Long-Term Treatment With Dipeptidyl Peptidase IV Inhibitor Improves Hepatic and Peripheral Insulin Sensitivity in the VDF Zucker Rat

A Euglycemic-Hyperinsulinemic Clamp Study

John A. Pospisilik,1 Sara G. Stafford,1 Hans-Ulrich Demuth,2 Christopher H.S. McIntosh,1 and Raymond A. Pederson1

Upon release into circulation, the potent insulin secretagogues glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are rapidly cleaved and inactivated by the enzyme dipeptidyl peptidase IV (DP IV). Long-term administration of specific DP IV inhibitors, so as to enhance circulating active GIP and GLP-1 levels, has been shown to improve glucose tolerance and β-cell glucose responsiveness and to reduce hyperinsulinemia in the Vancouver diabetic fatty (VDF) rat model of type 2 diabetes. Using the VDF model, the current study was undertaken to examine the effects of long-term DP IV inhibitor treatment on insulin sensitivity. Euglycemic-hyperinsulinemic clamp studies were performed on two sets of conscious VDF rats operated with or without the DP IV inhibitor P32/98 (20 mg · kg⁻¹ · day⁻¹ for 12 weeks). The protocol consisted of three sequential 90-min periods with insulin infusion rates of 0, 5, and 15 mU · kg⁻¹ · min⁻¹ and included a constant infusion of [³H]glucose for measure of hepatic and peripheral insulin sensitivity. Relative to untreated littersmates, the treated animals showed a left shift in the sensitivity of hepatic glucose output to insulin (average reduction ∼6 mol · kg⁻¹ · min⁻¹) and a marked gain in peripheral responsiveness to insulin, with glucose disposal rates increasing 105 and 216% in response to the two insulin steps (versus 2 and 46% in controls). These results provide the first demonstration of improved hepatic and peripheral insulin sensitivity after DP IV inhibitor therapy, and coupled with apparent improvements in β-cell function, they offer strong support for the utility of these compounds in the treatment of diabetes. Diabetes 51:2677–2683, 2002

From the 1Department of Physiology, University of British Columbia, Vancouver, British Columbia, Canada; and 2Probiodrug AG, Halle (Saale), Germany. Address correspondence and reprint requests to Dr. R.A. Pederson, Department of Physiology, University of British Columbia, 2146 Health Sciences Mall, Vancouver, BC, Canada V6T 1Z3. E-mail: pederson@interchange.ubc.ca.

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A.U.C., area under the curve; CISI, composite insulin sensitivity index; DP IV, dipeptidyl peptidase IV; EC₅₀, half-maximal concentration; FFA, free fatty acid; GDR, glucose disposal rate; GIP, glucose-dependent insulinotropic polypeptide; GIR, glucose infusion rate; ISI, insulin sensitivity index; GLP-1, glucagon-like peptide-1; HGO, hepatic glucose output; OGTT, oral glucose tolerance test.

Glucose-dependent insulinotropic polypeptide (GIP)₃–42 and glucagon-like peptide-1 (GLP-1)₉–36amide make up the endocrine arm of the enteroinsular axis, a concept describing the pathways that relay the presence of luminal nutrients in the small intestine to the endocrine pancreas, eliciting insulin release (1). Recent studies have highlighted the importance of GIP and GLP-1 not only as potent insulin secretagogues, but also as enhancers of β-cell function and stimulators of β-cell growth, survival, and differentiation (2–7). Upon release into the circulation, GIP and GLP-1 are rapidly degraded by the ubiquitous serine protease dipeptidyl peptidase IV (DP IV; EC 3.4.14.5), resulting in a circulating half-life of ∼1–2 min for the parent compounds (8–11). The NH₂-terminally truncated peptide products GIP₃–42 and GLP-1₉–36amide have been shown in vitro to be inactive at the receptor level (antagonist and partial agonist, respectively) and nonsulinotropic in both β-cell models and the perfused rat pancreas (12–14). DP IV–mediated NH₂-terminal cleavage has since been established as the primary mechanism of incretin inactivation, with a number of research groups making significant contributions (8–11,15). Identification of this physiological regulatory system has given rise to the development of specific DP IV inhibitors, a promising therapeutic paradigm involving protection of the full-length active forms of endogenously secreted GIP and GLP-1, and subsequent enhancement of their numerous antidiabetic effects (16–20).

Recently, we showed in the Vancouver diabetic fatty (VDF) rat (fa/fa, a model of type 2 diabetes) that long-term treatment with the DP IV inhibitor P32/98 produced lasting improvements in glucose tolerance, hyperglycemia, hyperinsulinemia, and β-cell glucose responsiveness (21). These findings were corroborated by two recent examinations of the long-term effects of DP IV inhibition in the ZDF rat (22) and type 2 diabetic humans (23). Oral glucose tolerance tests (OGTTs) as well as ex vivo soleus glucose uptake data from our previous study provided strong, yet indirect, evidence that insulin sensitivity was heightened in the DP IV inhibitor–treated animals (21). The following study was therefore undertaken to more accurately define the effects of DP IV inhibitor treatment on insulin sensitivity.
Toward these ends, a euglycemic-hyperinsulinemic clamp was performed on conscious VDF rats after 3 months of treatment with oral DP IV inhibitor (P32/98). The results provide the first demonstration that DP IV inhibitor therapy increases both hepatic and peripheral insulin sensitivity in the VDF model, providing strong support for the use of incretin-based therapies in the treatment of diabetes.

RESEARCH DESIGN AND METHODS

Materials. The DP IV inhibitor P32/98 (di-[25,38]-2-amino-3-methyl-phenylalanine-1,3-thiazolidine fumarate) was synthesized as previously described (24). Glucose for intravenous infusion was obtained from Abbott Laboratories (Montreal, PQ, Canada). All other chemicals were obtained from Sigma Canada (Toronto, ON, Canada) unless otherwise stated.

Animals. Twelve pairs of male VDF (fa/fa) littermates were randomly assigned to a control or treatment (P32/98) group at 400 g body weight (10 ± 0.5 weeks of age). Animals were housed on a 12-h light/dark cycle (lights on at 6:00 a.m.) and allowed ad libitum access to standard rat food and water. The treatment group received P32/98 (10 mg/kg) by oral gavage twice a day (8:00 a.m. and 8:00 p.m.) for 12 weeks, whereas the control animals received concurrent doses of 1% cellulose vehicle. The techniques used in this study were in compliance with the guidelines of the Canadian Council on Animal Care and were approved by the University of British Columbia Council on Animal Care (certificate A90-0006).

Assessment of oral glucose tolerance. After 11 weeks of treatment, an OGTT (1 g/kg) was performed after a 16-h fast and complete drug washout (~11 h after inhibitor P32/98) to (10 mmol/l Tris-HCl, 1 mmol/l EDTA, 0.25 mmol/l sucrose, and 50 mmol/l KCl, pH 7.2) and centrifuged for 30 min at 10,000g (4°C). After protein determination (BCAprot; Pierce, Rockville, MD), samples containing 100 μg protein were mixed with reaction buffer (50 mmol/l Tris-HCl, 2 mmol/l MnCl2, 2.5 mmol/l phosphoenolpyruvate, 10 mmol/l NaHCO3, 5 units/ml malate dehydrogenase, and 0.15 mmol/l β-NADH) in a 96-well plate. The reaction was initiated with the addition of 0.4 mmol/l dGDP (final concentration) and followed at 340 nm on a microtiter plate reader. One unit of PEPCCK activity corresponds to the conversion of 1 μmol β-NADH in 1 min. Plasma glucagon levels were measured using a COOH-terminally directed glucagon radioimmunoassay kit (Ab 1032K; Linco Research, St. Charles, MO).

Calculations and analysis. Hepatic glucose output (HGO) and glucose disposal rate (GDR) were calculated according to the method of Steele (27). In summary, HGO was calculated by subtracting the glucose infusion rate (GIR) from the tracer-determined rate of glucose appearance into the plasma compartment. Similarly, at steady state, GDR is equal to the sum of the rates of endogenous (HGO) and exogenous (GIR) glucose entry into the plasma compartment. Data (means ± SE) were compared using Prism 3.02 data analysis software (GraphPad Software, La Jolla, CA) within groups using a paired t test and between groups using a Student’s t test (P < 0.05).

RESULTS

In the current study, two groups of 11 VDF rats were treated with the DP IV inhibitor P32/98 for 12 weeks, after which they underwent a euglycemic-hyperglycemic clamp. Fourteen animals (seven per group) were successfully clamped (the remainder failed due to loss of aortic cannula patency). The data presented below are compiled from these 14 animals.

Oral glucose tolerance. One week before surgical preparation (week 11), an OGTT was performed on all animals. Animals were fasted, and inhibitor dosing was discontinued for 16 h, an interval sufficient to ensure complete drug washout. Plasma DP IV activity measurements made to confirm inhibitor washout showed a 41% increase in DP IV activity in the treated animals versus controls (33.3 ± 0.9 and 23.6 ± 0.6 nU/ml, respectively), a finding consistent with our previous study. Fasting plasma glucose levels in the treated animals were ~2 mmol/l lower than in control littermates (6.5 ± 0.3 vs. 8.6 ± 0.4 mmol/l), despite comparable fasting insulin levels (1.71 ± 0.23 and 1.69 ± 0.19 mmol/l, respectively) (Fig. 1). The difference in fasting blood glucose increased to nearly 4 mmol/l over the 120-min course of the OGTT, with glucose levels peaking at 10.4 ± 0.4 mmol/l in the treated group compared with 14.2 ± 0.7 mmol/l in the control group. Significant insulin responses were elicited in both groups despite marked hyperinsulinemia; however, the early-phase insulin response in the treated animals measured 260% of that of the controls (Fig. 1). Calculation of AUC revealed no difference in total insulin secretion between the two groups (data not shown). Analysis of these data according to a composite insulin sensitivity index (ISI) revealed mean ISI scores (arbitrary units) of 0.93 ± 0.07 and 1.46 ± 0.13 (P < 0.05) for the control and treated animals, respectively, suggesting enhanced insulin sensitivity after DP IV inhibitor treatment (Fig. 1, inset).

Euglycemic-hyperinsulinemic clamp. Baseline glucose, insulin, and DP IV activity (treated 35.6 ± 1.7, control 2678 DIABETES, VOL. 51, SEPTEMBER 2002

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\text{CISI} = \frac{1,000/(\text{FBG} \times \text{FPI})}{(\text{MG} \times \text{MI})^{1/2}}
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where FBG is the fasting blood glucose concentration, FPI is the fasting plasma insulin concentration, MG is the mean glucose concentration, and MI is the mean insulin concentration (area under the curve [AUC]/120 min) over the course of the OGTT.

Euglycemic-hyperinsulinemic clamps. After 12 weeks of treatment, animals were anesthetized with sodium pentobarbital (Somnotol; 36 mg/kg) and a midline incision was made on the ventral aspect of the neck. Chronic cœlia branches were then inserted into the left carotid artery (PE-50 cannula; Clay Adams) and the right jugular vein (dual PE-10 cannula encased in Silastic tubing; Dow Corning), brought around the neck subcutaneously, and passed through a small skin incision at the base of the neck. After ≥4 days of recovery with at least 2 days of consecutive weight gain, the animals underwent a euglycemic-hyperinsulinemic clamp. Treatment was discontinued the day of catheter implantation and re-initiated 2 days later; the final bolus of inhibitor was given at 5:00 p.m. the day before the clamp. The protocol consisted of three sequential 90-min periods: priming, which involved a bolus (2 μCi) followed by continuous infusion of [3-3H]glucose (0.03 μCi/min; Amersham), and two insulin infusion steps (HumulinR, Eli Lilly Canada, Montreal, PQ, Canada) of 5 and 15 mU · kg⁻¹ · min⁻¹, respectively (30 and 90 pmol · kg⁻¹ · min⁻¹). Blood samples (30 μl) were taken every 5 min and centrifuged briefly, and the plasma was analyzed for glucose using the glucose oxidase method (Beckman Glucose Analyzer 2; Beckman Instruments, Palo Alto, CA). During the final 30 min of each period, three 150-μl blood samples were taken at 15-min intervals for determination of plasma tracer and insulin levels. Samples were deproteinized (Ba(OH)2/ZnSO4) and evaporated to dryness (to remove [3H]H2O), and tracer levels were measured using a liquid scintillation counter after resuspension in distilled H2O.

Inhibition of lipolysis in adipocytes. Samples of epididymal adipose tissue (~3 cm³) were obtained under anesthesia 3 days after the clamp procedure. After a 10-min collagenase digestion (0.5 mg/ml), recovered adipocytes were washed three times and allowed to stabilize for 1 h in 37°C Krebs buffer repetitively gassed with 95% O2, 5% CO2. Aliquots of adipocyte suspension (2 ml) were then prestimulated for 10 min with 0, 0.3, 0.6, 1.5, or 4.75 mmol/l insulin, after which lipolysis was stimulated with a maximally stimulating dose of isoproterenol (10⁻⁷ mol final concentration). The reaction was allowed to proceed for 30 min at 37°C, after which the samples were boiled for 10 min and centrifuged at 4°C for 15 min (12,000g). The aqueous phase of the supernatant was recovered and stored at ~70°C. Glycerol determinations were made using a colorimetric glycerol kit (Boehringer Mannheim).

Measurement of PEPCCK activity and plasma glucagon levels. PEPCCK activity was measured as described: liver samples (0.5 g) were homogenized in 1 ml homogenization buffer (10 mmol/l Tris-HCl, 1 mmol/l EDTA, 0.25 mmol/l sucrose, and 50 mmol/l KCl, pH 7.2) and centrifuged for 30 min at 10,000g (4°C). After protein determination (BCAprot; Pierce, Rockville, MD), samples containing 100 μg protein were mixed with reaction buffer (50 mmol/l Tris-HCl, 2 mmol/l MnCl2, 2.5 mmol/l phosphoenolpyruvate, 10 mmol/l NaHCO3, 5 units/ml malate dehydrogenase, and 0.15 mmol/l β-NADH) in a 96-well plate. The reaction was initiated with the addition of 0.4 mmol/l dGDP (final concentration) and followed at 340 nm on a microtiter plate reader. One unit of PEPCCK activity corresponds to the conversion of 1 μmol β-NADH in 1 min. Plasma glucagon levels were measured using a COOH-terminally directed glucagon radioimmunoassay kit (Ab 1032K; Linco Research, St. Charles, MO).
24.4 ± 1.0 mU/ml) values measured at the outset of the clamp were comparable to those observed during the OGTT 10 days earlier; the treated group exhibited reduced fasting plasma glucose concomitant with unchanged plasma insulin values (Fig. 2). Fasting plasma glucose levels appeared slightly elevated under clamp conditions, a phenomenon attributable to the difference in measurement technique, and in keeping with our own observations and with the literature on measurement of whole blood versus plasma glucose levels (28). Figure 2 clearly shows that steady-state insulin levels between groups were comparable, rising 0.7 nmol/l during the first insulin step and a further 2 nmol/l during the second. These values, although hyperinsulinemic, correspond closely to plasma values measured both during the OGTT (Fig. 1) and during normal feeding (21). P32/98-treated animals displayed an immediate requirement for exogenous glucose infusion (GIR) in response to 5 mU · kg⁻¹ · min⁻¹ insulin; under the same conditions, control animals showed a reduced and significantly delayed (~25 min) response to the same stimulus (Fig. 2). Elevation of the insulin infusion rate to 15 mU · kg⁻¹ · min⁻¹ elicited a further increase in GIR in both groups, with plateau levels reaching 39.2 ± 5.3 and 26.8 ± 4.5 μmol · kg⁻¹ · min⁻¹ in the treated and control groups, respectively.

HGO, calculated from plasma tracer levels, was significantly reduced in the treated animals compared with controls during each stage of the clamp (Fig. 3A and B). Basal HGO averaged 6.5 ± 0.9 μmol · kg⁻¹ · min⁻¹ less in the treated than in the control group (12.7 ± 0.9 μmol · kg⁻¹ · min⁻¹). A difference of similar magnitude was found at each insulin infusion level (Fig. 3A and B). Calculation of GDR revealed little or no response to insulin in the control animals, consistent with previous clamp studies of obese Zucker rats (29). GDR in the treated group, however, showed a return of insulin responsiveness, with steady-state levels 105 and 216% above basal during each of the two insulin steps, respectively (Fig. 4). Basal GDR in the treated animals was reduced relative to controls (Fig. 4).

PEPCK and glucagon determinations. To further investigate the changes in hepatic insulin sensitivity, liver PEPCK levels and fasting plasma glucagon levels were measured. Measurement of PEPCK activity in liver samples obtained at termination showed no significant difference between the treated and untreated animals, suggesting a non-PEPCK-dependent pathway for the left shift in insulin sensitivity. PEPCK activity averaged 25.9 ± 2.9 and 31.5 ± 4.3 mU/mg tissue in the treated and control groups, respectively (Fig. 3C). Values for the treated animals were comparable to those obtained in control Wistar rats (24.7 ± 0.2 mU/mg).

Inhibition of lipolysis in adipocytes. Using glycerol release as an indicator, inhibition of isoproterenol-
stimulated lipolysis by insulin was examined in isolated epididymal adipocytes (Fig. 5). Concentration response of inhibition by insulin showed a left shift in adipocytes isolated from treated animals compared with controls; estimated half-maximal concentrations (EC50 values) were 0.29 ± 0.01 and 1.11 ± 0.01 nmol/l, respectively (*P < 0.01).

**DISCUSSION**

The protection of full-length GIP1-42 and GLP-17-36 amide (incretins) in the circulation using DP IV inhibitors represents a significant advancement in the search for new and effective alternative treatments for diabetes. Since the early 1990s, numerous studies have revealed a pleiotropy of antidiabetic effects triggered by interaction of the incretins with their respective cell-surface receptors. Among them are stimulation of β-cell glucose competence, proliferation, differentiation, growth, and cell survival; inhibition of glucagon secretion; and several reports indicating stimulation of glucose uptake in muscle cells (2-7,30,31). Recently, we showed that long-term DP IV inhibitor therapy caused marked improvements in glucose tolerance, hyperinsulinemia, and β-cell function in the VDF rat, findings that highlight the potential utility of these compounds in diabetes therapy. In the same study, OGTT data and an in vitro determination of insulin-stimulated glucose uptake in isolated muscle provided indirect evidence for a treatment-induced improvement in insulin sensitivity. The present study was carried out to characterize the effects of DP IV inhibitor therapy on insulin sensitivity in the same model, using a euglycemic-hyperinsulinemic clamp. The data collected constitute the first conclusive demonstration that DP IV inhibitor therapy improves both hepatic and peripheral insulin sensitivity.

Extensive literature exists on the obese Zucker rat, including numerous studies examining insulin resistance by means of euglycemic-hyperinsulinemic clamp (32). In one such study, Terrettaz et al. (29) tested HGO and GDR responses in obese Zucker rats over a wide range of insulin concentrations, allowing a comprehensive evaluation of both responsiveness (the efficacy or magnitude of the response to insulin) and sensitivity (the potency or EC50 of the response to insulin) to the hormone. The authors concluded that the obese animals demonstrate marked hepatic insulin resistance (characterized by a fully responsive yet right-shifted HGO and severely impaired glucose disposal (characterized by a total lack of responsiveness over a wide range of insulin concentrations) compared with lean littermates (29). As in these early reports, the control VDF rats in the current study showed a responsive yet right-shifted hepatic glucose response to insulin and an extremely blunted peripheral response (Figs. 3 and 4). It is apparent that long-term treatment with the DP IV inhibitor P32/98 partially reversed both of these...

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**FIG. 3.** Steady-state hepatic glucose output (A and B) calculated during a euglycemic-hyperinsulinemic clamp in conscious VDF rats (n = 7) treated for 12 weeks with the DP IV inhibitor P32/98 (○, ○) or a 1% cellulose control solution (■). At termination, liver biopsies were obtained from the same animals for measurement of PEPCK activity (C). Fasting plasma glucagon levels (D) were measured at the outset of the experiment as well as 1 week before the clamp. [Insulin]pl, plasma insulin. *P < 0.05, **P < 0.01 vs. controls.

**FIG. 4.** Steady-state GDR calculated during a euglycemic-hyperinsulinemic clamp in conscious VDF rats (n = 7) treated for 12 weeks with the DP IV inhibitor P32/98 (○) or a 1% cellulose control solution (■). Raw data (A) and data normalized to basal (B) are shown. *P < 0.05, **P < 0.01 vs. controls; #P < 0.05 vs. basal.
Adipocytes were isolated from epididymal fat pads by collagenase.

The finding that P32/98 treatment improves hepatic insulin sensitivity supports our previous work showing reduced hyperinsulinemia in the basal fed state and reduced fasting plasma glucose in the same model (21). Similar reductions in insulinemia and fasting plasma glucose have been shown in a number of studies using long-term GLP-1 (or GLP-1 mimetic) treatment (36–38). In keeping with the previous discussion of HGO sensitivity to insulin, the defining feature of P32/98 treatment on hepatic insulin sensitivity appeared to be a left shift in insulin responsiveness. Because fasting insulin levels did not differ between groups, a reduction in basal HGO in the treated animals is implicit. And because basal (fasting) glucose output from the liver is primarily determined by the rates of gluconeogenesis and glycogenolysis (both of which are potently stimulated by glucagon), a potential underpinning for these results might have been a reduction in gluconogenic enzyme expression, a group of enzymes that exhibit coordinate hormonal expression with respect to insulin and glucagon. Because PEPCK does not undergo short-term regulation via phosphorylation or allosteric effectors and because, physiologically, it is rate limiting for gluconeogenesis, PEPCK activity measurement provides an indicator of gluconogenic potential. Although a nonsignificant (~15%) decrease in PEPCK activity was demonstrated in the treated animals, the exact mechanisms responsible for the HGO sensitivity shift remain unclear and warrant further study (Fig. 3C).

Whereas treatment-induced alterations in HGO preferentially affected sensitivity to insulin rather than responsiveness, alterations in GDR appeared to comprise shifts in both responsiveness and sensitivity to insulin. Control animals displayed a basal GDR of 12.6 ± 1.1 μmol · kg⁻¹ · min⁻¹, nearly twofold that of the treated group (7.1 ± 1.2 μmol · kg⁻¹ · min⁻¹). Further, the P32/98-treated group displayed two- and threefold responses to 5 and 15 mU · kg⁻¹ · min⁻¹ insulin, respectively (Fig. 4B), whereas the control animals showed their first sign of peripheral insulin responsiveness (46%) only during the latter infusion step. These data suggest a marked left shift in insulin-stimulated peripheral glucose uptake and are consistent with our previous demonstration of increased glucose uptake in soleus muscle (21) and with several reports of incretin-stimulated increases in glucose uptake (30,39). A number of studies using GLP-1 receptor agonists over the long term have suggested similar improvements in insulin sensitivity (38). It is unclear whether direct acute incretin effects on glucose disposal, as reported by some groups (40,41) and refuted by others (42), play a role.

On that note, although the literature on DP IV inhibition focuses primarily on a mechanism of action involving the enhancement of circulating active GIP and GLP-1, the role of other peptide substrates of DP IV in the improvements evidenced here, and previously, should not be discounted. Natural substrates of DP IV include all tested members of the glucagon/vasoactive intestinal polypeptide (VIP) superfamily of polypeptides (43), including glucagon (44,45), as well as a number of neuroendocrine and immune factors (46). Many of these peptides play significant roles in the regulation of energy metabolism and are likely to contribute toward the improvements resulting from DP IV inhibitor treatment. For instance, recent work by Huypens et al. (47) has highlighted the importance of the counter-regulatory hormone glucagon in the maintenance of glucose competence of the β-cell and in proper insulin secretion. Considering that blood glucose is maintained by circulating glucagon levels for approximately two-thirds of the 24-h cycle, DP IV inhibitor–induced enhancement of NH₂-terminally intact glucagon (active; glucagon₁₋₂₉) is likely to contribute toward the reported improvements in β-cell function.

In addition to the examination of HGO and GDR in vivo, an in vitro examination of adipocyte insulin sensitivity was performed 3 days after the clamp. The VDF Zucker rat displays excessive fat accumulation and pronounced hyperlipidemia (including elevated free fatty acids [FFAs]), pathologies intimately associated with human type 2 diabetes. It has been suggested that >50% of the insulin resistance in diabetic patients is FFA-induced, with elevated plasma FFAs stimulating insulin secretion, peripheral glucose underutilization, and hepatic glucose overproduction (increased gluconeogenesis) (48,49). Physiologically, these FFA effects likely serve to preserve glucose stores when supply is limited. However, in times of plenty (e.g., a typical Western diet), these effects become counterproductive, inhibiting the utilization of glucose (48,49). The demonstration that insulin-induced inhibition of lipolysis is sensitized in adipocytes from DP IV inhibitor–treated

**FIG. 5.** Inhibition of isoproterenol-stimulated lipolysis by insulin in adipocytes isolated from VDF rats (n = 7) treated for 12 weeks with the DP IV inhibitor P32/98 (○) or a 1% cellulose control solution (■). Adipocytes were isolated from epididymal fat pads by collagenase digestion.

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**Graph:**

- **Glycerol Release (% basal)**
- **Insulin Concentration (nmol/l)**

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**Legend:**

- ○: P32/98 treatment
- ■: 1% cellulose control solution

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**Data:**

- Basal GDR for treated group: 12.6 ± 1.1 μmol · kg⁻¹ · min⁻¹
- Basal GDR for control group: 7.1 ± 1.2 μmol · kg⁻¹ · min⁻¹
- Two- and threefold responses to 5 and 15 mU · kg⁻¹ · min⁻¹ insulin, respectively (Fig. 4B)

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**Figure Description:**

Graph showing inhibition of isoproterenol-stimulated lipolysis by insulin. The graph displays two- and threefold responses to 5 and 15 mU · kg⁻¹ · min⁻¹ insulin, respectively (Fig. 4B), whereas the control animals showed their first sign of peripheral insulin responsiveness (46%) only during the latter infusion step. These data suggest a marked left shift in insulin-stimulated peripheral glucose uptake and are consistent with our previous demonstration of increased glucose uptake in soleus muscle (21) and with several reports of incretin-stimulated increases in glucose uptake (30,39). A number of studies using GLP-1 receptor agonists over the long term have suggested similar improvements in insulin sensitivity (38). It is unclear whether direct acute incretin effects on glucose disposal, as reported by some groups (40,41) and refuted by others (42), play a role.

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**Conclusion:**

- Treatment-induced alterations in HGO preferentially affected sensitivity to insulin rather than responsiveness, alterations in GDR appeared to comprise shifts in both responsiveness and sensitivity to insulin.
- Treatment with DP IV inhibitor P32/98 improved hepatic insulin sensitivity in VDF Zucker rats, as evidenced by decreases in hyperinsulinemia and fasting plasma glucose.
- In vitro examination of adipocyte insulin sensitivity showed increased sensitivity to insulin stimulation following DP IV inhibitor treatment.

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**Additional Information:**

- PEPCK activity was measured as an index of gluconogenic enzyme expression, which exhibits coordinate hormonal expression with respect to insulin and glucagon.
- Treatment with P32/98 led to a reduction in basal HGO, implicating potential changes in gluconogenic enzyme expression.
- The graph shows inhibition of isoproterenol-stimulated lipolysis by insulin, with significant differences observed between treated and control groups.

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animals suggests a potential mechanism of action for the improvements in hepatic and peripheral insulin sensitivity discussed above. A reduction in FFA release from adipocytes, secondary to a sensitized inhibition of lipolysis by insulin, might attenuate the glucose sparing effects of plasma FFAs, thereby reducing the severity of insulin resistance. These findings warrant further investigation into the effects of DP IV inhibition on lipid metabolism.

The success of DP IV inhibitors as a therapeutic strategy in the treatment of diabetes is owed in great part to the pleiotropic nature of its primary effectors, the incretins GIP and GLP-1. Previously believed to be mere enhancers of β-cell function, the incretins are being shown to possess numerous non-insulin-dependent functions, including stimulation of cell survival and modulation of peripheral energy disposal (liver and muscle) (50). The findings of this study corroborate such reports and further exemplify the importance of the non-insulinotropic effects of GIP and GLP-1 in the regulation of glucose homeostasis. In conclusion, the addition of improved hepatic and peripheral insulin sensitivity to the list of beneficial metabolic effects of long-term DP IV inhibitor therapy provides strong support for the use of these compounds in the treatment of diabetes.

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