Improved Glucose Regulation on a Low Carbohydrate Diet in Diabetic Rats Transplanted With Macroencapsulated Porcine Islets

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Islet xenografts from porcine donors can reverse diabetes in experimental animal models and may be an alternative to human islet transplantation. We have recently reported the ability of porcine islets encapsulated in a double layer of hydrophilic agarose to maintain in vitro functional ability for >6 months. Although β-cells are capable of adapting their secretory capacity in response to glucose levels, evidence has shown that prolonged hyperglycemia can compromise this ability. The aim of the present study was to determine the effects of diet manipulation on the long-term regulation of blood glucose levels, and the preservation of functional islet in the macrobead. Twenty-one streptozotocin-induced diabetic Wistar-Furth male rats were randomly assigned to two diets containing 64% carbohydrate (CHO) or 20% CHO. Groups of five to six animals assigned to either diet were implanted with either empty (EM) or porcine islet-containing macrobeads (PIM) and followed for 333 days. Observations included general condition, body weight, blood glucose, and food and water intakes. Monthly blood samples were collected for insulin and C-peptide analysis. The 20% CHO diet significantly lowered blood glucose values when compared with those of the 64% CHO groups for both the empty (14.94 ± 0.41 vs. 16.26 ± 0.42 mmol/L, respectively, p < 0.001) and islet macrobead recipients (12.88 ± 0.39 vs. 15.57 ± 0.85 mmol/L, respectively, p < 0.001). The different diets, however, had no statistically significant effects on the preservation of islet mass in the macrobead. Serum porcine C-peptide was detected throughout the experiment in animals receiving porcine islet macrobeads, regardless of diet. Diabetic rats fed a low carbohydrate level diet and transplanted with porcine islet macrobeads had improved total tissue glucose disposal and improved clinical parameters associated with diabetes, although macrobead islet mass did not differ between the two diet groups.

Key words: Porcine islets; Xenotransplantation; Encapsulation; Diet

INTRODUCTION

The use of animal-derived cells and organs for transplantation to replace missing physiological function in humans such as the lack of insulin for glucose control in type 1 diabetes is a promising alternative to the use of human donor tissue as a replacement source, because the supply of the latter is very limited. With a similar physiology to that of humans, including an insulin molecule that differs by only one amino acid, and their virtually unlimited availability, pigs are widely considered to be the most likely animal donor for the cellular replacement of pancreatic islets to treat type 1 diabetes. With many of the concerns regarding the safety and immunological aspects of xenotransplantation now considered to be or becoming manageable, the use of porcine islets in the treatment of human diabetes now appears to be a realistic objective.

In an effort to address both the biological safety and immune rejection challenges facing xenotransplantation in the treatment of insulin-dependent diabetes, The Rogosin Institute has designed a two-layer 6–8-mm-diameter ovoid agarose macrobead that not only enables the detection over time of any microbiological contaminants, but also provides a protective barrier for porcine islets against a direct xenogeneic immunologic host response. The islets themselves are embedded within the inner layer of agarose (1% concentration), which enables their maintenance in culture for at least 2 years. The outer layer of agarose, which is more concentrated...
(5%), provides structural integrity to the macrobead and also serves as a protective barrier against the host immune system, thus enabling the host to avoid the necessity of immunosuppressive agents.

As previously reported, xenografts of porcine and rat islets embedded in the agarose–agarose macrobeads have been shown to normalize blood glucose in spontaneously diabetic rodents (9,12), as well as in streptozotocin-induced diabetic animals (13,14). Importantly, the animals in these previous studies were insulin free throughout the study periods in the absence of immunosuppression. Eventually, blood glucose levels did increase over time, with islet macrobead recipients becoming mildly hyperglycemic. They continued, however, to show evidence of improved control of their diabetic state as evidenced by weight gain, lack of ketotic episodes, lack of polyuria, and improved responses to glucose loading.

The rise in serum glucose levels, as well as histological examination of the porcine islet macrobeads after implantation indicates the in vivo loss of islet cells, including β-cells, over time despite the protective macrobead environment. This fact prompted us to look at this phenomenon of β-cell loss and ask whether anything can be done to reduce it.

As substantial bodies of data indicate, following implantation, porcine islet macrobeads function dynamically, responding to changing demands for insulin in response to factors such as weight gain and carbohydrate load. There are limitations to this responsiveness, however, in that, if insulin production and secretion are already maximal, the implanted islets will not be able to meet still greater demands. For example, Unger and Grundy have reported that hyperglycemia may be provoked by an environmental perturbation that increases insulin demand and/or decreases insulin supply in a previously normoglycemic individual whose β-cells are already secreting at a maximal level (21). What may be even more important is the fact that such hyperglycemia, if it is sustained over time, can negatively affect β-cell survival following islet transplantation (15,16). In fact, in vitro incubation of islets and isolated β-cells with glucose induces a process of apoptotic β-cell death that is glucose concentration dependent (5). Increased insulin demand can be met through compensatory hypersecretion to a point, but any β-cell loss will put a considerable strain on the surviving cells and thus begin a cycle of progressive metabolic deterioration. Based on these data, it seems reasonable to conclude that reducing the demands on implanted islets, whether in an agarose macrobead or not, is likely to preserve islet mass and function over the long term.

Because carbohydrates are the major stimulants of insulin production and release (2), and thus a major factor in the metabolic demands made on islets, some form of carbohydrate restriction is a key candidate for dietary control of diabetes. In fact, before the discovery of insulin, dietary carbohydrate restriction was the recommended treatment for diabetes management. Diets containing 50–60% calories from carbohydrates have been the standard recommendation for patients with type 2 diabetes and metabolic syndrome (18). Dietary carbohydrates (CHO) are the major determinants of postprandial glucose levels (22), and low CHO diets have been reported to lower postprandial glucose levels directly, as well as indirectly over time by way of weight loss (6). Evidence from clinical and metabolic studies demonstrates aggravation of glycemic control and dyslipidemia in diabetic patients with a high-carbohydrate diet (6,7,10), whereas a low-carbohydrate diet may reverse these serious metabolic abnormalities (4).

In the study reported here, we have evaluated the effect of carbohydrate restriction on the function of porcine islet macrobeads implanted in rats with streptozotocin-induced diabetes over a period of 333 days in a test of the hypothesis that dietary carbohydrate restriction, by virtue of the reduced metabolic stress on the islets, would lead to improved preservation of β-cell mass and/or improved glucose homeostasis, as evidence by serum glucose levels and elimination of exogenous insulin requirements.

MATERIALS AND METHODS

Animals

Inbred male Wistar Furth rats (Harlan Sprague Dawley, Indianapolis IN), aged 5–6 weeks on arrival, were maintained on a 12-h light/dark cycle (lights on 0700 h) in a temperature/humidity-controlled room. Rats were housed individually per cage in poly-sulfone cages, 483 × 267 × 203 mm (L × W × H), with a rim wire lid and polyester filter tops (Allentown Caging Equipment Co., Inc., Allentown, NJ) within a Micro-FLO Environmental Systems High Efficiency Particulate Class 100 Air (HEPA) cabinet (Allentown Caging Equipment Co., Inc.). Room temperature was maintained within 18–26°C with a relative humidity of 30–70%. All animals had free access to food and clean tap water (Xenia, OH). All study protocol procedures were approved by The Rogosin Institute Animal Care and Use Committee (TRIACUC).

After macrobead implantation, rats were maintained on either of two types of purified diet (Test Diet, Division of LabDiet® a Purina Mills, Richmond, IN): #T57: 20% energy from carbohydrates (low-CHO diet: 67.79% energy from protein and 11.99% energy from fat) or #T56: 64% energy from carbohydrates (regular-CHO dies: 24.08% energy from protein and 11.97% energy from fat). Each experimental diet was consumed for 333 days.
**Diabetes Induction**

Rats were anesthetized with isoflurane, then injected intravenously (tail vein) with 65 mg/kg streptozotocin (VWR, West Chester, PA) dissolved in sodium acetate buffer (pH 4.5) for diabetes induction. Following the administration of streptozotocin, animals exhibited a significant loss of body weight, polyuria, polydipsia, and hyperglycemia. Diabetes was clinically confirmed with blood glucose readings of >27 mmol/L for 2–3 consecutive days. Rats were started and maintained on prontamine-zinc insulin (PZI Insulin, Blue Ridge Pharmaceuticals, Inc., Greensboro, NC) prior to macrobead implantation.

**Experimental Groups**

After diabetes was confirmed and daily exogenous insulin requirements determined, the animals were randomly assigned to four groups—group 1: 20% low-CHO diet implanted with empty macrobeads (20% CHO control, \( n = 5 \)); group 2: 20% low-CHO diet implanted with porcine islet macrobeads (20% CHO PIM, \( n = 6 \)); group 3: 64% regular-CHO diet implanted with empty macrobeads (64% CHO control, \( n = 5 \)); group 4: 64% regular-CHO diet implanted with porcine islet macrobeads (64% CHO PIM, \( n = 5 \)). Scheduled sacrifice occurred on day 333 post-macrobead implantation. Two animals from group 1 were euthanized shortly after macrobead implantation due to deteriorating body condition. One animal from each group was euthanized before the scheduled necropsy. However, in some cases this happened close to the study termination and the data obtained to that point were used for interpretation.

**Islet Isolation**

Donor islets were prepared from Newsham sows that were over 2 years of age and had a history of multiple parities as previously described (8). Islet counts were expressed as equivalent islet numbers (EIN), based on a standard islet size of 150 \( \mu \)m, and 500 EIN were encapsulated in agarose–agarose macrobeads as previously described (8).

**Porcine Islet Macrobead Implantation**

Macrobeads were collected the day prior to implant and stored overnight at room temperature (19–27°C) in RPMI + 1% A/A. Immediately prior to implant, macrobeads were washed three times with RPMI + 1% A/A.

All animals received an implant of either islet macrobeads at a dose equivalent to 1.0 times daily insulin requirements (45.8 ± 3.0 macrobeads) with an average total weight of 12.0 ± 1.0 g or a comparable number of empty macrobeads (47.35 ± 5.7 EM) with an average weight of 14.5 ± 1.7 g. The mean age of the islet macrobeads at implantation was 8 weeks with an average insulin secretion of 43 mU/macrobead/24 h. Empty macrobeads implanted into the control rats were 5 weeks of age.

Animals were anesthetized with a mix of 2 ml ketamine, 1 ml xylazine, and 0.3 ml butorphanol (ketamine 100 mg/ml, xylazine 20 mg/ml, and butorphanol 10 mg/ml) (Fort Dodge Animal Health, Fort Dodge, IA; Ben Venue Laboratories, Bedford, OH; Baxter Healthcare Corporation, Deerfield, IL, respectively) at a dose of 1.0–1.5 ml/kg (60 mg/ml ketamine, 6 mg/ml xylazine, and 3 mg/ml butorphanol) given intramuscularly.

During the implantation surgery, macrobeads were gently placed into the peritoneal cavity by use of a sterile plastic spoon (Scienceware, Pequannock, NJ). Musculature was closed with 2.0 metric Polymend ES sutures (VPL, Phoenix, AZ) and skin was closed with Autoclip wound clips (Becton Dickinson Primary Care Diagnostics, Sparks, MD).

**Clinical Observations**

Clinical observations from individual animals were recorded daily. Observations included general condition (good, fair, or poor), body weight, nonfasting blood glucose (Accu-Chek® Simplicity™ BG monitor and Chemstrips, Roche Diagnostics), urine glucose, and urine ketones (Keto-Diastix® Bayer, Eikhart, IN). Food and water intakes were monitored only during the weekdays (an average value was obtained for the weekend). Blood samples from saphenous vein (300–450 \( \mu \)l) were collected monthly in microcentrifuge tubes, stored at room temperature for 30 min then centrifuged, and the serum collected. Samples were stored at −70°C for subsequent insulin and C-peptide analysis.

**Insulin, Glucagon, and Porcine C-Peptide Assays**

Nonfasting blood for the detection of insulin and porcine C-peptide was collected monthly throughout the study.

Standard radioimmunoassays from Linco Research, Inc. (St. Charles, MO) were used for the detection of insulin (sensitivity of 2 \( \mu \)U/ml), glucagon (sensitivity of 20 pg/ml), and porcine C-peptide (sensitivity of 0.1 ng/ml). All samples were frozen at −20°C prior to assay and were run with samples in duplicate, and reference standards and controls in triplicate.

**Oral Glucose Tolerance Tests**

Oral glucose tolerance tests (OGTT) were performed using 50% dextrose in sterile water at a dose of 1.0 g dextrose/kg body weight. An oral bolus of glucose (1 g/kg, 50% dextrose injection, USP, Baxter, Deerfield, IL) was administered to nonanesthetized rats with a 16-gauge curved gavage needle (Ejay International, Glendora, CA) shortly after macrobead implantation on day 5 and again on day 181 and on day 314. Venous tail
blood samples were obtained for determination of blood glucose values at −5, 15, 30, 60, and 90 min relative to the glucose bolus administration (0 min).

Means and SDs were calculated for all dependent measures. Glucose area under the curve (AUC) was calculated using the total AUC with respect to ground method (20). To determine if there were any significant differences between groups, the original values were entered into a Student t-test.

Necropsy

Necropsies were performed on day 333 post-macrobead induction (352 days post-streptozotocin induction) for 15 of 21 animals. Weights of liver, kidneys, and spleen were recorded and macrobeads were collected in a sterile fashion and randomly selected for histopathology or for in vitro culture to assess islet hormone production (insulin, glucagon, and porcine C-peptide).

Histopathology

At necropsy, the following tissues were collected and fixed in 10% neutral buffered formalin for 24 h followed by storage in 70% ETOH: eyes, brain, heart, spleen, liver, kidneys, brain, testes, lungs, diaphragm, pancreas, abdominal musculature, sciatic nerve. All histopathology was performed by Pathology Associates International (PAI, A Charles River Company; West Chester, OH). Tissues were embedded in paraffin, and 5-µm sections were stained with hematoxylin and eosin (H&E). Samples were analyzed by a Diplomat of the American College of Veterinary Pathology, and the macroscopic and histopathological findings documented. Where appropriate, findings received a severity grade based upon a scale where: N = tissue within normal histological limits, 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked.

Porcine Islet Macrobead Cell Mass

Porcine islet macrobeads (PIM) retrieved at necropsy were fixed in 10% neutral buffered formalin overnight and then stored in 70% ethanol until further treatment. Five porcine islet macrobeads per animal were embedded in paraffin, sectioned at 5 µm, and stained with H&E. Cell nuclei from two slides containing five porcine islet macrobead sections were counted at 400× magnification using light microscopy, averaged, and considered as one separate observation with each observation representing an individual animal.

Statistics

Statistical analysis was performed using Microsoft Excel (Redmond, WA), StatSoft Statistica (Tulsa, OK), and data plotting was performed using Systat Software, Inc.—SigmaPlot 2001 (Point Richmond, CA). The data were analyzed using Student’s t-test and ANOVA. Calculations resulting in probability values of $p < 0.05$ were considered statistically significant.

RESULTS

Insulin Requirements

The average daily insulin requirements of each rat were established over a 16-day period (1.8 ± 0.15 U/day PZI Insulin) following diabetes induction.

For all animals receiving islet macrobeads, insulin therapy was discontinued beginning on the day of transplantation. They received no exogenous insulin for the duration of the study, regardless of which diet they were receiving.

Following empty macrobead implantation, average exogenous insulin requirements over the entire implantation period were 1.72 ± 0.38 U/day insulin (5% decrease from baseline) for the 20% CHO diet group and 1.89 ± 0.50 U/day insulin (5% increase over baseline) for the 64% CHO diet group ($p < 0.05$).

Blood Glucose

After streptozotocin administration, rats became hyperglycemic with average blood glucose levels of 25.33 ± 0.31 mmol/L for the 14-day period immediately prior to transplantation. Mean plasma glucose concentration declined to 7.92 ± 0.68 mmol/L during the first 7 days posttransplantation for those rats receiving islet macrobeads regardless of CHO group ($p < 0.001$). The control groups demonstrated hyperglycemia throughout the remainder of the study.

The daily blood glucose values of all porcine islet macrobead recipients gradually increased over the first 60 days posttransplantation. While the 20% CHO group stabilized at this time, the blood glucose values of the 64% CHO group continued to increase gradually over the next 200 days. Their food and water intake increased in parallel with this rise in blood glucose levels.

For animals receiving empty macrobeads, the 20% CHO diet lowered the average blood glucose levels over the entire implantation period to a statistically significant level compared to the 64% CHO control group (14.94 ± 0.41 vs. 16.26 ± 0.42 mmol/L, respectively, $p < 0.001$). For those animals that received islet macrobeads, the 20% CHO diet also significantly lowered the average blood glucose levels compared to animals maintained on the 64% CHO diet (12.88 ± 0.39 vs. 15.57 ± 0.86 mmol/L, respectively, $p < 0.001$) (Fig. 1).

Blood glucose variations expressed as percentage of baseline were calculated for each group using the following formula: $\%B_{\text{G}}i = 100 \times (B_{\text{G}i} - B_{\text{G0}})/B_{\text{G0}}$, where $\%B_{\text{G}}$ variation is blood glucose at time of determination expressed in percentage of baseline, $B_{\text{G}i}$ is absolute blood glucose value at time $i$, and $B_{\text{G0}}$ is the baseline blood glucose value before streptozotocin in-
Figure 1. Nonfasting blood glucose of streptozotocin-induced diabetic Wistar-Furth rats. Weekly mean blood glucose ± SD is shown for rats implanted with either (A) empty macrobeads or (B) porcine islet macrobeads and followed for 333 days. Rats received either 20% CHO diet (closed circles) or 64% CHO (open circles).

Body Weight

Overall, the 64% CHO diet promoted weight gain on all animals independent of the macrobead type (64% CHO CTR 393.6 ± 44.3 g vs. 20% CHO CTR 364.15 ± 0.77g and 64% CHO PIM 352.8 ± 27.4 g vs. 20% CHO PIM 332.2 ± 25.31 g at sacrifice, p < 0.05). Regardless of the experimental group, all animals exhibited good body conditions for the duration of the experiment, indicating that their general health was satisfactory.

Food Intake

Food consumption was monitored daily (Fig. 2). Islet macrobead recipients fed the 20% CHO diet maintained a lower average food intake during the experiment compared to that of the animals fed the 64% CHO (14.66 ± 0.43 vs. 20.13 ± 0.74 g/day, respectively, p < 0.0001). Control rats that received empty macrobeads and were maintained on the 20% CHO diet also demonstrate a significantly reduced food intake compared to that of the 64% CHO control group (15.26 ± 0.22 vs. 16.94 ± 0.53 g/day, respectively, p < 0.001).

Water Intake

As shown in Figure 3, average daily intake of water for diabetic Wistar Furth rats implanted with empty macrobeads and fed either a 20% or 64% CHO diet was significantly different (42.32 ± 0.68 and 32.53 ± 1.69 ml/day, respectively, p < 0.05). Diabetic rats implanted with islet macrobeads and maintained on a 64% CHO diet consumed significantly more water per day than islet macrobead implanted rats fed a 20% CHO diet (60.48 ± 4.75 and 42.33 ± 2.20 ml/day, respectively, p < 0.05).

Oral Glucose Tolerance Test

The animals underwent a preimplant oral glucose tolerance test (OGTT) to confirm the clinical diagnosis of diabetes on day −22. At this time the AUCg was 518.2 mmol L⁻¹ h⁻¹. A second preimplant OGTT was performed on day −9 prior to macrobead implantation but after streptozotocin induction of diabetes. An AUCG of 2819.6 mmol L⁻¹ h⁻¹ was recorded at that time.

Following macrobead implantation, the ability of the islet macrobeads to respond to a glucose challenge was maintained throughout the study period (Fig. 4). Although all islet macrobead-implanted study animals eventually became moderately hyperglycemic, OGTTs performed over the course of the study continued to demonstrate an initial rise in blood sugar, followed by a return to baseline glycemia over 90 min.

Diet did not affect glucose tolerance on day 5 post-transplantation (20% CHO diet 672.7 mmol L⁻¹ h⁻¹ vs. 64% CHO diet 770 mmol L⁻¹ h⁻¹) as reflected by the AUCg. Statistically significant differences, however, were observed for the porcine islet macrobead-implanted animals on days 181 (20% CHO diet 1440.0 mmol L⁻¹ h⁻¹ vs. 64% CHO diet 1724.2 mmol L⁻¹ h⁻¹, p < 0.05) and 314 (20% CHO diet 1277.7 mmol L⁻¹ h⁻¹ vs. 64%
Figure 2. Food intake of streptozotocin-induced diabetic Wistar-Furth rats. Weekly mean food intake ± SD is shown for rats implanted with either (A) empty macrobeads or (B) porcine islet macrobeads and followed for 333 days. Rats received either 20% CHO diet (closed circles) or 64% CHO (open circles).

CHO diet 1492.6 mmol L⁻¹ h⁻¹, \( p < 0.05 \)). The 20% CHO diet group experienced in general a delayed glucose response after OGTT. Additionally, no significant differences in the total AUCG were observed for the EM groups, regardless of diet, except on day 5 posttransplantation when the 20% CHO group had a lower AUCG (20% CHO diet 1436.8 mmol L⁻¹ h⁻¹ vs. 64% CHO diet 2334.4 mmol L⁻¹ h⁻¹, \( p < 0.05 \)).

Serum C-Peptide Levels

Blood was collected monthly from study rats to assess for the presence of porcine C-peptide. Porcine C-peptide was not detected in any animal prior to macrobead implantation, or from any empty macrobead recipients throughout the study. In contrast, porcine C-peptide was routinely detected in the serum of islet macrobead-implanted rats. Average serum C-peptide levels from 10 readings between 9 and 333 days posttransplantation did not differ between the rats maintained on a 64% CHO diet (0.58 ± 0.10 ng/ml) and rats maintained on a 20% CHO diet (0.47 ± 0.12 ng/ml, \( p < 0.05 \)).

Retrieved Porcine Islet Macrobead Hormone Production

Insulin, glucagon, and C-peptide were detected in the culture media from PIM retrieved from all study animals.

Figure 3. Water intake of streptozotocin-induced diabetic Wistar-Furth rats. Weekly mean water intake ± SD is shown for rats implanted with either (A) empty macrobeads or (B) porcine islet macrobeads and followed for 333 days. Rats received either 20% CHO diet (closed circles) or 64% CHO (open circles).
Figure 4. Oral glucose tolerance test results. Mean blood glucose values ± SD are shown for Wistar Furth rats after challenge with an oral gavage of 1.0 g dextrose/kg of body weight. Rats received either 20% CHO diet (closed circles) or 64% CHO (open circles). Glucose challenge was performed: (A) Pre- and post-streptozotocin induction, (B) day 5 posttransplantation, (C) day 181 posttransplantation, (D) day 314 posttransplantation.
at necropsy. To establish a reliable assessment for the retrieved porcine islet macrobead insulin production, the macrobeads were maintained in culture for 7 weeks. Insulin production per macro bead was decreased by 45% in animals fed a 64% CHO diet (43 mU/macro bead preimplantation; 23.51 ± 6.0 mU/macro bead posttransplantation). Retrieved macro bead insulin production was reduced by 63% (43 mU/macro bead preimplantation; 15.33 ± 6.0 mU/macro bead posttransplantation) in animals maintained on the 20% CHO diet.

**Histopathology**

With the exception of the expected mild to moderate pancreatic inflammation associated with this streptozotocin-induced diabetes animal model, inflammation, fibrosis, and/or hypertrophy/hyperplasia of the peritoneum covering the pancreas, abdominal wall, and/or diaphragm were thought to be related to the presence of the macrobeads. This response was minimal to mild in degree. The changes seen were not thought to have altered the normal function of the tissues/organs concerned. No clear differences among the groups were identified.

Macroscopically, the peritoneal cavities of the rats appeared normal. The only significant finding during necropsy that was confirmed by histological exam was the development of atypical renal tubule hyperplasia (2/8 animals from the control group and 3/12 animals from the PIM group), renal tubule adenoma (2/8 animals from the control group and 2/12 animals from the PIM group), and renal tubule carcinoma (5/8 animals from the control group and 6/12 animals from the PIM group). These findings are highly correlated with the mutagenic and cytotoxic streptozotocin that was used for diabetes induction, as previously reported by others (3, 11, 17, 19).

**Macrobead Islet Cell Mass**

Interestingly, total islet cell mass did not appear to differ as an average of 204.7 ± 40.6 cells were counted from macro bead sections retrieved from the 20% CHO group while 159.2 ± 13.3 cells were counted from sections retrieved from the 64% CHO group (p > 0.05). The data were normally distributed with no statistical significance between groups (p > 0.05).

**DISCUSSION**

The results of this study demonstrate that following the transplantation of porcine islet macrobeads, rats fed a 20% CHO diet had significantly lower daily blood glucose values and less daily variation, expressed as percentage of baseline, when compared to those in rats fed a regular diet containing 64% CHO. In fact, animals maintained on the 20% CHO diet, regardless of the type of macrobeads implanted, also had lower food and water intake values under similar conditions than animals maintained on the 64% CHO diet. These results were observed under conditions of increased secretory demand (growth), and this pattern persisted even as islet macro bead recipients became moderately hyperglycemic. Significant levels of porcine C-peptide were found in the serum of islet macro bead-implanted animals throughout the study, confirming long-term islet macro bead function. In contrast, rats that received empty macro beads required daily insulin injections and had no evidence of porcine C-peptide.

We hypothesized that chronic in vivo exposure of porcine islet macro beads to hyperglycemia might affect the preservation of islet cell mass or the development of secretory defects. However, no significant differences were observed in the levels of circulating serum porcine C-peptide. Furthermore, no significant differences in hormone production levels or macro bead β-cell mass were found for the islet macro beads retrieved at necropsy when the two carbohydrate diets were compared.

We take the lack of islet macro bead differences between the low and high CHO groups in terms of β-cell mass, serum porcine C-peptide levels, and insulin production from retrieved macro beads to be evidence of equivalent islet macro bead function. However, the marked differences in food/water intake, blood glucose levels, and OGTT data for the 20% CHO PIM group are consistent with the notion that the effects of the lower CHO diet lie downstream of insulin secretion.

Our data demonstrate a correlation between low to moderate hyperglycemia and a low intake of the 20% CHO diet. This decreased consumption may be due to various mechanisms. For example, a higher protein diet could have increased satiety and reduced hunger following ingestion without conditioned taste aversion (1). Alternatively, and the mechanism that we favor, is that the insulin sensitivity of recipient animals maintained on the low carbohydrate diet was at least preserved. In our study, a much higher intake of the 64% CHO diet, even in the presence of hyperglycemia, demonstrates a pronounced reduction in insulin sensitivity without a significant alteration in insulin secretion (as extrapolated from serum porcine C-peptide values). In fact, the number of β-cells in the retrieved macro beads from the higher CHO group is similar to the mass from the 20% CHO group. Furthermore, the functional capacity of the 64% CHO islet macro bead group is 50% or more of the 20% carbohydrate islet macro bead group, as shown by retrieved macrobeads’ insulin and C-peptide data.

These findings emphasize that postprandial glucose levels are primarily determined by the amount of ingested carbohydrates and that a low-carbohydrate diet improves insulin/glucose ratios. Whether similar improvements in glycemic control can be achieved by increasing the number of islet macro beads to account
for the increased insulin requirements associated with a higher CHO diet remains to be investigated. The use of a hyperinsulinemic euglycemic clamp could also address the effects of diet on streptozotocin-induced diabetic rats implanted with porcine islet macrobeads on whole-body insulin sensitivity, as well as alterations in basal and insulin-stimulated glucose metabolism.

The results of this study suggest that strategies aimed at reducing the deleterious effect of CHO-induced stress and insulin resistance on transplanted islets may be appropriate and beneficial in the clinic. Such strategies may be applicable in the use of free islets transplanted to the liver but certainly for islet replacement strategies using encapsulation technologies.

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