitagliptin is via an increase in the circulating incretin hormone glucagon-like peptide (GLP)-1, which is well known to suppress glucagon secretion and enhance insulin secretion (3, 15). In addition, in animal models of diabetes, sitagliptin showed favorable actions on islet and β-cell mass, morphology, and survival (21). Islet inflammation is an important contributor to islet and β-cell dysfunction in type 2 diabetes (17, 23). Several inflammatory pathways were reported to have relevance for impairment of islet and β-cell function and survival, including the lipoxigenase pathway (12, 36) and toll receptor activation (18, 24). A recent report indicates that GLP-2 receptor impacts on the susceptibility of islet damage in response to systemic inflammation (6). This raises the possibility that sitagliptin, in addition to the already known mechanisms, may act to improve islet and β-cell function via a reduction of systemic and/or local inflammation.

Whereas DPP IV inhibition is well known to improve glycemic control in type 2 diabetes by mechanisms targeting mainly the pancreatic islet or liver function, considerably less is known about the effect of DPP IV inhibition on peripheral tissues that are key for the control of insulin sensitivity, such as the adipose tissue. Increasing evidence indicates that adipose tissue inflammation is an important determinant of insulin resistance, diabetes, and cardiovascular disease (31, 53). In particular, visceral adiposity in obesity is associated with increased inflammation and altered adipocyte metabolism in both mice and humans. DPP IV has been identified in human and mouse adipose tissue, and both the GLP-1 and gastric inhibitory polypeptide (GIP) receptors were reported for murine adipocytes (51, 59). Recently, a role for DPP IV was emphasized in potentiating the action of neuropeptide Y in isolated human adipocytes (32). Collectively, the data suggest a potentially important role for DPP IV (CD26) in the regulation of adipocyte metabolism by modulation of lipolysis and insulin sensitivity. Immune cell infiltration, notably of different populations of macrophages and T cells, was recently shown to correlate with the development and maintenance of insulin resistance and diabetes in obesity (8, 26, 41, 56).

In this study, we determined the concurrent effect of sitagliptin treatment on inflammation in pancreatic islets and adipose tissue and effects on pancreatic function and adipose tissue inflammation and reduced pancreatic β-cell function are key issues in the development of cardiovascular disease and progressive metabolic dysfunction in type 2 diabetes mellitus. The aim of this study was to determine the effect of the DPP IV inhibitor sitagliptin on adipose tissue and pancreatic islet inflammation in a diet-induced obesity model. C57Bl/6J mice were placed on a high-fat (60% kcal fat) diet for 12 wk, with or without sitagliptin (4 g/kg) as a food admix. Sitagliptin significantly reduced fasting blood glucose by 21% as well as insulin by ~25%. Sitagliptin treatment reduced body weight without changes in overall body mass index or in the epididymal and retroperitoneal fat mass. However, sitagliptin treatment led to triple the number of small adipocytes despite reducing the number of the very large adipocytes. Sitagliptin significantly reduced inflammation in the adipose tissue and pancreatic islet. Macrophage infiltration in adipose tissue evaluated by immunostaining for Mac2 was reduced by sitagliptin (P < 0.01), as was the percentage of CD11b+/F4/80+ cells in the stromal vascular fraction (P < 0.02). Sitagliptin also reduced adipocyte mRNA expression of inflammatory genes, including IL-6, TNFα, IL-12(p35), and IL-12(p40), 2.5- to fivefold as well as 12-lipoxygenase protein expression. Pancreatic islets were isolated from animals after treatments. Sitagliptin significantly reduced mRNA expression of the following inflammatory cytokines: MCP-1 (3.3-fold), IL-6 (2-fold), IL-12(p40) (2.2-fold), IL-12(p35) (5-fold, P < 0.01), and IP-10 (2-fold). Collectively, the results indicate that sitagliptin has anti-inflammatory effects in adipose tissue and in pancreatic islets that accompany the insulinotropic effect.

visceral fat; cytokines; macrophages; 12/15-lipoxygenase; insulin resistance
tissue remodeling in a model of diet-induced obesity. We measured inflammatory cytokine expression in pancreatic islets and adipocytes and determined macrophage infiltration in adipose tissue. The results indicate that sitagliptin improves glucose tolerance and ameliorates inflammation in both pancreatic islets and adipose tissue by reducing inflammatory cytokine expression and macrophage infiltration. Our study is the first to address the anti-inflammatory effects of sitagliptin treatment on both islets and adipose tissue in a mouse model of diet-induced obesity and insulin resistance.

EXPERIMENTAL PROCEDURES

Animals and treatments. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Eastern Virginia Medical School. Male C57Bl/6j mice, 8 wk of age, were purchased from The Jackson Laboratory (Bar Harbor, ME) and randomly divided into two groups, those on a high-fat diet (60% kcal fat; Research Diets, New Brunswick, NJ) supplemented with 4 g/kg des-fluoro-sitagliptin (a sitagliptin analog provided by Merck Research Laboratories, Rahway, NJ) or on a high-fat diet only. Treatments (diet and sitagliptin) were continued for 12 wk for all mice. Mice on a low-fat diet, isocaloric with the high-fat diet (11% kcal fat; Research Diets), were treated with the same dose of sitagliptin or left untreated and were used as controls in some of the experiments. Mice were housed in a pathogen-free facility and caged individually, and food and water were provided ad libitum throughout the experiment. Body weight and food intake were measured weekly. Two separate trials were performed under identical experimental conditions, with each trial containing six to eight mice per experimental group. The body mass index (BMI) was calculated as described by others (42, 48).

Metabolic measurements. Glucose and insulin tolerance tests were performed as described previously (9, 43). Two tests were performed in both of the mouse groups, the first after 1 wk on a high-fat diet and the second after 12 wk on a high-fat diet. For glucose tolerance tests (GTT), mice were fasted overnight and injected the following morning intraperitoneally (ip) with 2 g/kg glucose. Blood glucose was measured with an UltraTouch glucometer from cut tail tips at 0, 10, 20, 30, 60, 90, and 120 min following glucose injection. The insulin tolerance test (ITT) was performed in randomly fed mice by 1 U/kg ip insulin injection. Blood glucose was measured in tail vein samples collected at baseline and following insulin injection at 15, 30, 45, and 60 min. GTT and ITT were performed on the same set of mice 5 days apart. Plasma insulin was measured using a commercially available ELISA kit from Merodia (Uppsala, Sweden) according to the manufacturer’s instructions.

Pancreatic islet isolation and in vitro glucose-stimulated insulin secretion. Pancreatic islets were isolated as described previously (43). Briefly, the pancreas was perfused through the common bile duct with 1.4 mg/ml collagenase P (Roche Applied Science) and then incubated briefly at 37°C in a shaking water bath in KRH buffer supplemented with 1% BSA, 20 nmol/l adenosine, and 1 mg/ml collagenase I. The resulting cell suspension was filtered through a 0.4-mm Nitex nylon mesh (Sefar Filtration, New York, NY). The floating adipocytes were collected and washed, and the infranatant was removed and centrifuged at 500 g for 5 min to pellet the stromal vascular fraction (SVF). Adipocytes were further purified for real-time PCR cytokine expression, and SVF was used for flow cytometry to determine macrophage content.

Flow cytometry. The SVF pellet obtained following adipose tissue digestion was suspended in fluorescence-activated cell sorting buffer following erythrocyte lysis. Counted SVF cells were incubated for 30 min at room temperature with the following fluorophore-conjugated primary antibodies: CD11b-FITC (BD Pharmingen, San Jose, CA), CD45-PerCP (BD Pharmingen), I-AgPE (BioLegend, San Diego, CA), and F4/80-Alexa 647 (BioLegend). Cells were analyzed on a BD upgraded fluorescence-activated cell sorting Caliber Flow Cytometer (8 colors) using FlowJo software (Tree Star, Ashland, OR). The macrophage content was defined by both F4/80- and CD11b-positive cells in a CD45-positive gate.

Real-time PCR. Adipocytes isolated from epididymal samples (as described above) were lysed in Trizol (Invitrogen, Carlsbad, CA), and RNA was isolated using a RNeasy kit from Qiagen (Valencia, CA) according to the manufacturer’s instructions. Isolated pancreatic islets were lysed in Trizol, and RNA was separated using the RiboPure kit (Ambion, Austin, TX). cDNA was prepared using a protocol, enzymes, and reagents provided by the Promega Reverse Transcription System (Promega, Madison, WI). Real-time PCR was performed using Taqman probes from Applied Biosystems (Carlsbad, CA). For assessing leukocyte 12-lipoxygenase expression, SYBR green with custom design primers was used. (forward: 5'-ctcaaggtctctgagga-3'; reverse: 5'-gtctgattcctgccagat-3'). All thermal cycling was performed in the CFX96 Thermal Cycler (Bio-Rad, Hercules, CA). β-Actin was used to normalize the data. Results were expressed by the 2—ΔΔCt method, with the nontreated group used as a control.

Immunohistochemistry. Epididymal adipose tissue was fixed in 10% buffered formalin overnight, then embedded in paraffin, and, following antigen retrieval, incubated with one of the following primary antibodies overnight at 4°C: rat anti-MAC2 antibody (1:100 dilution; Cedarlane Laboratories, Burlington, NC) or rabbit anti-12-lipoxygenase (1:100 dilution; Santa Cruz Biotechnology, Santa Cruz, CA). Detection was performed using the avidin-biotin peroxidase complex method, and slides were counterstained using Mayer’s hematoxylin. Tissue sections incubated with goat IgG (Pierce) instead of the primary antibody were used as method controls. To quantify MAC2 immunostaining, labeled cells were counted in eight to 10 different fields for each sample and expressed as number of positive cells/area unit. All pictures were captured with an Olympus microscope using ×200 magnification. To determine 12/15-lipoxygenase staining intensity, MetaMorph software version 6.3 (Molecular Devices, Downingtown, PA) was used, and data were normalized/section area analyzed.

Statistical analysis. Statistical analysis was performed using GraphPad Prism Software (GraphPad Software, La Jolla, CA). Student’s t-test paired analysis was used for GSIS data and unpaired analysis for all
other experiments. Data were expressed as means ± SE, and the null value was rejected for a P value <0.05.

RESULTS

Effects of sitagliptin on body weight, adiposity, and glucose homeostasis. C57Bl/6J male mice were placed on a 60% kcal fat diet for 12 wk, and sitagliptin (0.4 g/kg) was simultaneously administered as a food admix to one of the groups. Body weight was measured weekly. As shown in Fig. 1A, starting with week 5 of diet, sitagliptin-treated mice had significantly lower body weights compared with the untreated high-fed group. There was no difference in the major visceral fat pad weights, as shown in Fig. 1B, although there was a strong trend for a lower total visceral adiposity in the sitagliptin-treated group (n = 0.067). The comparable adiposity was also reflected in the BMI, which shows no significant difference between the treated and control groups (Fig. 1C). In a group of lean mice on a low-fat diet, sitagliptin did not have a significant effect on body weight, and body weight curves were comparable with the high-fat sitagliptin-treated group (Supplemental Fig. S1A; Supplemental Material for this article can be found online at the AJP-Endocrinology and Metabolism web site). Also, food intake was not significantly different between the treated and nontreated groups (Supplemental Fig. S1B). To determine whether sitagliptin exerted insulinotropic effects or affected insulin sensitivity, we measured glucose and insulin tolerance in mice on a high-fat diet with or without sitagliptin treatment. Fasting blood glucose was significantly lower in sitagliptin-treated mice, with no difference in the blood glucose between groups in randomly fed mice (Fig. 2A). Also, plasma insulin concentrations were significantly lower in the randomly fed mice treated with sitagliptin, whereas the fasting insulin levels tended to be lower in the sitagliptin-treated group (Fig. 2B). The postprandial hyperinsulinemia and increased fasting plasma glucose in high-fat-fed obese mice indicates the presence of impaired glucose tolerance that is ameliorated by sitagliptin treatment. These findings were confirmed by the GTT. GTT showed a significant difference in the area under the curve (AUC) between the beginning and end of the high-fat diet in the control group of mice (Fig. 3, A and C). However, in the sitagliptin-treated mice there was no significant difference in glucose tolerance between weeks 1 and 12 (Fig. 3, A and C). Also, the AUC was significantly higher in the control vs. sitagliptin-treated group at the end of the study (Fig. 3, B and C). The result suggests that endogenous insulin secretion is impaired in response to a glucose challenge in the high-fat group, and this impairment is not seen in the sitagliptin-treated group. Insulin tolerance tests showed no significant difference between the treated and nontreated groups either at the beginning or the end of the dietary regimens (Fig. 3, D–F). The results indicate that sitagliptin lowered circulating insulin in randomly fed mice and reduced fasting hyperglycemia in the obese mice on the high-fat diet.

Sitagliptin improves GSIS in isolated islets. To directly assess whether sitagliptin treatment improves insulin secretion in response to an increase in glucose, isolated islets were incubated in 3 and 28 mmol/l glucose. The basal insulin secretion (3 mmol/l) was not different between sitagliptin-treated and control groups (Fig. 4A). The islets from control mice responded with an increase in insulin secreted in the medium when challenged with 28 mmol/l glucose; however, the increase was not significant (Fig. 4A). In contrast, islets from sitagliptin-treated mice showed a significantly greater increase in insulin release during a 28 mmol/l glucose exposure. To further determine whether sitagliptin had effects on
islet morphology or number, paraffin-embedded sections were stained with hematoxylin and eosin and analyzed for islet number and area, neither of which showed differences between sitagliptin-treated and control groups (Fig. 4B). However, size distribution analysis showed a decrease in the percentage of large islets in the sitagliptin-treated group vs. nontreated high-fat control (area >0.02 mm$^2$, $P < 0.05$) and a relative increase in the percentage of small islets (area <1.000 $\mu$m$^2$, $P < 0.05$) (Fig. 4C).

**Sitagliptin reduces inflammatory cytokine expression in the pancreatic islets.** We next examined mRNA expression of several key proinflammatory cytokines in islets isolated from sitagliptin and control groups. This set of cytokines was chosen on the basis of previous data on C57Bl6/J mice showing an elevation of the proinflammatory cytokines in the IL-6/IL-12 pathways and a potential pathogenic role in insulin resistance and type 2 diabetes. The mRNA expression of the cytokines and chemokines measured in this study were all significantly increased in the high-fat diet-fed mice compared with lean, low-fat diet-fed counterparts (Supplemental Fig. S2A). For all of the cytokines tested, islets from sitagliptin-treated mice on the high-fat diet showed significantly reduced expression of the proinflammatory cytokines compared with the control group (Fig. 5). The most prominent decreases in mRNA expression were found for monocyte chemoattractant protein-1 (MCP-1; 3.3-fold), IL-12(p35) (5-fold), and IL-12(p40) (2.2-fold) ($P < 0.01$). Also, approximately twofold decreases in expression were detected for IL-6 and IP-10 ($P < 0.05$). In mice on the low-fat diet, sitagliptin treatment did not affect mRNA expression of the cytokines examined, suggesting a specific diet-related effect of the drug (Supplemental Fig. S2B).

**Effect of sitagliptin on adipocyte inflammation.** To investigate whether sitagliptin reduces inflammation in tissues other than pancreatic islets that are potential contributors to impaired glucose homeostasis, we examined proinflammatory cytokine expression in visceral adipocytes isolated from mice with or without sitagliptin treatment (Fig. 6). Also, leukocyte 12/15-lipoxygenase expression was measured (Fig. 7) since previous work from our group indicated a role of the enzyme in the adipose tissue inflammation and insulin resistance (43). Of the cytokines examined, there were significant ~2.5-fold decreases in IL-6 and TNF$\alpha$ expression ($P < 0.05$) and a 4.5- to fivefold decrease in expression of IL-12(p35) and IL-12(p40) ($P < 0.05$) in the adipocytes isolated from the sitagliptin-treated group vs. controls (Fig. 6). There was no significant difference in mRNA expression of the chemokines MCP-1 and C-X-C motif chemokine 10 (IP-10) (Fig. 6). In mice on the low-fat diet, mRNA expression of TNF$\alpha$, IL-6, IL-12(p35), and IL-12(p40) were significantly lower, by two- to 3.4-fold, compared with obese mice on the high-fat diet (Supplemental Fig. S3A). In addition, sitagliptin treatment of mice on the low-fat diet did not significantly change expression of any of the cytokines examined (Supplemental Fig. S3B). We also examined protein expression of 12/15-lipoxygenase enzyme in total adipose tissue by immunohistochemistry and mRNA expression by real-time PCR in isolated adipocytes (Fig. 7). The immunopositive cells were seen in both adipocytes (Fig. 7, dotted arrows) and immune cells localizing in crown-like structures (Fig. 7, solid arrows). Morphometric analysis revealed a significantly reduced level ($P < 0.01$) of 12/15-lipoxygenase protein in the adipose tissue from the sitagliptin-treated group (Fig. 7). Also, leukocyte 12/15-lipoxygenase levels were significantly reduced in adipocytes isolated from adipose tissue of sitagliptin-treated obese mice compared with untreated controls. Moreover, 12-lipoxygenase protein expression in adipose tissue of lean mice was not changed by sitagliptin treatment, showing a selective effect of the latter in adipose tissue of obese mice (Supplemental Fig. S4).

**Sitagliptin reduces macrophage infiltration in adipose tissue.** A hallmark of adipose tissue inflammation is immune cell infiltration. Macrophages are among the most abundant immune cells infiltrating adipose tissue after chronic high-fat feeding. We determined macrophage relative abundance in adipose tissue from high-fat-fed mouse groups using both immunohistochemistry and flow cytometry (Fig. 8, A and B). Immunopositive cells for Mac2 were significantly more numerous in the control compared with the sitagliptin-treated group, suggesting reduced inflammation by sitagliptin treatment (Fig. 8A). Furthermore, we digested the adipose tissue and analyzed the stromal vascular fraction component for the presence of macrophages and lymphoid cells. Macrophages were identified as cells positive for CD45/F4/80/CD11b (Fig. 8B). The relative percentage of macrophages of the total CD45$^+$ cells was 71.95 $\pm$ 2.96 in the high-fat group and was significantly reduced in the sitagliptin-treated group to 62.52 $\pm$ 5.85 com-

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**Fig. 2.** Effect of sitagliptin treatment on plasma glucose and insulin. **A:** fasted and fed plasma glucose were determined using a glucometer as baseline values in the insulin and glucose tolerance tests and at the end of the study. **B:** plasma insulin concentration was determined at the end of the study by immunoassay. Results are from $n = 12–14$ mice/group and expressed as means $\pm$ SE.
pared with controls ($P = 0.019$). The number of CD45$^+$ cells normalized to total adipose tissue was not significantly different between the two groups (data not shown). Also, the lymphoid cells characterized as CD45$^+/I$-Ab$^-/F4/80^-$ were comparable between the two groups (Fig. 8B). This indicates that, in addition to ameliorating inflammation in adipocytes, sitagliptin also reduced infiltration of macrophages in the adipose tissue in response to the high-fat feeding. In control lean mice, the number of macrophages infiltrating the adipose tissue was significantly lower compared with obese mice (1.22 ± 0.38 vs. 6.8 ± 1.1), and sitagliptin treatment did not significantly change the macrophage number (Supplemental Fig. S5A). This finding was confirmed by flow cytometry analysis of the CD11b$^+/F4/80^+$ double-positive cells that were not significantly changed by sitagliptin treatment in the lean mice (Supplemental Fig. S5B).

**Effect of sitagliptin on adipose tissue morphology.** Adipocyte hyperplasia is a known response to high-fat feeding in rodents and is correlated with altered metabolic function such as reduced insulin sensitivity and elevated lipolysis. We ana-
lyzed adipocyte number and size to establish whether sitagliptin treatment was associated with changes in adipose tissue morphology (Fig. 9). There was a trend for a reduced mean adipocyte size in the sitagliptin-treated group that was borderline significant ($P \approx 0.058$; Fig. 9C). Adipocyte number was not significantly changed by sitagliptin treatment (Fig. 9C). Analysis of adipocyte size distribution revealed an evident reduction in the number of very large adipocytes (area $>4,000 \, \mu m^2$) in the sitagliptin-treated mice compared with controls (Fig. 9, A and B). Also, the number of the very small adipocytes (area $<500 \, \mu m^2$) was higher in the sitagliptin-treated group (Fig. 9D). As shown in Fig. 1, the total visceral adipose tissue was not changed, which suggests that sitagliptin induced remodeling of visceral fat with lower numbers of very large adipocytes and larger numbers of small adipocytes, which is suggestive of an improved adipocyte metabolic phenotype. Sitagliptin had no significant effect on adipose tissue morphology in lean control mice (data not shown).

Taken together, these results show for the first time that sitagliptin can prevent or attenuate inflammation in both pancreatic islets and adipose tissue, which may in part explain the improved glucose tolerance in diet-induced obese mice.

**DISCUSSION**

Our study shows that in a high-fat-diet model of obesity and insulin resistance, sitagliptin reduces pancreatic islet and adipose tissue inflammation, concurrent with improved glucose metabolism. Also, sitagliptin improves islet function and reduces inflammation in visceral adipose tissue. The data suggest that sitagliptin has anti-inflammatory effects that enhance positive effects for metabolic and vascular function.
intraperitoneal glucose tolerance test in our study. One reason for the discrepancy in the results may be the longer treatment time in our study of 12 wk vs. 8 wk in the study by Lamont and Drucker (33). Sitagliptin improves blood glucose control by 10.220.32.247 on August 15, 2017 http://ajpendo.physiology.org/ Downloaded from

Sitagliptin significantly reduced body weight in high-fat-fed mice compared with untreated controls. This effect was somewhat surprising since the majority of the diabetic patients treated with sitagliptin do not experience significant weight loss (2). However, one other report in a similar animal model on high-fat diet reported significant loss of weight following sitagliptin treatment (33). In the same report, the authors found no difference in the oxygen consumption, locomotor activity, or food intake in mice after prolonged DPP IV inhibition by sitagliptin (33). Interestingly, mice with genetic ablation of the Dpp4 gene are resistant to the development of obesity (14). In our study, the reduction in body weight is not accompanied by a reduction in adiposity, although there is a strong trend for reduced adipose mass in sitagliptin-treated mice. Importantly, the effect of sitagliptin is present only in mice fed a high-fat diet. In lean mice, sitagliptin has no effect on body weight, indicating that the drug specifically attenuates body weight gain in response to high-fat feeding.

Several human and animal studies showed that sitagliptin treatment improves glucose tolerance and stimulates pancreatic insulin secretion in response to a glucose load in humans and animal models of type 2 diabetes, insulin resistance, and dietary obesity (1, 20–22, 33, 39). In accord with previous data, our study shows that 12-wk sitagliptin treatment in mice on 60% kcal fat diet improved glucose tolerance, reduced fasted blood glucose, and lowered plasma insulin in randomly fed mice compared with untreated insulin-resistant obese mice. However, in contrast to the study by Lamont and Drucker (33), we did find a significant reduction in glucose excursion during an intraperitoneal glucose tolerance test. One reason for the discrepancy in the results may be the longer treatment time in our study of 12 wk vs. 8 wk in the study by Lamont and Drucker (33). Sitagliptin improves blood glucose control by potentiating the effects of GLP-1 and GIP (21). Continuous, sustained activation of the incretin axis appears to be critical for optimal blood glucose control (34). Therefore, the length of the treatment may be critical in achieving better glucose control by long-term persistence of circulating GLP-1 and explain sitagliptin effect to improve glucose excursion in the intraperitoneal glucose tolerance test in our study.

To determine whether sitagliptin improved glucose-stimulated insulin secretion, we measured insulin secretion in response to low and high glucose in isolated islets (Fig. 4). This result reflects preserved insulin secretory response to a glucose challenge in sitagliptin-treated mice, consistent with an insulinotropic effect of GLP-1, likely elevated by sitagliptin. Also, extensive and sustained sitagliptin treatment may improve pancreatic insulin secretion by preserving islet mass in type 1 and type 2 diabetes and preventing β-cell apoptosis (39, 44). Interestingly, we found a change in the islet size distribution showing a significantly higher percentage of the small islets and reduced relative percentage of the very large islets. Islet hyperplasia is induced by very high-fat diets and is associated with reduced insulin sensitivity (37). This result may explain the better insulin secretory response of the islets from sitagliptin-treated mice in response to an in vitro glucose challenge. It is also possible that improvement in glucose tolerance by sitagliptin is also the result of reduced glucagon production and reduced hepatic glucose production and outputs, as shown previously (22).

Obesity, insulin resistance, and type 2 diabetes are associated with the presence of an inflammatory phenotype in both pancreatic islets and insulin target tissues, notably adipose tissue, in animal models and humans (19, 54).

Accumulating evidence shows that inflammation in the pancreatic islets impairs function and reduces insulin secretion in type 2 diabetes (17, 23). Production of proinflammatory lipid mediators and cytokines has been shown to induce islet apoptosis, reduce insulin secretion, and lead to progressive islet loss (18, 24). Importantly, increased cytokine production was reported in human islets treated in vitro with 12/15-lipoxygenase products such as 12-hydroxyeicosatetraenoic acid generated via the 12-lipoxygenase pathway (36). Also, laser-captured β-cells from patients with type 2 diabetes compared with nondiabetic controls have elevated levels of various cytokines and chemokines (38). Previous findings from our group, as well as results from this study, showed increased production of inflammatory cytokines in the islets of C57Bl/6 mice on high-fat diets (13, 43). Our present results are the first ones to show that prolonged DPP IV inhibition by sitagliptin reduces proinflammatory cytokine expression in adipocytes, pancreatic islets, and liver. Data are normalized to β-actin, and the fold change in expression in the sitagliptin vs. control group was calculated using the $2^{-\Delta \Delta Ct}$ method. Results are from $n = 7–10$ mice/group. *Statistically significant ($P < 0.05$) compared to HF group.
demonstrate that islets from mice treated with sitagliptin have reduced expression of several proinflammatory cytokines and chemokines compared with control untreated mice. Expression of both IL-12 subunits, as well as IL-6 expression, was reduced. IL-12 is a key cytokine driving Th1 lineage via production of IFNγ. Although IFNγ expression was not significantly decreased by sitagliptin, there was a strong trend showing reduced expression (data not shown). The effect of sitagliptin was selective in obese mice, with no effect in lean mice with lower levels of cytokine expression. Interestingly, a recent report shows that IL-12 is increased in serum of type 2 diabetic patients and is correlated with BMI and homeostatic model assessment of insulin resistance (52). Therefore, sitagliptin may have beneficial effects on insulin secretion by also reducing islet IL-12 expression and production. However, additional studies are needed to determine mechanisms that may impact on islet function due to increased expression of the IL-12 pathway in obesity and type 2 diabetes. IL-6 may have both positive and negative metabolic effects, and its role in obesity and type 2 diabetes remains controversial (4, 54). Although the majority of IL-6 is produced by the adipose tissue, pancreatic islets also express low levels of IL-6. We found a significant reduction of IL-6 in the pancreatic islets of sitagliptin-treated mice. In transgenic mice overexpressing IL-6 in pancreatic β-cells, a hyperplastic response and altered islet architecture was reported (10).

In addition to reduced cytokine expression, we also found a significant decrease in expression of the chemokines MCP-1 and IP-10 in islets from sitagliptin-treated mice compared with controls. Islets from patients with type 2 diabetes are infiltrated with macrophages, and human islets exposed to metabolic stress (elevated glucose and palmitate) release increased levels of cytokines and chemokines (25). An increased macrophage infiltration was also reported for the C57Bl/6 mice on high-fat
diets (25). MCP-1 is a potent macrophage chemoattractant and contributes to increased inflammation via macrophage islet homing and further cytokine production. MCP-1 can be produced by both the β-cells and the immune cells infiltrating the islets. Also, IP-10 is produced by the β-cell and by immune and vascular cells and is a potent chemoattractant for the T cells. Interestingly, IP-10 is elevated in diabetes (40, 46), and high glucose levels increase IP-10 production by human monocytes (16). Therefore, sitagliptin may reduce IP-10 islet expression indirectly via reducing blood glucose, attenuation of islet immune infiltration, or more direct effects on the β-cell. Consequently, multiple pathways may contribute to reduced chemokine production by sitagliptin in pancreatic islets. Additional studies would be important in elucidating the mechanisms accounting for the effects of sitagliptin on cytokine and chemokine production in pancreatic islets.

Adipose tissue inflammation is a major contributor to insulin resistance seen in central obesity. In accord with previous findings, we showed that proinflammatory cytokines as well as the 12/15-lipoxygenase pathway are increased in C57Bl/6J mice on high-fat diets (13, 43). In this study, we found that sitagliptin treatment reduces adipocyte expression of proinflammatory cytokines IL-6, TNFα, IL-12(p35), and IL-12(p40). These cytokines are known to act locally to impair adipocyte insulin sensitivity as well as systemically affect whole body insulin sensitivity (47, 58). Unlike the effect of sitagliptin in pancreatic islets, expression of MCP-1 and IP-10 was not changed in adipocytes by sitagliptin treatment. However, MCP-1 expression was reduced significantly in total adipose tissue (data not shown), suggesting that nonadipose cells, most likely immune cells, present in adipose tissue are a likely target for sitagliptin. Further studies will be needed to
evaluate whether sitagliptin has incretin-dependent or -independent effects on the adipocytes or on the immune cells infiltrating the adipose tissue. DPP IV (CD26) is known to modulate immune function, T cell proliferation, and macrophage migration (28, 45), and DPP IV inhibition reportedly reduced inflammatory cytokine production by the T cells and inhibited transendothelial migration of the latter (27, 45). Interestingly, a recent study showed that sitagliptin decreases NOD mice CD4+ T cell migration through incretin-dependent and -independent pathways (30). We showed that sitagliptin reduced macrophage infiltration in adipose tissue. Reduction of adipose tissue macrophages in obese rodents is positively associated with improved glucose tolerance and insulin sensitivity (47). Importantly, a recent study showed that DDP IV expression is increased in blood monocytes of obese human subjects (35). Therefore, DPP IV inhibition may have an additional therapeutic effect by limiting monocyte migration into adipose tissue and reducing local inflammation.

Previous data showed that 12-lipoxygenase expression is increased in adipose tissue of high-fat-fed, obese, insulin-resistant C57Bl/6J mice, and global deletion of the 12-lipoxygenase reduced adipose tissue inflammation and macrophage infiltration, concurrent with improved glucose tolerance and insulin sensitivity (43). In this study, we showed reduced immunopositive cells for 12/15-lipoxygenase in adipose tissue in sitagliptin-treated obese mice localized mainly in the crown-like structures but also in adipocytes, as further confirmed by mRNA expression data in purified adipocytes. 12-Lipoxygenase impairs insulin signaling in both adipocytes and macrophages (11) and enhances proinflammatory cytokine production (55). Therefore, it is conceivable that the 12-lipoxygenase pathway is one of the targets of sitagliptin and may in part explain the reduction of cytokine production in adipocytes and macrophage infiltration in adipose tissue.

We also found that in sitagliptin-treated mice the relative number of very large hypertrophic adipocytes is reduced, whereas the very small adipocyte population is relatively increased compared with control mice. The functional characteristics of adipose tissue apparently depend on the finer details of the adipose cell distribution (29), with a prevalence of large adipocytes conducive to increased proinflammatory cytokine production (49) and reduced adipocyte insulin sensitivity (5).
A recent report also showed that sitagliptin reduces adipocyte hypertrophy in C57Bl/6J mice on high-fat diets (50). The remodeling of adipose tissue in obese mice treated with sitagliptin suggests improved insulin sensitivity and correlates well with the reduced adipose tissue inflammation.

Collectively, our results indicate that sitagliptin treatment in obese insulin-resistant mice is associated with an improved metabolic phenotype and concurrent reduction of inflammation in pancreatic islets and adipose tissue. Therefore, DPP IV reduction may emerge as a beneficial treatment not only for improving insulin sensitivity but also for associated complications of type 2 diabetes characterized by chronic inflammation in adipose tissue and β-cells.

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DISCLOSURES

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REFERENCES


