Exenatide (Exendin-4) Improves Insulin Sensitivity and β-Cell Mass in Insulin-Resistant Obese fa/fa Zucker Rats Independent of Glycemia and Body Weight

Bronislava R. Gedulin, Svetlana E. Nikoulina, Pamela A. Smith, George Gedulin, Loretta L. Nielsen, Alain D. Baron, David G. Parkes, and Andrew A. Young

Amylin Pharmaceuticals, Inc., San Diego, California 92121

The effects of the incretin mimetic exenatide (exendin-4) on metabolic parameters, insulin sensitivity, and β-cell mass were examined in nondiabetic, insulin-resistant obese fa/fa Zucker rats. After 6 wk of treatment, ad libitum-fed exenatide-treated (EX) and pair-fed vehicle control (PF) rats had comparable food intake, body weight, hemoglobin A1c (HbA1c), and fasting plasma concentrations of glucose, insulin, and lipids. Concurrent decreases in food intake and weight gain were observed in EX and PF rats, compared with ad libitum-fed vehicle control (CON) rats (P < 0.001). The increases in HbA1c and fasting plasma insulin concentrations that occur during the normal progression of this disease model were significantly reduced in EX and PF rats, compared with CON rats (P < 0.001). The insulin sensitivity index (ISI; glucose infusion rate to plasma insulin concentration) measured during a hyperinsulinemic euglycemic clamp was 224% higher in EX rats than CON rats (P < 0.001) and 61% higher in EX rats than PF rats (P < 0.004). The latter difference was despite comparable HbA1c, fasting glucose, fasting insulin, total cholesterol, high-density lipoprotein, and daily food consumption between EX and PF animals. In the absence of exenatide, β-cell mass was hyperbolically related to ISI (β-cell mass ∝ ISI was constant). Analogous to the disposition index, the β-cell mass ∝ ISI product was 63% greater in EX than PF rats (P < 0.05). Thus, exenatide increased β-cell mass to a greater extent than would be expected in animals of comparable insulin resistance, suggesting a direct trophic effect on islet neogenesis in obese fa/fa rats independent of body weight and glycemia. (Endocrinology 146: 2069–2076, 2005)
However, in contrast to the ZDF rat strain, plasma glucose concentrations in nondiabetic obese fa/fa Zucker rats are normal or only mildly elevated, compared with age-matched lean Zucker rats, because they are able to accommodate insulin resistance with a compensatory islet hypertrophy and hyperinsulinemia. Thus, the nondiabetic obese fa/fa Zucker rat can be used to study effects on insulin resistance that are somewhat independent of glycemic improvement.

Loss of glucose in the urine of diabetic ZDF rats, reversal of this caloric loss with glucose-lowering doses of exenatide, and a simultaneous reduction in caloric intake, are factors that confound the identification of insulin-sensitizing mechanisms in the diabetic rat model. To help identify the contribution of a decreased caloric intake, effects in exenatide-treated animals were compared with effects in pair-fed animals restricted to the same food intake. The effect of exenatide on β-cell mass was also examined. Because β-cell mass is sensitive to glycemia and insulin demand, effects needed to be considered in a context in which these influences were equalized or otherwise accommodated. We show here that in the nondiabetic fa/fa rat model of insulin resistance, exenatide exhibits an insulin-sensitizing effect that is partially, but not fully, attributable to changes in food intake and largely independent of glycemic improvement. Independent of effects on metabolic parameters, exenatide also exhibits a trophic effect on β-cell mass.

Materials and Methods

Animals

Male obese fa/fa Zucker rats were housed at 22 ± 1°C in a reversed 12-h light, 12-h dark cycle and allowed ad libitum access to a standard rodent diet (LM-485; Teklad, Madison, WI) and water. All studies were approved by the Institutional Animal Care and Use Committee and conformed to U.S. Department of Agriculture guidelines.

Treatment regimen

Studies were initiated when the rats were approximately 9 wk of age, after at least 1 wk of acclimatization to the animal facility. Fasting plasma samples for measurement of glucose, insulin, lipids, and hemoglobin A1c (HbA1c) were collected from the tail in anesthetized (Hurricane: 20% topical benzocaine solution, Beutlich, Waukegan, IL) on d 0, 14, 28, and 41 from rats fasted approximately 15 h. Food intake and body weight were measured daily. On d 0, animals were distributed on the basis of matched HbA1c into the following three treatment groups: vehicle, 100 µl of 0.9% saline twice daily (BID) fed ad libitum (CON, n = 11); exenatide, 5 µg/kg BID fed ad libitum (EX, n = 10); and pair-fed vehicle, 100 µl of 0.9% saline BID pair fed with EX rats (PF, n = 10). Treatment was continued for 6 wk and discontinued 24 h before the animals underwent a euglycemic hyperinsulinemic clamp procedure. The purpose of the 24-h delay was to ensure that a negligible fraction of the estimated 600 pM maximum concentration after sc dosing remained, thereby allowing the residual effect of treatment on insulin sensitivity and β-cell function to be measured, unconfounded by ongoing incretin activity. For the sc exenatide dose used in the present study, a 24-h period encompasses approximately 16 half-lives (6), leaving approximately 0.01 pmol (a concentration unassociated with any reported response) at the time of the clamp.

Hyperinsulinemic euglycemic clamp procedure

Clamp procedures (38) were performed after an overnight fast (CON, n = 9; EX, n = 9; PF, n = 8). Five rats that completed 6 wk of treatment and contributed plasma analytes did not complete the clamp procedure. The rats were tracheotomized and catheterized via the femoral artery to record arterial pressure (Biopac Systems, Santa Barbara, CA) and for glucose/lactate measurements (YSI 2300 STAT analyzer; YSI Inc., Yellow Springs, OH) and via the femoral vein for infusion of recombinant human insulin (Humulin R, Eli Lilly, Indianapolis, IN) and 20% glucose. Animals were maintained under isoflurane anesthesia for the entire procedure, starting 60 min before glucose infusion (t = −60 min). Saline (0.9% at 1 ml/h) was infused into the femoral artery to maintain fluid homeostasis, starting at t = −60 min. At t = −30 min, the rats were administered an iv 180 nmol/kg (30 U/kg) bolus of insulin, followed by a continuous infusion (14.5 mmol/liter; 2.4 U/ml) at a rate of 0.6 mmol/kgmin (100 mU/kg/min). Plasma glucose concentrations were monitored every 10 min. When glucose concentrations approached euglycemia, 20% glucose was infused at a variable rate to maintain glucose concentrations of 5.8 mmol (105 mg/dl) (start at t = 0 min). The clamp was continued for a further 3 h. Samples for glucose were taken at intervals starting at t = −60 min and ending at t = 180 min. Samples for insulin measurements were taken at t = 0, 45, 150, and 180 min. The insulin sensitivity index (ISI) at 180 min was calculated by dividing the glucose infusion rate required to maintain euglycemia by the steady-state plasma insulin concentration.

Assays

For the 6-wk treatment period, endogenous rat insulin was quantified using a rat insulin RIA (Linco Research Inc., St. Charles, MO). For the clamp studies, human insulin was quantitated using a human insulin RIA (Linco). The Cobas Mira Plus (Roche Diagnostics, Indianapolis, IN) was used for fasting glucose and lipid measurements. The DCA2000 (Bayer Corp., Elkhart, IN) was used for measuring HbA1c.

Histological assessment of β-cell mass

A subset of rats completing the clamp procedure was killed by isoflurane overdose and cervical dislocation (CON, n = 5; EX, n = 6; PF, n = 5). The pancreas was removed from each animal, cleared of fat and lymph nodes, weighed, and fixed in 3.7% formaldehyde in PBS (pH 7.4), for histology in a flattened position. Each pancreas was separated into gastrosplenic (head) and duodenal/biliary (body) portions, and tissue was sequentially transferred to 15 and 30% sucrose, and Tris-buffered saline tissue-freezing media (Fisher Scientific, Los Angeles, CA) and frozen at −70°C before sectioning.

Frozen tissue sections 15 µm thick were prepared from pancreata using a model OT cryostat (Bright Instruments Co. Ltd., Cambridge, UK) and placed on Superfrost/Plus slides (Fisher Scientific) at −20°C. Fifty consecutive sections from each block of tissue (two per pancreas) were prepared. Insulin was detected immunohistochemically using a guinea pig antihuman insulin serum (Linco) as the primary antibody. Relative to human insulin, this serum is 46% cross-reactive to rat insulin and does not detectably react with glucagon, somatostatin, or pancreatic polypeptide. An antirat insulinkin antibody (Southern Biotechnology Associates) was used at a 1:100 dilution, and the respective antihuman insulin serum at a 1:100 dilution. The antirat insulinkin antibody was conjugated with CY2 (Jackson Immunoresearch Laboratories Inc., West Grove, PA) on the tissue sections (each sixth specimen of consecutive sections). Each section was also counterstained with hematoxylin (S3309, Dako, Carpenteria, CA) and mounted in aqueous media with antifading agents (Biomedica BM-811, Fisher Scientific). Slides were read using a BH2 fluorescent microscope with a SpotRT digital camera (Olympus, Temecula, CA) and analyzed using Image-Pro Plus 4.1 software (DataCell Ltd., North Chelmsford, MA). Ten sections for each pancreas were stained and examined blindly by two investigators. Six representative sections for each pancreas were chosen for analysis. Each whole section was assayed for the presence and quantity of β-cells, and a minimum of 120 images of β-cells per pancreas were assayed.

β-Cell area was quantitated using the total area immunostained for insulin in multiple representative tissue sections from both portions of the pancreas, similar to the methodology described in Finegood et al. (39). This system consisted of an Olympus BH2 fluorescent microscope (×10 objective) equipped with a SpotRT digital camera (Olympus) and Image-Pro Plus 4.1 software (DataCell Ltd.). The system was pixel calibrated. The relative cross-sectional area of β-cells was determined by quantification of the area occupied by fluorescently labeled β-cells (all fields with β-cells present, a minimum of 20 images) and the cross-sectional area of all tissue determined on hematoxylin-stained slides. β-Cell areas were determined by marking the threshold of the captured image for green fluorescence and the total tissue for blue color. β-Cell

Gedulin et al. • Exenatide and Insulin Sensitivity


Downloaded from https://academic.oup.com/endo/article-abstract/146/4/2069/287820 by guest on 19 May 2018
mass per piece was estimated as the product of the cross-sectional area of β-cell/tissue and the weight of the pancreas piece before fixation. Total β-cell mass was the sum of the estimates from the two pieces of pancreas. β-Cell mass determinations were performed in a blinded fashion by two individuals. Total β-cell mass was calculated by multiplying total pancreatic weight by the fraction of the pancreatic cross-section occupied by insulin-containing cells.

**Statistical analysis**

Statistical significance between groups was evaluated by unpaired Student’s t test or testing for general effects by one-way ANOVA followed by Tukey-Kramer multiple comparisons (InStat, version 3.06, GraphPad Software Inc., San Diego, CA). Monotonic association was tested using Spearman’s rank correlation (InStat, version 3.06, GraphPad Software). All values are presented as mean ± SEM.

**Results**

**Metabolic parameters**

Changes in plasma analytes after 6 wk treatment are shown in Table 1. By design, all treatment groups had similar baseline HbA1c values. After 6 wk of treatment, EX and PF obese fa/.fa Zucker rats remained well matched with regard to average daily food intake, body weight, HbA1c, fasting plasma glucose concentration, fasting plasma insulin concentration, and fasting plasma total cholesterol concentration (P > 0.05 for each analyte). In contrast, food intake, body weight, HbA1c, and fasting plasma insulin concentration were all significantly reduced in EX and PF, compared with the corresponding values in the CON group (P < 0.05 for each analyte). Weight gain was significantly less in EX and PF rats (166 ± 8 and 181 ± 11 g, respectively), compared with weight gain in CON rats (237 ± 6 g; P < 0.001 for each comparison).

Whereas HbA1c increased in CON rats after 6 wk of saline treatment (P < 0.001), increases from baseline in EX and PF rats were not significant (P > 0.05). EX and PF thereby decreased HbA1c relative to CON at 6 wk (P < 0.001 for each comparison). Fasting plasma glucose concentrations did not differ among treatment groups (P > 0.05).

Fasting plasma insulin concentration similarly increased in CON rats (P < 0.001 vs. baseline). Values in EX and PF rats did not change and thus became different from CON at 6 wk (P < 0.001 for each comparison).

Fasting plasma total cholesterol concentrations increased over 6 wk in all treatment groups (P < 0.03 vs. baseline) but did not differ among treatment groups (P > 0.05 for each comparison). Neither were there differences among groups in posttreatment fasting plasma high-density lipoprotein (HDL) concentrations (P > 0.05 for each comparison), even though plasma HDL concentrations increased in all groups (P < 0.001 vs. baseline).

**Effects on insulin sensitivity measured by euglycemic clamp**

Results from hyperinsulinemic euglycemic clamps performed approximately 24 h after the last treatment and after an overnight fast are shown in Fig. 1. Plasma insulin concentrations during euglycemia (time weighted for t = 0–180 min) did not differ among EX, PF, and CON rats (180 ± 11, 184 ± 7, and 175 ± 6 nm, respectively; P > 0.05). Plasma

---

**TABLE 1. Effects of 6 wk of exenatide treatment on metabolic parameters in nondiabetic, insulin-resistant obese fa/.fa Zucker rats**

<table>
<thead>
<tr>
<th></th>
<th>CON (n = 11)</th>
<th>EX (n = 10)</th>
<th>PF (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>356 ± 6</td>
<td>353 ± 12</td>
<td>349 ± 5</td>
</tr>
<tr>
<td>wk 6</td>
<td>593 ± 8</td>
<td>519 ± 16a</td>
<td>530 ± 11b</td>
</tr>
<tr>
<td>Difference</td>
<td>+237 ± 6</td>
<td>+166 ± 8</td>
<td>+181 ± 11</td>
</tr>
<tr>
<td>Within-group comparison</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Food Intake (g/d) wk 6</td>
<td>33.2 ± 0.7</td>
<td>29.2 ± 1.1b</td>
<td>27.9 ± 0.7a</td>
</tr>
<tr>
<td>HbA1c (% baseline)</td>
<td>2.93 ± 0.13</td>
<td>2.87 ± 0.12</td>
<td>2.79 ± 0.12</td>
</tr>
<tr>
<td>wk 6</td>
<td>3.78 ± 0.17</td>
<td>2.98 ± 0.03b</td>
<td>2.9 ± 0.06b</td>
</tr>
<tr>
<td>Difference</td>
<td>+0.85 ± 0.11</td>
<td>+0.11 ± 0.14</td>
<td>+0.11 ± 0.13</td>
</tr>
<tr>
<td>Within-group comparison</td>
<td>P &lt; 0.001</td>
<td>P = 0.4600</td>
<td>P = 0.4300</td>
</tr>
<tr>
<td>Plasma glucose (mmol/liter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>10.11 ± 0.35</td>
<td>10.66 ± 0.44</td>
<td>9.91 ± 0.46</td>
</tr>
<tr>
<td>wk 6</td>
<td>9.15 ± 0.46</td>
<td>8.51 ± 0.33</td>
<td>10.1 ± 0.99</td>
</tr>
<tr>
<td>Difference</td>
<td>−0.96 ± 0.61</td>
<td>−2.16 ± 0.56</td>
<td>+0.19 ± 0.92</td>
</tr>
<tr>
<td>Within-group comparison</td>
<td>P = 0.1400</td>
<td>P &lt; 0.004</td>
<td>P = 0.8400</td>
</tr>
<tr>
<td>Insulin (nmol/liter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.27 ± 0.16</td>
<td>1.54 ± 0.31</td>
<td>1.23 ± 0.23</td>
</tr>
<tr>
<td>wk 6</td>
<td>2.96 ± 0.36</td>
<td>1.13 ± 0.16a</td>
<td>1.43 ± 0.18a</td>
</tr>
<tr>
<td>Difference</td>
<td>+1.68 ± 0.35</td>
<td>−0.41 ± 0.21</td>
<td>+0.20 ± 0.17</td>
</tr>
<tr>
<td>Within-group comparison</td>
<td>P &lt; 0.001</td>
<td>P = 0.0900</td>
<td>P = 0.2600</td>
</tr>
<tr>
<td>Total cholesterol (mmol/liter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.29 ± 0.36</td>
<td>3.33 ± 0.22</td>
<td>3.31 ± 0.35</td>
</tr>
<tr>
<td>wk 6</td>
<td>5.37 ± 0.6</td>
<td>4.12 ± 0.42</td>
<td>3.98 ± 0.27</td>
</tr>
<tr>
<td>Difference</td>
<td>+2.08 ± 0.31</td>
<td>+0.79 ± 0.32</td>
<td>+0.66 ± 0.23</td>
</tr>
<tr>
<td>Within-group comparison</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.03</td>
<td>P &lt; 0.02</td>
</tr>
<tr>
<td>HDL (mmol/liter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.18 ± 0.08</td>
<td>1.24 ± 0.05</td>
<td>1.22 ± 0.09</td>
</tr>
<tr>
<td>wk 6</td>
<td>1.86 ± 0.11</td>
<td>1.75 ± 0.10</td>
<td>1.84 ± 0.14</td>
</tr>
<tr>
<td>Difference</td>
<td>+0.68 ± 0.09</td>
<td>+0.52 ± 0.07</td>
<td>+0.62 ± 0.09</td>
</tr>
<tr>
<td>Within-group comparison</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

a P < 0.001 vs. CON; b P < 0.05 vs. CON.
glucose concentrations during the 60- to 180-min steady-state interval also did not differ among EX, PF, and CON rats (5.9 ± 0.1, 5.9 ± 0.1, 6.0 ± 0.1 mM, respectively; P > 0.05).

The exogenous glucose infusion rate required to maintain euglycemia over the 60- to 180-min clamp interval was 2.5-fold greater in EX rats, compared with CON rats (53.3 ± 3.8 vs. 21.3 ± 3.6 μmol/kg/min; P < 0.001). This glucose infusion rate was also higher than that required in PF rats (40.0 ± 3.8 μmol/kg/min; P < 0.05), despite the two groups being matched across multiple measures, as described above. Arterial pressure, monitored as an index of depth of anesthesia and general health of the animal, did not differ among treatment groups.

The ISI (glucose infusion rate to plasma insulin concentration) in EX rats was 224% higher than that in CON rats (P < 0.0001) and 61% higher than in PF rats (P < 0.003). ISI was inversely related to fasting plasma insulin concentrations measured before the clamp procedure (see Table 1).

**Effects on pancreas**

Total pancreatic weight did not differ among treatment groups (1.98 ± 0.09, 1.95 ± 0.09, 1.76 ± 0.12 g; Fig. 2A). Absolute β-cell mass was decreased by 36% in EX rats (115 ± 9 mg/pancreas, P < 0.02) and 26% in PF rats (134 ± 30 mg/pancreas, P > 0.05), compared with CON rats (181 ± 24 mg/pancreas), in which HbA1c, fasting insulin, and body weight were higher (Fig. 2B). Absolute β-cell mass mirrored changes in fasting insulin levels (Spearman rank correlation R = 0.485, P = 0.057). In the absence of exenatide treatment (i.e. with CON and PF groups combined), there was a reciprocal relationship between insulin sensitivity index and β-cell mass (see Fig. 3A). β-Cell mass was least in the most insulin-sensitive animals (those with least insulin demand), and was greatest in those with greatest insulin demand (the most insulin-resistant animals). The reciprocity of these two factors was manifest in the product of β-cell mass * ISI in CON and PF groups being similar. To accommodate the contribution of observed changes in insulin sensitivity in the analysis of the influence of exenatide on β-cell mass, the product of β-cell mass / ISI in EX was compared with that in CON and PF and was found to be greater (P < 0.02 and P < 0.03, respectively; Fig. 3B). Treatment-specific changes in unadjusted β-cell mass occurred in parallel with changes in fasting insulin levels (Table 1). The 63% increase in (β-cell mass * ISI) in EX rats, compared with PF rats, well matched in other metabolic measures, therefore signifies a greater β-cell mass than would be predicted for animals of comparable insulin sensitivity.

![Fig. 1. Hyperinsulinemic euglycemic clamp in nondiabetic, insulin-resistant obese fa/fa Zucker rats after 6 wk of exenatide treatment. A, Plasma glucose concentration, rate of exogenous glucose infusion required to maintain euglycemia, plasma insulin concentration, and mean arterial pressure. CON, open circles (n = 9); EX, filled circles (n = 9); PF, open triangles (n = 8). B, ISI (glucose infusion rate to plasma insulin concentration) calculated after 3 h of hyperinsulinemic clamp at 5.8 mM plasma glucose concentration. Asterisk denotes significant difference (P < 0.05). Mean ± SEM.](https://academic.oup.com/endo/article-abstract/146/4/2069/2878820)
The present study sought to identify an insulin-sensitizing effect of exenatide in a rodent model of insulin resistance, isolating effects that were attributable to glycemic change and changes in food intake and body weight. In addition, we examined the effects of exenatide on β-cell mass that were dissociable from changes in glycemia and insulin sensitivity. PF animals that consumed the same (reduced) quantity of food as that consumed by EX rats doubled their insulin sensitivity, as measured in hyperinsulinemic euglycemic clamps, and showed a reduction in β-cell mass that was commensurate with the reduced insulin demand. Part of the effect of exenatide may thus be attributable to a reduction in caloric intake. However, the 224% increase in insulin sensitivity in EX animals was beyond that observed with pair feeding, and β-cell mass was greater than that expected in animals of comparable glycemia and insulin sensitivity, indicating additional independent effects of exenatide.

Chronic exenatide treatment for 6 wk reduced the mild increases in HbA₁c, fasting endogenous insulin, and plasma cholesterol concentrations that occur in the normal progression of this disease model, with minimal effects on fasting plasma concentrations of glucose. The matching between PF and EX groups was sufficiently precise and global to allow a robust identification of an effect of exenatide on increased insulin sensitivity independent of body weight and glycemic changes. The large increase in insulin sensitivity observed in the present study with exenatide is reminiscent of a previous report of an 88% increase in clamp insulin sensitivity and a 2.4-fold increase in muscle glucose uptake after chronic administration of GLP-1 in rats (40). Thus, chronic administration of exenatide and GLP-1 appears to be associated with increases in insulin sensitivity.

The present study is the first to show that exenatide can improve insulin sensitivity, independent of decreasing glucose and body weight, pointing to mechanisms other than reversal of glucotoxicity and lipotoxicity. A number of studies have examined the abilities of exenatide to increase pancreatic β-cell mass in vivo (21). In a partial pancreatectomy (90–95%) rat model of β-cell insufficiency, the daily administration of exenatide for 10 d post pancreatectomy attenuated the development of diabetes and stimulated an expansion of β-cell mass and cell proliferation (41). In the Goto-Kakizaki rat, a genetic model of type 2 diabetes in which a deficiency in neonatal β-cell mass results in nonfasting hyperglycemia, neonatal treatment with exenatide enhanced pancreatic insulin content and total β-cell mass, and decreased basal plasma glucose levels (42). In neonatal Wistar rats injected with streptozotocin at birth to induce β-cell destruction, the response to early exenatide exposure was improved islet function and mass (43). In other experiments using neonatal Sprague Dawley rats, Stoffers et al. (44) induced intrauterine growth retardation to produce neonatal rats with deficient islet cell function and mass. Exenatide treatment restored β-cell mass and glucose tolerance to normal levels. This effect was reportedly due to enhanced β-cell proliferation and pancreatic duodenal homeobox-1 (PDX-1) expression, compared with vehicle-treated growth-retarded rats. One report described increased β-cell proliferation in diabetic db/db mice treated with exenatide for 15 d (45), although another study reported improved glucose tolerance and increased insulin secretion and a 1.4-fold increase in β-cell mass in db/db mice treated with exenatide (46). Treatment with exenatide increased pancreatic expression of PDX-1, in association with enhanced islet neogenesis, stimulated insulin secretion, lowered blood glucose, and reduced HbA₁c in db/db mice (47).

Insulin sensitivity and insulin secretion are mutually related such that insulin resistance is compensated by increased insulin secretion. Mathematical analyses of the relationship between insulin sensitivity and insulin secretion has revealed a hyperbolic function, such that the product of the two variables is constant (48, 49). The product (insulin secretion * insulin sensitivity) is usually called the disposition index (50), which is a measure of the power (open-loop gain) of insulin-mediated glucose control. This reciprocal relationship describes the compensatory increase in β-cell function in the insulin resistance of obesity, pregnancy, and glucocorticoid administration, whereas deviation below the hyperbola identifies defective β-cell function as an underlying cause of impaired glucose tolerance and type 2 diabetes. The converse analysis of insulin sensitivity and insulin secretion has shown a down-regulation of β-cell function after increases in insulin sensitivity with weight reduction.
FIG. 3. Relationship between insulin sensitivity and β-cell mass in nondiabetic, obese fa/fa Zucker rats after 6 wk of exenatide treatment. A, Pancreatic β-cell mass increased hyperbolically with decreasing insulin sensitivity in both vehicle-treated groups, independent of food intake and weight gain. Treatment groups: CON, open circles; EX, filled circles; PF, open triangles. B, β-Cell mass adjusted by ISI was significantly greater in the EX group than either vehicle-treated group. Asterisk denotes significant difference (P < 0.02 vs. CON; P < 0.03 vs. PF). Mean ± SEM.

and exercise (48). Correct judgment of changes in insulin secretory function therefore needs to acknowledge this inverse relationship and requires interpretation in the context of changes in insulin sensitivity and, hence, insulin demand.

To our knowledge, the present work is the first to relate histologically imaged β-cell mass to insulin sensitivity, quantified in clamp experiments. To the extent that β-cell mass is an index of insulin secretory capacity, the (β-cell mass * ISI) product used in the present work is analogous to disposition index, and in the two saline-treated groups (i.e. in the absence of exenatide) was constant (see Fig. 3B). The effect of exenatide in the present experiments was to increase β-cell mass above that appropriate for the prevailing insulin sensitivity, resulting in a near doubling of the (β-cell mass * ISI) product. This result is analogous to the increase in disposition index that has been previously reported for GLP-1 (51) and a GLP-1 analog (52) and predicts more powerful glucoregulatory control.

The mechanisms underlying the increase in insulin sensitivity with exenatide remain elusive. Neither exenatide (53) nor GLP-1 (54) has an acute effect on insulin sensitivity measured by clamp in humans, and because neither compound reproducibly activates insulin-sensitive tissues (2), chronic insulin-sensitizing effects are likely to be indirect. A possible effect in liver (55) is debatable, based on the absence of GLP-1 receptors in liver (56) and absence of a specific effect of intraportal (vs. peripheral) GLP-1 on hepatic substrate fluxes (57). The association between exenatide dose and reduction in fasting lactate (9) does, however, point to lactate-generating tissues (especially muscle as the predominant carbohydrate sink) as being involved in the changes in insulin sensitivity.

Summary

In summary, in a nondiabetic rodent model of hyperphagic insulin resistance, we have shown combined effects of exenatide to increase whole-body insulin sensitivity and increase insulin sensitivity-adjusted β-cell mass. It is likely that the combination of these effects contributes to the antidiabetic actions of exenatide in animal models of diabetes (58) and possibly humans with type 2 diabetes (59, 60).

Acknowledgments

We thank Julie Hoyt, Raywin Huang, Scott Barnhill, Luis Alvarado, and Serge Putvinski for their research contributions; Suzann Hahs for editorial assistance; and Miriam Weyer for graphics assistance.

Received October 13, 2004. Accepted December 14, 2004.

Address all correspondence and requests for reprints to: Andrew A. Young, M.D., Ph.D., Amylin Pharmaceuticals, Inc., 9360 Towne Centre Drive, Suite 110, San Diego, California 92121. E-mail: ayoung@amylin.com.

This work was supported by Amylin Pharmaceuticals, Inc. (San Diego, California). All authors are employees of Amylin Pharmaceuticals, Inc. and hold stock and/or stock options in the company.

References

7. Kieffer TJ, McIntosh CH, Pederson RA 1995 Degradation of glucose-depen-
dent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. Endocrinology 136:3585–3596


Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.