Original Article

β-Cell Deficit Due to Increased Apoptosis in the Human Islet Amyloid Polypeptide Transgenic (HIP) Rat Recapitulates the Metabolic Defects Present in Type 2 Diabetes

Aleksey V. Matveyenko and Peter C. Butler

Type 2 diabetes is characterized by defects in insulin secretion and action and is preceded by impaired fasting glucose (IFG). The islet anatomy in IFG and type 2 diabetes reveals an ~50 and 65% deficit in β-cell mass, with increased β-cell apoptosis and islet amyloid derived from islet amyloid polypeptide (IAPP). Defects in insulin action include both hepatic and extrahepatic insulin resistance. The relationship between changes in β-cell mass, β-cell function, and insulin action leading to type 2 diabetes are unresolved, in part because it is not possible to measure β-cell mass in vivo, and most available animal models do not recapitulate the islet pathology in type 2 diabetes. We evaluated the HIP rat, a human IAPP transgenic rat model that develops islet pathology comparable to humans with type 2 diabetes, at age 2 months (nondiabetic), 5 months (with IFG), and 10 months (with diabetes) to prospectively examine the relationship between changes in islet morphology versus insulin secretion and action. We report that increased β-cell apoptosis and impaired first-phase insulin secretion precede the development of IFG, which coincides with an ~50% defect in β-cell mass and onset of hepatic insulin resistance. Diabetes was characterized by ~70% deficit in β-cell mass, progressive hepatic and extrahepatic insulin resistance, and hyperglucagonemia. We conclude that IAPP-induced β-cell apoptosis causes defects in insulin secretion and β-cell mass that lead first to hepatic insulin resistance and IFG and then to extrahepatic insulin resistance, hyperglucagonemia, and diabetes. We conclude that a specific β-cell defect can recapitulate the metabolic phenotype of type 2 diabetes and note that insulin resistance in type 2 diabetes may at least in part be secondary to β-cell failure. *Diabetes* 55:2106–2114, 2006

Type 2 diabetes is a complex and progressive metabolic disorder. Insulin resistance, the most common consequence of obesity (1,2), is a well-recognized risk factor for type 2 diabetes (3,4). However, most obese individuals do not develop hyperglycemia (5), adequately compensating by appropriately increasing insulin secretion (6,7). Therefore, defective β-cell function is also required to develop type 2 diabetes. Glucose-induced insulin secretion is defective in individuals at risk of developing type 2 diabetes (8,9), a defect that becomes more pronounced in those who develop type 2 diabetes (7,10,11). Moreover, there is an ~70% deficit in β-cell mass in type 2 diabetes (12). Since there is already an ~50% deficit in β-cell mass in individuals with impaired fasting glucose (IFG) (12), it seems likely that the loss of β-cell mass is important in the pathophysiology of type 2 diabetes.

However, the relationship among insulin resistance, deficient β-cell mass, and impaired insulin secretion and the sequence in which they develop is poorly understood. This is in large part because in humans, it is still not possible to assess β-cell mass in vivo. Most animal models of type 2 diabetes are poorly representative of type 2 diabetes, often requiring either extreme obesity (13,14) or rarely recapitulating the pathology present in the islet in humans with type 2 diabetes (15,16), which is characterized by islet amyloid derived from islet amyloid polypeptide (IAPP) (12), a protein coexpressed and secreted with insulin by pancreatic β-cells (17). There is increasing recognition that abnormal toxic oligomers play a major role in the increased cell death characteristic of diseases such as Alzheimer's disease, Parkinson's disease, prion-related diseases, and the islet in type 2 diabetes (18). The propensity for IAPP to form amyloid fibrils depends on the hydrophobicity of amino acid residues 20–29 (19,20). $IAPP_{20–29}$ is closely homologous, hydrophobic, and will readily form fibrils in cats, humans, and nonhuman primates (19). A proportion (~20%) of individuals in these species are prone to spontaneously develop type 2 diabetes, particularly if they have a positive family history and are obese (21–23). In contrast, $IAPP_{20–29}$ in rats and mice is much less hydrophobic because of three proline substitutions; therefore, rat and mouse IAPP is soluble in an aqueous solvent and does not form amyloid fibrils (19). Consistent with this, wild-type rodents do not spontaneously develop type 2 diabetes characterized by islet amyloid. Several mouse models transgenic for human IAPP have been reported (24–27). In general, these show propensity for developing diabetes if the expression rate of the human IAPP transgene is sufficiently increased, whether by crossbreeding (26), pharmacological induction of insulin resistance (27), or obesity-induced insulin resistance (24,25). However, the evolution of metabolic defects

From the Larry Hillblom Islet Research Center, David Geffen School of Medicine, University of California, Los Angeles, California.

Address correspondence and reprint requests to Dr. Peter C. Butler, Larry Hillblom Islet Research Center, UCLA David Geffen School of Medicine, 900A Weyburn Pl., Los Angeles, CA 90095. E-mail: pbutler@mednet.ucla.edu.

Received for publication 22 December 2005 and accepted in revised form 24 March 2006.

IAPP, islet amyloid polypeptide; IFG, impaired fasting glucose.

DOI: 10.2337/db05-1672

in relation to islet pathology is limited by blood volume in mice.

We previously reported a transgenic human IAPP rat model, the HIP rat, that develops diabetes at ~10 months of age with islet pathology closely resembling that in humans with type 2 diabetes, including an ~70% deficit in β-cell mass, increased β-cell apoptosis, and islet amyloid (28). In the present study, we sought to make use of this rat model to determine whether a progressive decline in β-cell mass due to increased β-cell apoptosis caused by toxic IAPP oligomers recapitulates the defects in insulin secretion and insulin action present in humans with type 2 diabetes. Moreover, we sought to prospectively establish the relationship between the decline in β-cell mass and β-cell function and any changes in insulin action in the HIP rat.

RESEARCH DESIGN AND METHODS

The generation of the HIP rats has been previously described in detail (28). Rats were bred and housed at the University of California Los Angeles animal housing facility. All animals were housed in pairs (aged 2–5 months) or in individual cages (aged 5–10 months), fed Rodent Diet 8604 (50% carbohydrate, 24% protein, and 4% fat; Harlan Teklad, Madison, WI) ad libitum and subjected to the standard 12-h light-dark cycle. The University of California Los Angeles Institutional Animal Care and Use Committee approved all surgical and experimental procedures. One week before the study, rats were anesthetized (3:3:1 Ketamine HCl, Xylazine, Acepromizine malate [0.1 ml/0.1 kg body wt, intramuscularly]) and indwelling catheters were inserted into the right internal jugular vein (dual silastic cannula, 0.03 cm [internal diameter]) and left carotid artery (polyethylene tubing, PE-50; Clay Adams 0.058 [internal diameter]). All catheters were filled with 100 units/ml heparin/saline solution, exteriorized to the back of the neck, and incased in the infusion harness. Catheters were flushed daily with 100 units/ml heparin/saline solution except on the day of clamp experiments when catheters were flushed with saline only to avoid heparin "spill over" into systemic circulation. All experimental animals were housed individually following surgical procedure.

Hyperglycemic clamp and arginine injection. Rats were studied 5–7 days after catheter placement in the fasting conscious unrestrained state. To assess insulin secretion, HIP (n = 17) and wild-type (n = 19) rats underwent a hyperglycemic clamp followed by an arginine bolus injection at 2, 5, and 10 months of age. On the morning of the study, animals were weighed and the venous line was connected to an adjustable infusion pump (Harvard Apparatus, Holliston, MA) for glucose infusion. The carotid line was used for blood sampling. Following a 30-min equilibration period (−30 to 0 min), animals received an intravenous glucose bolus (375 mg/kg) followed by a variable 50% (wt/vol) glucose infusion to clamp arterial glucose at ~250 mg/dl (0–70 min). At 1 h, rats received a bolus injection of L-arginine solution (1 mmol/kg; Sigma, St. Louis, MO). Arterial blood samples (50 ul) were taken at baseline and every 10 min during the clamp for immediate determination of plasma glucose. Additional blood samples (100 ul) were collected in prechilled heparinized microcentrifuge tubes containing protease inhibitor cocktail solution (Sigma) at −30, 0, 5, 15, 30, 45, 60, 61, and 70 min and immediately centrifuged. Plasma was immediately frozen on dry ice and stored at −80°C for determination of plasma insulin, glucagon, and IAPP concentrations.

Hyperinsulinemic-euglycemic clamp. Using the same experimental setup, a different group of HIP (n = 21) and wild-type (n = 19) rats (aged 2, 5, and 10 months) underwent a hyperinsulinemic-euglycemic clamp to examine insulin sensitivity. Following an equilibration period, a constant infusion of regular human insulin (4 mU · kg^{-1} · min^{-1} Novolin; Novo Nordisk, Princeton, NJ) was initiated and continued until the end of the clamp (0–120 min). Plasma glucose levels were determined every 10 min, and glucose (50% wt/vol) was infused (5–120 min) to clamp plasma glucose levels at ~100 mg/dl. Additional blood samples (~100 μl) were collected at baseline (−30 min) and during the clamp (10, 30, 50, 80, and 120 min) for determination of plasma insulin and free fatty acid concentrations. Rates of exogenous glucose infusion were recorded, and whole-body insulin sensitivity was assessed. At 10 months of age in four of the eight HIP rats, blood glucose did not decrease during the insulin infusion at 4 mU · kg^{-1} · min^{-1} (i.e., they had severe hepatic and extrahepatic insulin resistance, and the glucose infusion rate would have been equal to zero). We did not include these rats in the clamp data in Table 2, which therefore report a conservative estimate of the extent of insulin resistance at 10 months. Once it was established that HIP rats develop insulin resistance by 5 months of age, basal and insulin-stimulated glucose turnover in six additional 5-month-old HIP versus five control rats were quantified using a [3-^{3}H]glucose infusion along with the same hyperinsulinemic-euglycemic clamp protocol. Briefly, rats received primed (3 μCi)- continuous (0.05 μCi/min) infusion of [3-^{3}H]glucose (Perkin Elmer, Boston, MA) for 90 min of an equilibration basal period followed by 120 min of tracer infusion (0.2 μCi/min) throughout the hyperinsulinemic-euglycemic clamp. Blood samples (~150 μl) for determination of tracer specific activity at basal were drawn at − 40, −30, −20, and −10 min and during insulin infusion at 90, 100, 110, and 120 min. All blood samples were immediately centrifuged and plasma stored at −80°C for subsequent analysis.

Analytical procedures. Plasma glucose concentrations were measured by the glucose oxidase method (Beckman Glucose Analyzer 2; Beckman Coulter, Fullerton, CA). Plasma insulin, IAPP, and glucagon levels at baseline and during the hyperglycemic clamp were measured using LINCO*plex* multiplex rat endocrine immunoassay panel (Linco Research, St. Charles, IL). Plasma insulin concentrations during the hyperinsulinemic-euglycemic clamp were measured using competitive colomiteric enzyme-linked immunosorbent assay (Linco Research). Plasma free fatty acid levels were measured using in vitro standard enzymatic colomiteric method (Wako Chemicals, Richmond, VA). To determine plasma glucose specific activity, plasma samples were deprotonized in duplicates with $ZnSO_4$ and $Ba(OH)_2$, evaporated to remove ^3H_2O, reconstituted with distilled H_2O, and assayed for ^3H activity (disintegrations per mintue [dpm] per minute) with a liquid scintillation counter (Packard Bioscience, Boston, MA) using Ecoscint-H liquid scintillation fluid (National Diagnostics, Atlanta, GA). Glucose specific activity reached steady state in both HIP rats and wild-type controls in the basal and clamp sampling periods (final 30 min of baseline and clamp periods). Hepatic glucose production at baseline was assumed to equal basal R_a and, during the hyperinsulinemic-euglycemic clamp, was determined by subtracting average exogenous glucose infusion rates during the last 30 min of the clamp from the isotopically measured R_a.

Islet morphology and immunohistochemistry. After the rats were killed, pancreata were rapidly removed and all nonpancreatic tissue was also removed. Then, each pancreas was weighed and immediately fixed in 4% paraformaldehyde overnight at 4°C. The pancreas was embedded in paraffin and, subsequently, complete longitudinal sections (4 μm) of pancreas (head, body, and tail) were obtained through its maximal width. These sections were stained for hematoxylin/eosin, insulin (guinea pig anti-insulin, 1:100; Zymed, Carlsbad, CA), glucagon (rabbit antiglucagon, 1:500; Immunostar, Hudson, WI), and Congo red (Sigma), as previously described (26). The β- and α-cell mass were measured by first quantifying pancreatic cross-sectional area positive for insulin or glucagon and multiplying this by pancreatic weight. In addition, to quantify β and α apoptosis, sections were costained by immunofluorescence for insulin (guinea pig anti-insulin, 1:100; Zymed) or glucagon (rabbit anti-glucagon, 1:500; Immunostar) along with terminal deoxynucleotidyl transferase biotin-dUTP nick-end labeling (TUNEL) method for apoptosis using the cell death detection kit TMR Red from Roche Diagnostics (Mannheim, Germany) and nuclear marker 4,6-diamidino-2-phenylindole. All islets per pancreatic section (three sections per animal) were examined in detail at ×200 magnification (×20 objective, ×10 ocular) for the total number TUNEL-positive β- or α-cells, which was expressed in relation to the total number of β- or α-cells. These analyses were performed in ~50 islets per rat (all islets on two slides per rat).

Statistical analysis. Statistical analysis was performed using ANOVA analysis and regression analysis using Statistica (version 6; Statsoft, Tulsa, OK). Data in graphs and tables are presented as means ± SE. Findings were assumed statistically significant at the $P < 0.05$.

RESULTS

Fasting plasma glucose, insulin, glucagon, and IAPP concentrations. There was a progressive increase in glucose concentrations in HIP rats from 2 to 10 months of age ($P < 0.05$; Fig. 1). At 2 months, glucose concentrations in HIP and wild-type rats were comparable; by 5 months, HIP rats had developed impaired fasting plasma glucose (128.3 ± 5.7 vs. 102.8 ± 2 mg/dl; $P < 0.05$); and by 10 months, overt diabetes had developed (185.3 ± 25 vs. 100.4 ± 1.5 mg/dl; $P < 0.05$). Fasting insulin concentrations were comparable in HIP and wild-type rats at 2 and 5 months of age, although, at 5 months, this was in the context of higher glucose concentrations (Fig. 1). By 10 months of age, insulin concentrations were deficient in HIP rats (182 ± 37.3 vs. 379.9 ± 45.6 pmol/l; $P < 0.05$)

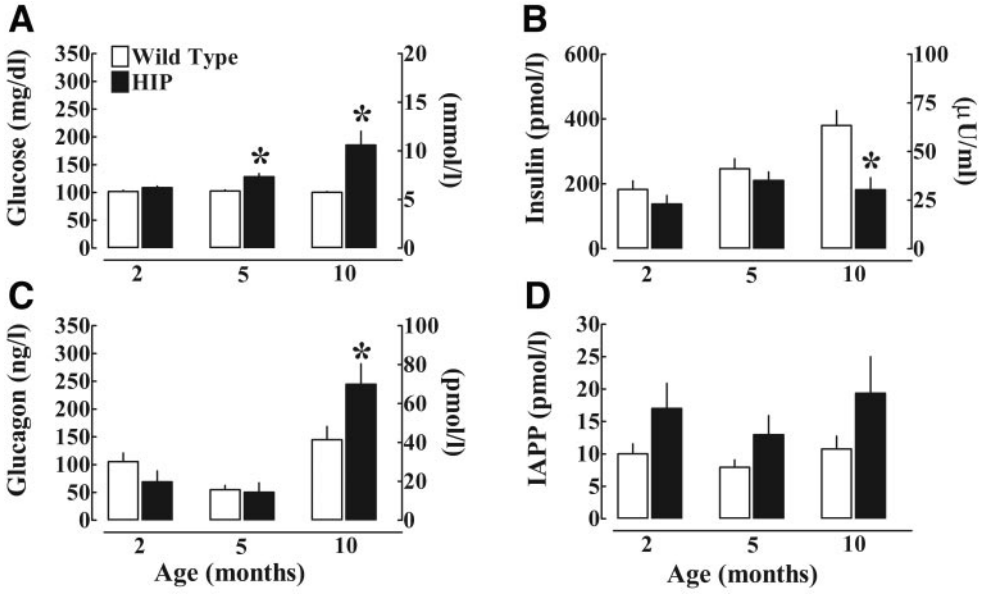

FIG. 1. The mean fasting plasma glucose (*A*), insulin (*B*), glucagon (*C*), and IAPP (*D*) concentrations in HIP and wild-type rats at 2, 5, and 10 months of age. Data are expressed as means ± SE. *HIP vs. wild type, $P < 0.05$.

despite higher glucose concentrations. Fasting glucagon concentrations were comparable in HIP and control rats at 2 and 5 months. HIP rats developed hyperglucagonemia coincident with onset of diabetes at 10 months (244.4 ± 37 vs.145.3 ± 23.8 ng/l; $P < 0.05$; Fig. 1). Fasting plasma IAPP levels were not significantly different between HIP and wild-type rats at any point from 2 to 10 months of age ($P > 0.05$ for all age-groups).

Islet morphology. Pancreas weight was not significantly different between HIP rats and controls at any age. β-Cell mass was comparable in HIP and wild-type rats at 2 months but subsequently declined progressively in HIP rats to ~50% of control at 5 months and to ~80% of control by 10 months of age (Fig. 2). As expected, the decline in β-cell mass in HIP rats was due to increased β-cell apoptosis, whereas α-cell mass and α-cell apoptosis were comparable in HIP rats and wild-type rats at all ages (Fig. 2).

Hyperglycemic clamp and arginine bolus. By design during the clamp, plasma glucose levels were maintained from 5 to 70 min at ~250 mg/dl, and clamp glucose levels were not significantly different between HIP and wild-type rats at any time point during hyperglycemia (Fig. 3). The mean glucose infusion rates required to achieve and maintain hyperglycemia were 40, 44, and 76% lower in HIP versus wild-type rats at 2, 5, and 10 months of age ($P < 0.05$ for all age-groups). Despite similar glucose concentrations, first- and second-phase insulin secretion to hyperglycemia was deficient and progressively declined in 2-, 5-, and 10-month-old HIP rats (~50, 70, and 80% deficit versus wild type, respectively; $P < 0.05$ for all age-groups) (Fig. 3 and Table 1). Insulin secretion to arginine was not significantly different in HIP rats at 2 months of age but thereafter demonstrated a progressive decline at 5 months (1,049.1 ± 265 vs. 2,421 ± 457 pmol/l; $P < 0.05$) and 10 months (740.6 ± 223.4 vs. 2,478.2 ± 432 pmol/l; $P < 0.05$) of age.

Hyperinsulinemic-euglycemic clamp. These studies were undertaken to evaluate whole-body insulin sensitivity in HIP and wild-type rats from 2 to 10 months of age. Table 2 provides corresponding mean plasma glucose, insulin, and free fatty acid concentrations during the hyperinsulinemic-euglycemic clamp. Plasma insulin concentrations were comparable during the insulin infusions

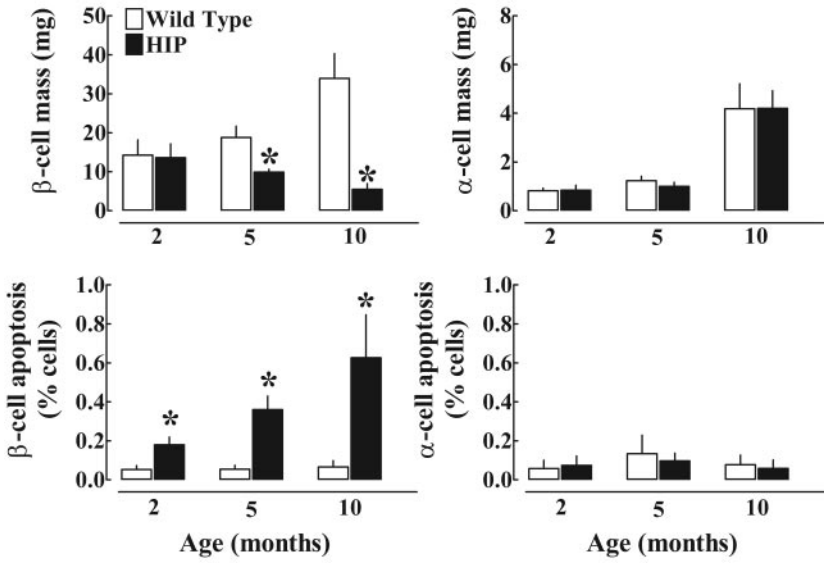

FIG. 2. The mean β- and α-cell mass and apoptosis in HIP and wild-type rats at 2, 5, and 10 months of age. Data are expressed as means ± SE. *HIP vs. wild type, $P < 0.05$.

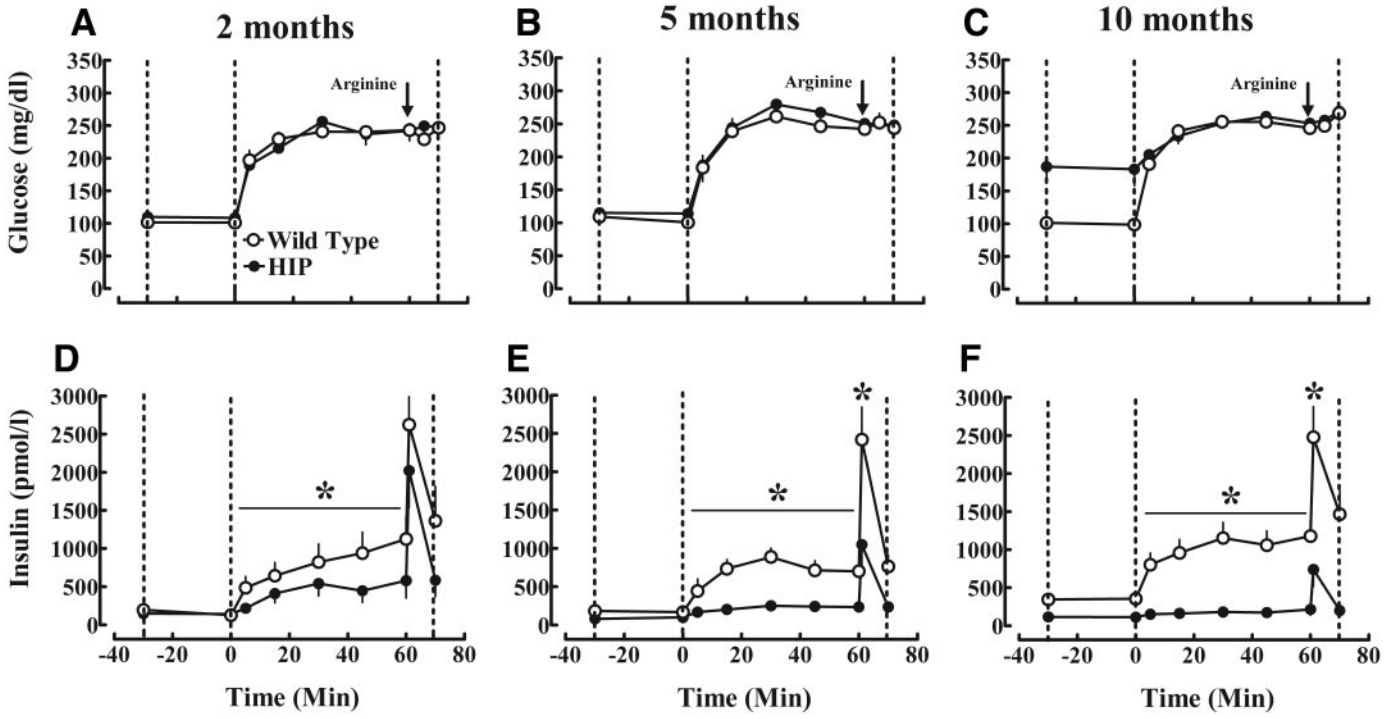

FIG. 3. Mean plasma glucose (*A–C*) and insulin (*D–F*) concentration profiles at fasting (−30 to 0 min) and during a hyperglycemic clamp (0–80 min) with arginine bolus injection given at 60 min in HIP and wild-type rats between 2 and 10 months of age. Data are expressed as means ± SE. *HIP vs. wild type, $P < 0.05$.

in HIP and wild-type rats at all age-groups. Whole-body insulin sensitivity, assessed by the mean glucose infusion rates during the hyperinsulinemic period, was comparable in HIP and control rats at 2 months of age (93.8 ± 7.1 vs. 100.1 ± 8.8 μmol · kg^{-1} · min^{-1}; P = NS). However, by 5 months of age, HIP rats had an ~40% deficit in insulin sensitivity (Table 2 and Fig. 4; $P < 0.05$). By 10 months of age, insulin sensitivity was markedly decreased such that the insulin infusion of 4 mU · kg^{-1} · min^{-1} had no effect on the blood glucose at ~250 mg/dl in four of the eight HIP rats. These rats are not included in the mean glucose infusion rates in Table 2, so the reported glucose infusion rate is a conservative estimate of the insulin resistance in the HIP rat by 10 months of age, since only the most insulin-sensitive half of rats was included. Despite this, insulin sensitivity was still further diminished by 10 months of age in HIP versus wild-type rats (46.8 ± 3 vs. 26.46 ± 7.4 μmol · kg^{-1} · min^{-1}; $P < 0.05$). When further clamp studies were performed with the isotope dilution technique in 5-month-old HIP versus wild-type rats to establish the site (hepatic versus extrahepatic) of this insulin resistance, as expected in control rats, hepatic glucose release was substantially suppressed by increased insulin during the hyperinsulinemic clamp (27.5 ± 3.25 vs. 7.52 ± 4.2 μmol · kg^{-1} · min^{-1}; $P < 0.001$, basal vs. clamp; Fig. 5). However, there was a marked defect in suppression of hepatic glucose release at the same insulin concentrations in the HIP rat (34.45 ± 2.7 vs. 27.90 ± 4.5 μmol · kg^{-1} · min^{-1}; $P > 0.05$, basal vs. clamp; Fig. 5), indicating hepatic insulin resistance. In contrast, at the same age, isotopically measured extrahepatic glucose uptake was comparable in HIP and wild-type rats (83.51 ± 8.5 vs. 87.48 ± 10 μmol · kg^{-1} · min^{-1}; $P > 0.05$; Fig. 5), implying that extrahepatic insulin sensitivity is normal in the HIP rat at 5 months of age. Since the same insulin infusion (4 mU · kg^{-1} · min^{-1}) had no effect on blood glucose at 10 months of age in four of the HIP rats and a much reduced effect in the four HIP rats with the least insulin resistance, by 10 months, they clearly had marked global (hepatic and extrahepatic) insulin resistance.

DISCUSSION

We report here that HIP rats and humans with type 2 diabetes not only share close islet morphology but also develop a very comparable profile of metabolic defects. In the HIP rat (and in first-degree relatives of type 2 diabetes), defective glucose-induced insulin secretion precedes hyperglycemia (8,9). In both humans and the HIP rat with IFG, there is an ~50% deficit of β-cell mass and hepatic insulin resistance (12,29). In both humans and the HIP rat, type 2 diabetes is characterized by an ~70% loss of β-cell mass due to increased β-cell apoptosis with a further decline in β-cell function and insulin sensitivity and hyperglucagonemia (7,12,29,30). The HIP rat is therefore an excellent model in which to explore the relationship between development of the islet pathology and metabolic changes characteristic of type 2 diabetes.

In both humans at risk of developing type 2 diabetes (8,9) and in the HIP rat, defective glucose-induced insulin secretion precedes development of type 2 diabetes. Defective glucose-induced insulin secretion was present in the HIP rat when β-cell mass was still comparable with that in wild-type rats, although the frequency of β-cell apoptosis was already increased. In the HIP rat, the increased frequency of β-cell apoptosis is related to aggregation of human IAPP (28), which has been shown to have its adverse action through toxic oligomers (18). The underlying basis of the defective glucose-induced insulin secretion in type 2 diabetes is complex (31). Once diabetes develops, hyperglycemia, per se, likely contributes to both loss of β-cell mass and function (32) in type 2 diabetes. Factors

TABLE 1
Hyperglycemic clamp at age 2, 5, and 10 months

	2 months	5 months	10 months
First-phase insulin (pmol/l)			
Wild type	618.2 ± 172.5	590.8 ± 138.8	719 ± 171.4
HIP	314.9 ± 74.7*	186.5 ± 30.3†	119.3 ± 50.4†‡
Second-phase insulin (pmol/l)			
Wild type	1,055.7 ± 272.9	768.6 ± 107	917.9 ± 207.7
HIP	523.2 ± 164.4*	243.1 ± 42.6†§	146.2 ± 65.7†‡
$AIR_{arginine}$ (pmol/l)			
Wild type	2,803.7 ± 361.5	2,421 ± 457.9	2,478.2 ± 432
HIP	2,022.6 ± 443.7	1,049.1 ± 265*	740.6 ± 223.4†§

Data are means ± SE. *HIP vs. wild type, $P < 0.05$; †HIP vs. wild type, $P < 0.01$; ‡10 vs. 2 months, $P < 0.05$; §5 vs. 2 months, $P < 0.05$. $AIR_{arginine}$, acute insulin response to arginine.

that have been implicated as mediators of this include oxygen free radical toxicity (33), expression of interleukin-1β (34), depletion of insulin stores (32), decreased flux of glucose through glucokinase (35), and mitochondrial dysfunction (36). β-Cell cytotoxic effects of free fatty acids have also been implicated, particularly in obese rodent models of diabetes, such as the diabetes-prone Zucker fatty rat (13) and the leptin-insensitive (37) or -deficient (14) mouse models. While these rodents also have a progressive defect in β-cell mass, they generally require a markedly obese phenotype before developing diabetes, and the islet does not develop the islet pathology (islet amyloid) characteristically present in most humans with type 2 diabetes. Also, the marked insulin resistance required to induce a β-cell defect in these models confounds studies of the evolving metabolic consequences of a deficit in β-cell mass.

The HIP rat has the advantage of developing diabetes with an islet pathology that is comparable with that in humans with type 2 diabetes and does not require extreme obesity. Furthermore, accumulation of misfolded proteins may provoke endoplasmic reticulum stress–induced apoptosis (38). Since protein synthesis of major client proteins by secretory cells is adaptively decreased in response to endoplasmic reticulum stress (39), this may provide a possible common explanation for the early deficit in glucose-mediated insulin secretion in the HIP rat and humans at risk of developing type 2 diabetes. Soluble IAPP may inhibit insulin secretion in a paracrine manner (40,41), and so increased expression of IAPP per cell in humans and in HIP rats could also contribute to impaired glucose-induced insulin secretion. In the HIP rat, while glucose-mediated insulin secretion was abnormal when β-cell was still unchanged (2 months), defective arginine-induced insulin secretion was not present until β-cell mass was defective (5 months), consistent with prior studies in pigs (42) reporting that arginine-induced insulin secretion more closely relates to β-cell mass than glucose-induced insulin secretion.

There is increasing interest in the pathophysiology of IFG and the transition from IFG to type 2 diabetes, since it is now possible to delay and/or prevent the development of type 2 diabetes in patients with IFG and/or glucose intolerance (43,44). In an effort to explain type 2 diabetes development, one model (45) proposed that insulin resistance eventually leads to β-cell failure and hyperglycemia. This model gains support from rodents characterized by genetic obesity that develop diabetes (13,14,37) and the fact that insulin resistance is a risk factor for type 2 diabetes (3). The problem with this model is that the majority of individuals with insulin resistance, most commonly due to obesity (1,2), adaptively increase insulin secretion (6,7) and do not develop diabetes (5). Therefore, the role of insulin resistance in the pathogenesis of type 2 diabetes appears to be to induce the β-cell defect in those who are genetically vulnerable. One mechanism by which

TABLE 2
Hyperinsulinemic-euglycemic clamp at age 2, 5, and 10 months

	2 months	5 months	10 months
Clamp glucose (mg/dl)			
Wild type	98 ± 4.2	98.3 ± 3.2	99.1 ± 7.1
HIP	97.3 ± 5.7	99.1 ± 7.1	102 ± 3
Clamp insulin (pmol/l)			
Wild type	663.8 ± 49.9	820.4 ± 52.2	907.2 ± 114.3
HIP	623.5 ± 68.2	835.3 ± 75.7*	981.2 ± 76.5†
Insulin clearance($ml \cdot kg^{-1} \cdot min^{-1}$)			
Wild type	27.7 ± 2	24.4 ± 2.6	29.27 ± 6.6
HIP	25.2 ± 2.6	23.8 ± 3.5	17.9 ± 2.1
Clamp FFA (mmol/l)			
Wild type	0.16 ± 0.01	0.24 ± 0.04	0.29 ± 0.02†
HIP	0.20 ± 0.04	0.21 ± 0.02	0.33 ± 0.01†‡
Glucose infusion rate($\mu mol \cdot kg^{-1} \cdot min^{-1}$)			
Wild type	93.8 ± 7.1	77.8 ± 7.3	46.8 ± 3†‡
HIP	100.1 ± 8.7	46.8 ± 7.4*§	26.5 ± 7.4†‖

Data are means ± SE. *5 vs. 2 months, $P < 0.05$; †10 vs. 2 months, $P < 0.05$; ‡10 vs. 5 months, $P < 0.05$; §HIP vs. wild type, $P < 0.01$; ‖HIP vs. wild type, $P < 0.05$.

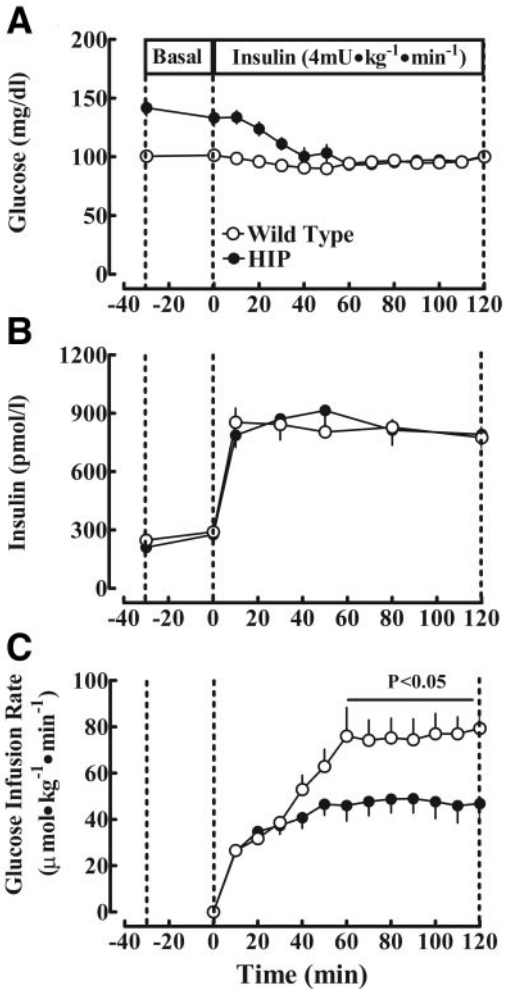

FIG. 4. Mean plasma glucose (*A*), insulin (*B*), and glucose infusion rates (*C*) at fasting (−30 to 0 min) and during the hyperinsulinemic-euglycemic clamp in 10 HIP and 11 wild-type rats at 5 months of age. Data are expressed as means ± SE.

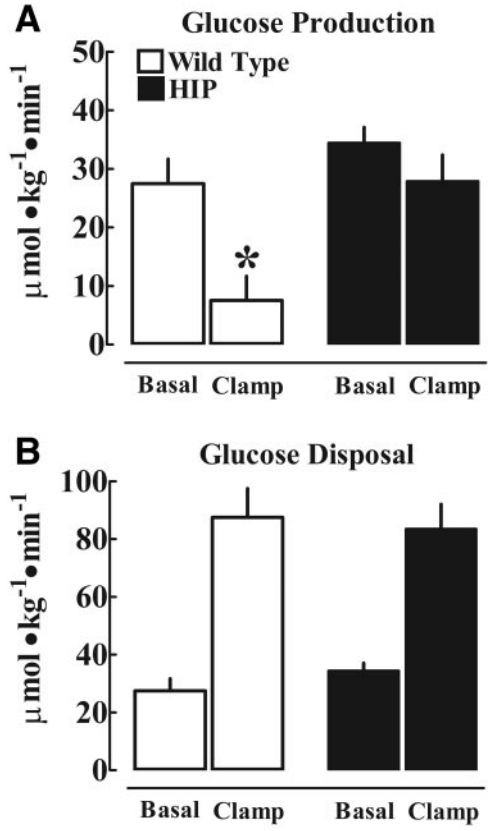

FIG. 5. Mean rates of hepatic glucose production (*A*) and extrahepatic glucose disposal (*B*) at basal and during final 30 min of the hyperinsulinemic-euglycemic clamp in six HIP and five wild-type rats at 5 months of age. Data are expressed as means ± SE. *Basal vs. clamp, $P < 0.05$.

this might theoretically be accomplished is the increased expression of IAPP that occurs in response to insulin resistance (46). Previously, we proposed that there might be a lower capacity to fold and traffic IAPP in genetically vulnerable individuals (e.g., as a result of polymorphisms in chaperone proteins); this concept is gaining acceptance in other similar amyloidogenic diseases such as Alzheimer's disease (47,48). The HIP rat provides a powerful tool to dissect out the importance of IAPP expression per se versus insulin resistance on progressive islet dysfunction. Thus, at 2 months of age, the HIP rat has increased human IAPP expression because of human IAPP transgenic expression rather than insulin resistance. Indeed, at 2 months of age, both blood glucose and insulin sensitivity are normal in the HIP rat, but by 5 months of age, the HIP rat develops hepatic insulin resistance coincident with the onset of IFG, and by 10 months of age, with further progression of insulin resistance, the HIP rat develops diabetes. These data imply that hepatic insulin resistance characteristic of IFG (29) and hepatic and extrahepatic characteristic of type 2 diabetes (30) might at least in part be due to β-cell failure. In the context of the population that develops type 2 diabetes most frequently already being insulin resistant (obese), we propose that the additional insulin resistance in individuals with IFG and type 2 diabetes versus comparably obese nondiabetic control subjects may be at least in part due to β-cell failure (29,30).

In healthy subjects, insulin is secreted in discrete insulin secretory bursts that present the liver with an insulin concentration profile that oscillates with an amplitude of ~1,000 pmol/l in the fasting state (49,50) and up to 5,000 pmol/l after meal ingestion (51). As β-cell mass is decreased in animal models, both basal and glucose-stimulated insulin secretion decline due to a selective defect in insulin pulse mass (52,53), a profile that has been observed in humans with type 2 diabetes (54). The pulsatile mode of insulin delivery to the liver increases hepatic insulin sensitivity (55,56), possibly by avoiding insulin receptor downregulation (57) and/or sustained activation of AKT with attendant feedback inhibition of insulin receptor substrate-2 (58). We postulate that the decline in hepatic insulin sensitivity in the HIP rat (and in humans) with IFG may relate at least in part to the decline in β-cell mass leading to an abnormal pattern of insulin delivery to the liver. Since hepatic insulin extraction depends on the amplitude of insulin pulses presented to the liver (59), this defect in insulin secretion would be largely offset in the systemic circulation by decreased hepatic insulin clearance. Another theoretical explanation for insulin resistance in the HIP rat is the actions of circulating IAPP, since high IAPP concentrations have been shown to cause insulin resistance (60). However, this effect has been shown in muscle rather than liver (61) and requires IAPP concentrations in the nanomolar versus low picomolar range observed here (Fig. 1). Moreover, the circulating IAPP concentrations were not increased in HIP versus

wild-type rats ($P > 0.05$ for 2–10 months of age). As expected from prior studies (62), once diabetes supervened at 10 months of age, extrahepatic insulin resistance was also present, presumably as a consequence of the effects of glucose toxicity (63). Indeed, once HIP rats developed overt hyperglycemia (>250 mg/dl) from 12 to 18 months of age, the insulin infusion rate (4 mU · kg^{-1} · min^{-1}) used during the hyperinsulinemic-euglycemic clamp was no longer sufficient to lower plasma glucose in these animals to ~100 mg/dl.

There are limited data regarding the importance of β-cell mass in the pathophysiology of IFG or type 2 diabetes because of the paucity of data on β-cell mass in humans. These data are only available from autopsy studies, revealing an ~50 and 70% deficit in β-cell mass in IFG and type 2 diabetes, respectively (12), but, by necessity, these data are only available at a single time point. When the relationship between blood glucose and β-cell mass in humans is examined, a β-cell mass of ~50% of normal level is the transition between hyperglycemia development vulnerability and a capacity to secrete sufficient insulin to maintain normal blood glucose concentrations (64). It is therefore of interest that the curve describing the relationship between β-cell mass and fasting blood glucose in the wild-type and HIP rat also shows a point of inflection at ~50% of the mean of β-cell mass in wild-type rats (Fig. 6). As in the human curve, there is a steep increase in blood glucose with each decrement of β-cell mass thereafter, consistent with the known adverse effects of hyperglycemia on β-cell function and survival (34). There are comparable cross-sectional data in dogs (65), monkeys (66), and pigs (53) showing that an ~50% deficit in β-cell mass is sufficient to lead to IFG. Also, living related donors of individuals with type 1 diabetes who had a 50% pancreatectomy had a high risk of subsequently developing diabetes (67). The problem with prior data is that they are cross-sectional, so it is not possible to be certain that humans developing type 2 diabetes progress from a normal β-cell mass through to ~50% deficit with IFG and ~70% deficit with diabetes. While it is not possible to be certain that the same sequence of defects in β-cell mass and metabolic dysfunction occur in humans with type 2 diabetes and HIP rats, before we can quantify β-cell mass in humans, the HIP rat allows us to examine the evolution of an islet pathology closely resembling that in humans with IFG and type 2 diabetes. Simultaneously, the HIP rat allows us to quantify insulin secretion and insulin action and imply that it is certainly possible that the β-cell deficit in humans with type 2 diabetes is both progressive and closely related to the development of the disease.

In the present study, we also report that in contrast to the β-cell mass, the α-cell mass in HIP rats does not differ from that in wild-type rats from 2 through 10 months of age (i.e., throughout the transition from the nondiabetic state to IFG and diabetes). Consistent with this finding, the measured frequency of α-cell apoptosis did not change throughout this period. These data are consistent with the selective deficit in β-cell mass reported in humans with type 2 diabetes (12). They also emphasize that IAPP-induced β-cell death and the associated islet amyloid does not influence viability of other islet cells, reinforcing the accumulating evidence that it is not extracellular amyloid that is cytotoxic but rather intracellular toxic oligomers of amyloidogenic proteins (18). An alternative explanation is that α-cells are resistant to amyloid-related toxicity, although this is unlikely since the addition of amyloidogenic proteins to many different cell types in culture has been reported to induce apoptosis (68–70). We also report here that in the HIP rat, hyperglucagonemia develops coincident with the development of diabetes. From these data alone, it is not possible to determine if this is due to declining intra-islet insulin-mediated inhibition of glucagon secretion or secondary to hyperglycemia per se. Nonetheless, this finding again provides a parallel to humans with type 2 diabetes who have hyperglucagonemia that contributes to postprandial hyperglycemia (29).

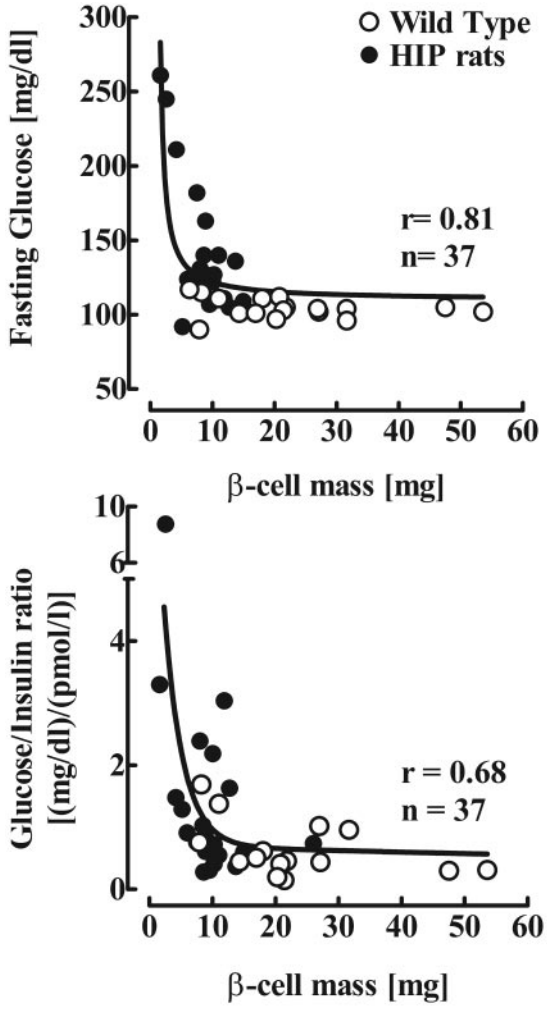

FIG. 6. The relationship between β-cell mass and the fasting plasma glucose and fasting plasma glucose–to–insulin concentration ratio in 22 HIP and 15 wild-type rats at 2–10 months of age.

In summary, in the HIP rat, we report that the β-cell human IAPP transgene leads first to increased β-cell apoptosis and β-cell dysfunction (age 2 months), then to hepatic insulin resistance leading to IFG (5 months), and finally to more severe insulin resistance, hyperglucagonemia, and diabetes (10 months). Through this transition, we document both a progressive decline in glucose-mediated insulin secretion (both first and second phase) and a relative decline in β-cell mass that is comparable in extent to that in humans with IFG (~50%) and type 2 diabetes (~70%), respectively (12). Of particular note, this selective deficit in β-cell function, and then mass, leads to hepatic insulin resistance. These data imply that the added insulin resistance characteristic of type 2 diabetes may be a consequence, rather than the cause, of β-cell dysfunction.

ACKNOWLEDGMENTS

We gratefully acknowledge funding for these studies from the U.S. Public Health Service (National Institutes of Health DK59579) and the Larry Hillblom Foundation.

We gratefully acknowledge the helpful suggestions from our collaborators at the Larry Hillblom Islet Research Center, UCLA Anil Bhushan, Alexandra Butler, Tatyana Gurlo, Leena Haataja, Kathrin Maedler, and Walter Soeller at Pfizer Research in Groton, Connecticut.

REFERENCES

1. Bjorntorp P: Abdominal obesity and the development of noninsulin-dependent diabetes mellitus. *Diabetes Metab Rev* 4:615–622, 1988
2. Carey DG, Jenkins AB, Campbell LV, Freund J, Chisholm DJ: Abdominal fat and insulin resistance in normal and overweight women: direct measurements reveal a strong relationship in subjects at both low and high risk of NIDDM. *Diabetes* 45:633–638, 1996
3. Yki-Jarvinen H: Role of insulin resistance in the pathogenesis of NIDDM. *Diabetologia* 38:1378–1388, 1995
4. DeFronzo RA, Bonadonna RC, Ferrannini E: Pathogenesis of NIDDM: a balanced overview. *Diabetes Care* 15:318–368, 1992
5. Chan JM, Rimm EB, Colditz GA, Stampfer MJ, Willett WC: Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. *Diabetes Care* 17:961–969, 1994
6. Polonsky KS, Given BD, Van Cauter E: Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. *J Clin Invest* 81:442–448, 1988
7. Perley MJ, Kipnis DM: Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic sujbjects. *J Clin Invest* 46:1954–1962, 1967
8. Boden G, Soeldner JS, Gleason RE, Marble A: Elevated serum human growth hormone and decreased serum insulin in prediabetic males after intravenous tolbutamide and glucose. *J Clin Invest* 47:729–739, 1968
9. van Haeften TW, Dubbeldam S, Zonderland ML, Erkelens DW: Insulin secretion in normal glucose-tolerant relatives of type 2 diabetic subjects: assessments using hyperglycemic glucose clamps and oral glucose tolerance tests. *Diabetes Care* 21:278–282, 1998
10. Seltzer HS, Allen EW, Herron AL Jr, Brennan MT: Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. *J Clin Invest* 46:323–335, 1967
11. Temple RC, Carrington CA, Luzio SD, Owens DR, Schneider AE, Sobey WJ, Hales CN: Insulin deficiency in non-insulin-dependent diabetes. *Lancet* 1:293–295, 1989
12. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC: β-Cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. *Diabetes* 52:102–110, 2003
13. Lee Y, Hirose H, Ohneda M, Johnson JH, McGarry JD, Unger RH: Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-beta-cell relationships. *Proc Natl Acad Sci U S A* 91:10878–10882, 1994
14. Tomita T, Doull V, Pollock HG, Krizsan D: Pancreatic islets of obese hyperglycemic mice (*ob/ob*). *Pancreas* 7:367–375, 1992
15. Donath MY, Gross DJ, Cerasi E, Kaiser N: Hyperglycemia-induced β-cell apoptosis in pancreatic islets of Psammomys obesus during development of diabetes. *Diabetes* 48:738–744, 1999
16. Portha B, Giroix MH, Serradas P, Gangnerau MN, Movassat J, Rajas F, Bailbe D, Plachot C, Mithieux G, Marie JC: β-Cell function and viability in the spontaneously diabetic GK rat: information from the GK/Par colony. *Diabetes* 50 (Suppl. 1):S89–S93, 2001
17. Butler PC, Chou J, Carter WB, Wang YN, Bu BH, Chang D, Chang JK, Rizza RA: Effects of meal ingestion on plasma amylin concentration in NIDDM and nondiabetic humans. *Diabetes* 39:752–756, 1990
18. Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG: Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* 300:486–489, 2003
19. Westermark P, Engstrom U, Johnson KH, Westermark GT, Betsholtz C: Islet amyloid polypeptide: pinpointing amino acid residues linked to amyloid fibril formation. *Proc Natl Acad Sci U S A* 87:5036–5040, 1990
20. Betsholtz C, Svensson V, Rorsman F, Engstrom U, Westermark GT, Wilander E, Johnson K, Westermark P: Islet amyloid polypeptide (IAPP): cDNA cloning and identification of an amyloidogenic region associated with the species-specific occurrence of age-related diabetes mellitus. *Exp Cell Res* 183:484–493, 1989
21. Howard CF Jr: Longitudinal studies on the development of diabetes in individual Macaca nigra. *Diabetologia* 29:301–306, 1986
22. O'Brien TD: Pathogenesis of feline diabetes mellitus. *Mol Cell Endocrinol* 197:213–219, 2002
23. King H, Aubert RE, Herman WH: Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections. *Diabetes Care* 21:1414–1431, 1998
24. Hoppener JW, Oosterwijk C, Nieuwenhuis MG, Posthuma G, Thijssen JH, Vroom TM, Ahren B, Lips CJ: Extensive islet amyloid formation is induced by development of type II diabetes mellitus and contributes to its progression: pathogenesis of diabetes in a mouse model. *Diabetologia* 42:427–434, 1999
25. Butler AE, Janson J, Soeller WC, Butler PC: Increased β-cell apoptosis prevents adaptive increase in β-cell mass in mouse model of type 2 diabetes: evidence for role of islet amyloid formation rather than direct action of amyloid. *Diabetes* 52:2304–2314, 2003
26. Janson J, Soeller WC, Roche PC, Nelson RT, Torchia AJ, Kreutter DK, Butler PC: Spontaneous diabetes mellitus in transgenic mice expressing human islet amyloid polypeptide. *Proc Natl Acad Sci U S A* 93:7283–7288, 1996
27. Couce M, Kane LA, O'Brien TD, Charlesworth J, Soeller W, McNeish J, Kreutter D, Roche P, Butler PC: Treatment with growth hormone and dexamethasone in mice transgenic for human islet amyloid polypeptide causes islet amyloidosis and β-cell dysfunction. *Diabetes* 45:1094–1101, 1996
28. Butler AE, Jang J, Gurlo T, Carty MD, Soeller WC, Butler PC: Diabetes due to a progressive defect in β-cell mass in rats transgenic for human islet amyloid polypeptide (HIP Rat): a new model for type 2 diabetes. *Diabetes* 53:1509–1516, 2004
29. Butler PC, Rizza RA: Contribution to postprandial hyperglycemia and effect on initial splanchnic glucose clearance of hepatic glucose cycling in glucose-intolerant or NIDDM patients. *Diabetes* 40:73–81, 1991
30. Butler PC, Kryshak EJ, Schwenk WF, Haymond MW, Rizza RA: Hepatic and extrahepatic responses to insulin in NIDDM and nondiabetic humans: assessment in absence of artifact introduced by tritiated nonglucose contaminants. *Diabetes* 39:217–225, 1990
31. Meier JJ, Butler PC: Insulin secretion. In *Endocrinology*. 5th ed. DeGroot LJ, Jameson JL, Eds. Philadelphia, Elsevier Saunders, 2005, p. 961–973
32. Robertson RP, Zhang HJ, Pyzdrowski KL, Walseth TF: Preservation of insulin mRNA levels and insulin secretion in HIT cells by avoidance of chronic exposure to high glucose concentrations. *J Clin Invest* 90:320–325, 1992
33. Tanaka Y, Gleason CE, Tran PO, Harmon JS, Robertson RP: Prevention of glucose toxicity in HIT-T15 cells and Zucker diabetic fatty rats by antioxidants. *Proc Natl Acad Sci U S A* 96:10857–10862, 1999
34. Maedler K, Sergeev P, Ris F, Oberholzer J, Joller-Jemelka HI, Spinas GA, Kaiser N, Halban PA, Donath MY: Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. *J Clin Invest* 110:851–860, 2002
35. Matschinsky F, Liang Y, Kesavan P, Wang L, Froguel P, Velho G, Cohen D, Permutt MA, Tanizawa Y, Jetton TL, et al.: Glucokinase as pancreatic beta cell glucose sensor and diabetes gene. *J Clin Invest* 92:2092–2098, 1993
36. Maechler P, Wollheim CB: Mitochondrial function in normal and diabetic beta-cells. *Nature* 414:807–812, 2001
37. Kawasaki F, Matsuda M, Kanda Y, Inoue H, Kaku K: Structural and functional analysis of pancreatic islets preserved by pioglitazone in *db/db* mice. *Am J Physiol Endocrinol Metab* 288:E510–E518, 2005
38. Harding HP, Ron D: Endoplasmic reticulum stress and the development of diabetes: a review. *Diabetes* 51 (Suppl. 3):S455–S461, 2002
39. Kaufman RJ: Orchestrating the unfolded protein response in health and disease. *J Clin Invest* 110:1389–1398, 2002
40. Ohsawa H, Kanatsuka A, Yamaguchi T, Makino H, Yoshida S: Islet amyloid polypeptide inhibits glucose-stimulated insulin secretion from isolated rat pancreatic islets. *Biochem Biophys Res Commun* 160:961–967, 1989
41. Tedstone AE, Nezzer T, Hughes SJ, Clark A, Matthews DR: The effect of islet amyloid polypeptide (amylin) and calcitonin gene-related peptide on glucose removal in the anaesthetized rat and on insulin secretion from rat pancreatic islets in vitro. *Biosci Rep* 10:339–345, 1990
42. Larsen MO, Rolin B, Wilken M, Carr RD, Gotfredsen CF: Measurements of insulin secretory capacity and glucose tolerance to predict pancreatic β-cell mass in vivo in the nicotinamide/streptozotocin Göttingen minipig, a model of moderate insulin deficiency and diabetes. *Diabetes* 52:118–123, 2003
43. Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M: Prevention of type 2 diabetes mellitus by changes

in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 344:1343–1350, 2001
44. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM: Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 346:393–403, 2002
45. Kahn CR: Banting Lecture: Insulin action, diabetogenes, and the cause of type II diabetes. *Diabetes* 43:1066–1084, 1994
46. Mulder H, Ahren B, Sundler F: Islet amyloid polypeptide and insulin gene expression are regulated in parallel by glucose in vivo in rats. *Am J Physiol* 271:E1008–E1014, 1996
47. Katayama T, Imaizumi K, Honda A, Yoneda T, Kudo T, Takeda M, Mori K, Rozmahel R, Fraser P, George-Hyslop PS, Tohyama M: Disturbed activation of endoplasmic reticulum stress transducers by familial Alzheimer's disease-linked presenilin-1 mutations. *J Biol Chem* 276:43446–43454, 2001
48. Katayama T, Imaizumi K, Manabe T, Hitomi J, Kudo T, Tohyama M: Induction of neuronal death by ER stress in Alzheimer's disease. *J Chem Neuroanat* 28:67–78, 2004
49. Song SH, McIntyre SS, Shah H, Veldhuis JD, Hayes PC, Butler PC: Direct measurement of pulsatile insulin secretion from the portal vein in human subjects. *J Clin Endocrinol Metab* 85:4491–4499, 2000
50. Porksen N, Munn S, Steers J, Vore S, Veldhuis J, Butler P: Pulsatile insulin secretion accounts for 70% of total insulin secretion during fasting. *Am J Physiol* 269:E478–E488, 1995
51. Porksen N, Munn S, Steers J, Veldhuis JD, Butler PC: Effects of glucose ingestion versus infusion on pulsatile insulin secretion: the incretin effect is achieved by amplification of insulin secretory burst mass. *Diabetes* 45:1317–1323, 1996
52. Larsen MO, Gotfredsen CF, Wilken M, Carr RD, Porksen N, Rolin B: Loss of beta-cell mass leads to a reduction of pulse mass with normal periodicity, regularity and entrainment of pulsatile insulin secretion in Gottingen minipigs. *Diabetologia* 46:195–202, 2003
53. Kjems LL, Kirby BM, Welsh EM, Veldhuis JD, Straume M, McIntyre SS, Yang D, Lefebvre P, Butler PC: Decrease in β-cell mass leads to impaired pulsatile insulin secretion, reduced postprandial hepatic insulin clearance, and relative hyperglucagonemia in the minipig. *Diabetes* 50:2001–2012, 2001
54. Laedtke T, Kjems L, Porksen N, Schmitz O, Veldhuis J, Kao PC, Butler PC: Overnight inhibition of insulin secretion restores pulsatility and proinsulin/insulin ratio in type 2 diabetes. *Am J Physiol Endocrinol Metab* 279:E520–E528, 2000
55. Komjati M, Bratusch-Marrain P, Waldhausl W: Superior efficacy of pulsatile versus continuous hormone exposure on hepatic glucose production in vitro. *Endocrinology* 118:312–319, 1986
56. Bratusch-Marrain PR, Komjati M, Waldhausl WK: Efficacy of pulsatile versus continuous insulin administration on hepatic glucose production and glucose utilization in type I diabetic humans. *Diabetes* 35:922–926, 1986
57. Goodner CJ, Sweet IR, Harrison HC Jr: Rapid reduction and return of surface insulin receptors after exposure to brief pulses of insulin in perifused rat hepatocytes. *Diabetes* 37:1316–1323, 1988
58. Hirashima Y, Tsuruzoe K, Kodama S, Igata M, Toyonaga T, Ueki K, Kahn CR, Araki E: Insulin down-regulates insulin receptor substrate-2 expression through the phosphatidylinositol 3-kinase/Akt pathway. *J Endocrinol* 179:253–266, 2003
59. Meier JJ, Veldhuis JD, Butler PC: Pulsatile insulin secretion dictates systemic insulin delivery by regulating hepatic insulin extraction in humans. *Diabetes* 54:1649–1656, 2005
60. Johnson KH, O'Brien TD, Betsholtz C, Westermark P: Islet amyloid, islet-amyloid polypeptide, and diabetes mellitus. *N Engl J Med* 321:513–518, 1989
61. Tabata H, Hirayama J, Sowa R, Furuta H, Negoro T, Sanke T, Nanjo K: Islet amyloid polypeptide (IAPP/amylin) causes insulin resistance in perfused rat hindlimb muscle. *Diabetes Res Clin Pract* 15:57–61, 1992
62. DeFronzo RA, Simonson D, Ferrannini E: Hepatic and peripheral insulin resistance: a common feature of type 2 (non-insulin-dependent) and type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 23:313–319, 1982
63. Yki-Jarvinen H: Glucose toxicity. *Endocr Rev* 13:415–431, 1992
64. Ritzel R, Butler AE, Rizza RA, Veldhuis JD, Butler PC: Relationship between β-cell mass and fasting blood glucose concentration in humans. *Diabetes Care* 29:717–718, 2006
65. Gotoh M, Monden M, Okamura J, Mori T, Shima K: Insulin and glucagon secretion after pancreatectomies: correlation of secretion and hormonal contents of remaining pancreas. *Diabetes* 38:861–867, 1989
66. Goodner CJ, Koerker DJ, Weigle DS, McCulloch DK: Decreased insulin- and glucagon-pulse amplitude accompanying β-cell deficiency induced by streptozocin in baboons. *Diabetes* 38:925–931, 1989
67. Kendall DM, Sutherland DE, Najarian JS, Goetz FC, Robertson RP: Effects of hemipancreatectomy on insulin secretion and glucose tolerance in healthy humans. *N Engl J Med* 322:898–903, 1990
68. Loo DT, Copani A, Pike CJ, Whittemore ER, Walencewicz AJ, Cotman CW: Apoptosis is induced by beta-amyloid in cultured central nervous system neurons. *Proc Natl Acad Sci U S A* 90:7951–7955, 1993
69. Pike CJ, Burdick D, Walencewicz AJ, Glabe CG, Cotman CW: Neurodegeneration induced by beta-amyloid peptides in vitro: the role of peptide assembly state. *J Neurosci* 13:1676–1687, 1993
70. Ritzel RA, Butler PC: Replication increases β-cell vulnerability to human islet amyloid polypeptide-induced apoptosis. *Diabetes* 52:1701–1708, 2003