Intrahepatic glucose flux as a mechanism for defective intrahepatic islet alpha cell response to hypoglycemia

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ABSTRACT

Objective: Glucagon responses to hypoglycemia from islets transplanted in the liver are defective. To determine whether this defect is related to intrahepatic glycogen, inbred Lewis rats islets were transplanted in hepatic sinus (H), peritoneal cavity (P), omentum (O) and kidney capsule (K) of Lewis rats rendered diabetic with streptozotocin.

Research Design and Methods: Glucagon responses to hypoglycemia were obtained before and after transplantation under fed conditions and after fasting for 16h and 48h to deplete liver glycogen.

Results: Glucagon (AUC) responses to hypoglycemia in H (8839 +/- 1988 pg/ml/90min) were significantly less than in normal rats (40,777 +/- 8,192, p<0.01). Fasting significantly decreased hepatic glycogen levels. Glucagon responses in H were significantly larger after fasting (fed = 8839 +/- 1988 vs. 16h fasting = 24,715 +/-5,210, 48h fasting = 29,639 +/- 4550, p<0.01). Glucagon response in H decreased after refeeding (48h fasting = 29,639 +/- 4550, vs. refed = 10,276 +/- 2750; p<0.01). There was no difference in glucagon response to hypoglycemia between H and normal control group after fasting for 48h (H = 29,639 +/- 4550 vs. Control = 37,632 +/- 5335 p=ns). No intragroup differences were observed in the P, O, and K groups, or normal control and STZ groups, when comparing fed or fasting states.

Conclusions: These data suggest that defective glucagon responses to hypoglycemia by intrahepatic islet alpha cells is due to dominance of a suppressive signal caused by increased glucose flux and glucose levels within the liver secondary to increased glycogenolysis caused by systemic hypoglycemia.
We and others have previously reported that diabetic recipients of successful intrahepatic pancreatic islet transplantation have defective intrahepatic alpha cell glucagon responses to hypoglycemia (1-6). This is an important clinical issue because patients who are chosen for islet transplantation are at especially high risk of severe hypoglycemia (7,8). Within several years after transplantation, most recipients usually return to insulin therapy and are once again at risk for hypoglycemia.

When the liver site is used, islets are infused via the hepatic portal vein and lodge in the hepatic sinusoids where they establish connections with arterioles from the central hepatic artery (9,10). Alpha cells on the periphery of the islets are in intimate contact with hepatocytes. Since the liver is a major site of glycogen storage and glucose production, we hypothesize that intrahepatic alpha cells are exposed to higher glucose concentrations than is delivered by the hepatic artery as a result of hypoglycemia. If so, it is possible that increased glucose flux within the hepatic parenchyma during hypoglycemia-induced glycogenolysis provides a strong inhibitory signal to the intrahepatic alpha cells.

To examine this hypothesis, we transplanted islets isolated from inbred Lewis rats into the hepatic sinus (H group), the peritoneal cavity (P group), the omental pouch (O group) and the left kidney capsule (K group) of recipient Lewis rats previously rendered diabetic with streptozotocin (STZ). Glucagon responses to hypoglycemia were analyzed under the conditions of fed, fasting for 16h and fasting for 48h to evaluate: 1) the degree to which alpha cell glucagon responses to hypoglycemia are defective; 2) whether glucagon responses in H group are significantly greater after liver glycogen storage is depleted; and 3) whether the alpha cell defective response returns after refeeding.

MATERIALS AND METHODS

Animals. Six weeks old inbred male Lewis rats were purchased from Charles River laboratories. Animals were placed in rooms with a 12-h light/dark cycle with constant temperature and given free access to food and water. Subcutaneous insulin pellets (0.5u/24h) were provided for STZ-treated animals to avoid excessive hyperglycemia. All experiments were approved by the Pacific Northwest Research Institute Institutional Animal Care and Use Committee.

Experimental design. Islets were transplanted into the hepatic sinus (H group, n=10), the peritoneal cavity (P group, n=7), the omental pouch (O group, n=5) and the left kidney capsule (K group, n=5) of STZ-induced diabetic recipients. Non-transplanted STZ-diabetic (S group, n=9) and normal animals (C group, n=6) were also studied. Blood glucose levels and body weights were measured in transplanted rats every day for two weeks after transplantation and once a week thereafter. Insulin pellets were removed from all the recipients within two weeks after transplantation. In some intraperitoneal transplanted recipients and STZ recipients without transplantation, insulin pellets were re-implanted if the blood glucose levels were higher than 350mg/dl.

One month after transplantation, intravenous insulin tolerance tests (ITT) were performed to evaluate glucagon responses to hypoglycemia. Each rat had ITT three times under fed, fasting 16h and fasting 48h conditions. Each ITT was one week apart for the H group and one month apart for the other groups. 4 rats from H group had ITTs again on the fourth week after returning to the fed condition. At the end of the experiment, animals were euthanized and liver samples were taken for glycogen staining and measurement by a chemical method.

Transplant recipient preparation. Seven week old Lewis rats were given an intraperitoneal injection of 120mg/kg STZ
Rats were considered diabetic when blood glucose levels exceeded 450mg/dl on two sequential measurements. Animals that did not develop diabetes by one day were given the same dose of STZ again. Insulin pellets (Linshin Canada, Scarborough, ON, Canada) delivering ~ 0.25 unit/24h were inserted under the neck skin to partially regulate blood glucose levels. ITT was performed in animals 2 weeks after diagnosis of diabetes. Only animals shown to have defective glucagon responses to hypoglycemia (peak delta glucagon less than 50 pg/ml) were accepted as recipients.

**Islet preparation.** Six week old male Lewis rats were used as donors. On the day of transplantation, pancreata were cannulated for infusion of collagenase and then removed from the animal for processing (11). After isolation, islets were hand picked and counted. 1800-2200 islets from 3-4 rats were pooled for culture in RPMI 1640 (Sigma-Aldrich, St. Louis, MO) with 11.1 mM glucose for 2 hours. Islets were then collected and washed with Hanks buffer 3 times. After the final wash, supernatant was removed as much as possible without losing islets. Using a rat serum coated 1 ml Eppendorf pipette tip, islets were transferred to rat serum coated microfuge tube. 1800 – 2200 islets were transplanted into each recipient within 20 minutes thereafter.

**Intrahepatic transplantation.** Recipients (10-12 week old male Lewis rats previously rendered diabetic with STZ) were anesthetized by inhalation of 2% isoflurane. Once anesthetized, the abdomens were shaved, disinfected and opened. Using rat serum coated glass pipette, islets were transferred to another siliconized, rat serum coated fine-tip glass pipette. One end of the glass pipette was connected to a 3ml syringe. The other tip of the pipet had been pulled to close to 0.7 mm OD and connected to a silastic tubing (Sigma – Aldrich, St. Louis, MO, 0.3mm ID 0.64mm OD). The other end of silastic tubing was connected to the metal part of a 23G needle. The portal vein was identified and punctured by the 23G needle. 1800-2200 islets in 1 ml volume were then infused by slow injection over 1-2 minutes into the portal vein. Checking islets by microscope using this technique confirmed no damage to islets. The puncture site was pressed with gauze for at least 5min to stop bleeding. Then the gauze was carefully removed and abdomen was closed. This technique permitted quantitative transfer islets to the liver in a short time.

**Kidney capsule transplantation.** Each recipient was anesthetized as described above. The left kidney was exposed through a flank incision. A small hole in the kidney capsule was made near the caudal pole. PE-50 tubing was advanced into the kidney capsule. 1800 – 2200 islets in less than 200µl rat serum were slowly infused under the kidney capsule using PE-50 tubing (Braintree, MA) connected to a 1-ml syringe. Then the flank incision was closed and animal was allowed to wake up under close observation.

**Intraperitoneal Transplantation.** The abdomen of the anesthetized recipient was opened by a 0.5cm incision. 1800 – 2200 islets in 400 µl rat serum were transplanted into the peritoneal cavity via rat serum coated 1 ml Eppendorf pipette tip. Then the incision was closed and animal was allowed to recover.

**Omental Transplantation.** To create an omental pouch, the greater omentum was spread out, and a 7-0 suture was run along the margin of the omentum. 1800 – 2200 islets in less than 200 µl rat serum were slowly dripped onto the exposed omentum using 200 µl eppendorf pipette. Then the suture was pulled up, tied, and the incision was closed.

**Jugular vein permanent cannulation.** Two weeks after transplantation, right jugular veins of H and C group recipients were permanently cannulated. On the day of cannulation, anesthesia was maintained by
inhalation of 2% isoflurane. The skin was incised on the top of the skull and 4 self-tapping skull screws were placed. Jugular vein was cannulated with silastic tubing (Sigma – Aldrich, St. Louis, MO, 0.51 mm ID 0.94 mm OD) and the tubing was secured to a silicone ring. The other end of the catheter was led subcutaneously toward the skin incision on the skull and connected to a piece of stainless steel tubing (1.5 cm) that was bent 90 °. This “elbow” was secured by dental acrylic to the skull screws. The cannula was flushed with saline, 0.05 ml of heparin (500 µl /ml) and refilled with 50 µl 50% solution of polyvinylpyrrolidone dissolved in heparin and a port plug was inserted into the free end of the cannula. The catheters were flushed once a week. Animals in the other groups did not have permanent jugular vein catheters and one month elapsed between fed, 16h, and 48h ITTs.

**Intravenous Insulin Tolerance Test.** Rats were kept anesthetized by inhalation of 1% isoflurane. 0.3 ml blood samples were taken from jugular vein. After two basal samples were taken, 0.5 u/ml Humulin regular insulin was infused into rats at a rate of 50 µl /min. Once blood glucose levels were lower than 60 mg/dl, insulin infusion was stopped. Blood was sampled for blood glucose and plasma glucagon and C-peptide at the time insulin infusion was stopped and 30, 45, 60 and 90 min later.

**Glycogen measurement.** Liver glycogen was measured using a modified method of Siu et al (12). In brief, at the time of sacrifice, small pieces of liver (100 mg or less) were taken, excess blood was blotted with gauze, and samples were weighed. Samples were placed into a cryovial, flash frozen with liquid nitrogen, and stored at -80 until the time of assay. Tubes were placed on ice and 0.5 ml 30% KOH saturated with Na2SO4 was added to each sample, making sure that the tissue was completely immersed. Samples were placed in a boiling water bath for 20 minutes and then cooled on ice. 0.55 ml cold 95% ethanol was added to each tube to precipitate the glycogen. Tubes were left on ice for 30 minutes and then centrifuged at 840 x g for 20 minutes. Supernatants were carefully aspirated. Glycogen precipitates were then dissolved in 1 ml distilled water. Triplicate 15-30 µl aliquots of glycogen samples were placed in 12 x 75 mm glass tubes and the volume was brought up to 150 µl with distilled water. 150 µl 5% phenol was added to all tubes. 0.75 ml sulfuric acid was rapidly added to all tubes, making sure to direct the stream to the sample surface. Samples were incubated at room temperature for 20 minutes. Absorbance was read at 490 on a Beckman DU64 spectrophotometer. Glycogen values were corrected by weight of liver samples.

**Glycogen staining** Pieces of liver tissues of fed-, 16 h-, and 48 h – fasted rats were fixed in 4% formaldehyde for 6 hours and paraffin-embedded. Tissue sections were stained for glycogen using the PAS technique. Briefly, sections were dewaxed in xylene and rehydrated through graded alcohol. They were incubated in 1% periodic acid solution for 5 minutes, washed in distilled water, and subsequently immersed in Schiff reagent (SurgiPath) for 15 minutes at room temperature. Slides were then washed in running water for 10 minutes, counterstained in Harris hematoxylin (Richard Allan Scientific), dehydrated, and mounted.

**Assays.** Plasma glucose was measured immediately using a Roche Accu-Chek glucose analyzer. Blood samples were collected into ice-chilled tubes. 1,000 IU/ml trasylool was added to prevent degradation of glucagon. Plasma C-peptide and glucagon were measured using a rat radioimmunoassay (Linco Research, St. Charles, MO).

**Statistics.** Data were presented as mean ± SE. Results were analyzed using Wilcoxon matched pair signed-rank test or ANOVA, as appropriate. A p value <0.05 was considered statistically significant.
RESULTS.

**Blood glucose after transplantation.** Blood glucose levels in diabetic rats after STZ treatment before insertion of the insulin pellets were $>$600 mg/dl. On the day of transplantation, the blood glucose levels in recipients were 350-550 mg/dl. The islet grafts were successful in establishing normoglycemia throughout the entire experimental period in O, K, and H groups (Fig 1). The mean time needed to normalize blood glucose levels after transplantation in O, K and H group were 7 +/- 2.9, 2.5 +/- 0.4, and 2.3 +/- 0.7 days, respectively. Islets transplanted into the peritoneal cavity failed to restore euglycemia. However, P group recipients still benefited from transplantation because they required fewer insulin pellets than the STZ controls and maintained lower blood glucose levels (304 +/- 57 vs. 438 +/- 68 mg/dl, respectively, at day 70; p < 0.05). No spontaneous normalization of blood glucose levels in S group was observed during the experiment period. The drop of blood glucose levels in S group at day 49 in Figure 1 was due to reinstitution of insulin pellets implants.

**Glycogen after fasting.** 16h and 48h fasting significantly decreased glycogen storage in the liver in intrahepatic transplanted rats (fed = 55.3 +/- 6.4, 16h fast = 2.23 +/- 0.84, 48h fast = 0.04 +/- 0.10; µg /mg of liver; p<0.0001). There were no significant differences in liver glycogen levels between normal rats and intrahepatic transplanted rats under fed, 16 h fasting, or 48 h fasting conditions (Fig. 2).

Although chemical measurements did not show significant difference in liver glycogen levels between 16h fasting and 48h fasting, glycogen histochemistry staining using paraffin sections demonstrated presence of glycogen in some but not all hepatocytes after 16h fasting (Fig 3). Islets were observed to be in intimate contact with hepatocytes (Fig 3D). Liver glycogen was completely depleted after 48h fasting in all rats.

**Glucagon responses to hypoglycemia in H group under fed, 16h fast, 48h fast and refed conditions.** The basal blood glucose levels were significantly higher when animals were fed compared to fasting (fed = 170 +/- 11, 16h fast = 110 +/- 6, 48h fast = 96 +/- 4; mg/dl; p<0.05, Fig. 4). After re-feeding, the basal blood glucose levels returned to the levels of their original fed state three weeks prior (fed = 170 +/- 11, refed = 180 +/- 19; mg/dl; p=ns). After intravenous insulin infusion, all groups achieved hypoglycemia. There was no significant difference in blood glucose levels among the fed, 16h fasting, 48h fasting and refed when insulin infusion were stopped (22 +/- 3, 21 +/- 3, 17 +/- 3 , and 26 +/- 3, mg/dl; respectively, p=ns).

A significant decline of basal C-peptide levels was observed after fasting (fed = 681 +/- 77, 16h fast = 388 +/- 80, 48h fast = 294 +/- 50; pM; p<0.01). After refeeding, basal C-peptide levels were increased to the levels of fed state. During hypoglycemic challenge, C-peptide decreased to immeasurable levels in all groups.

Significantly higher glucagon responses to hypoglycemia were observed under fasting conditions (fed = 8,777 +/- 2231, 16h fast = 24,217 +/- 5892, 48h fast = 30,790 +/- 5,062; pg/ml/90min AUC; p<0.05-0.01). After refeeding, glucagon responses to hypoglycemia were significantly lower than the responses observed under fasting conditions (refed = 10,276 +/- 2,750; p<0.05) and no difference was found when comparing glucagon responses under fed and refed conditions.

**Glucagon responses to hypoglycemia in C and S groups under fed, 16h fast and 48h fast conditions.** The fed basal blood glucose levels in C group after anesthesia were lower than those in H group (C = 132 +/- 10, H = 170 +/- 11; mg/dl; p=0.05). The basal blood glucose levels decreased significantly from...
fed levels after 16h- and 48h- fasting (132 +/- 10, 86 +/- 3, and 85 +/- 3; mg/dl; p<0.05; Fig. 5). The fed basal C-peptide levels were significantly higher in C group than in H group (C = 1,101 +/- 295, H = 681 +/- 77, pM; p<0.01). 16h- and 48h- fasting significantly decreased fed basal C-peptide levels (457 +/- 76, and 303 +/- 75 pM; respectively; p<0.01). Fasting or fed did not change the magnitude of the glucagon responses to hypoglycemia in normal control group (fed = 40,777 +/- 8,192, 16h fast = 48,810 +/- 13785, 48h fast = 37,632 +/- 5,335; p=ns). A comparison of glucagon responses in the hepatic transplant and the non-diabetic control group is provided in Fig 6.

In S control group, blood glucose levels were significantly lower after 48h fasting because insulin pellets were implanted (Table 1). After insulin infusion, blood glucose levels reached hypoglycemic levels in the fed, 16h- and 48h- fast groups and there was no significant difference between them (fed = 43 +/- 8, 16h fast = 36 +/- 5, 48h fast = 29 +/- 6; mg/dl; p=ns; Table 1). No glucagon responses to hypoglycemia were observed under any condition (Fig. 5D). C-peptide levels were immeasurable throughout the experiment.

Comparison of different transplantation sites and C, S groups. Fed blood glucose levels before ITT were normal in H, K and O groups, but higher in P group (Table 1.). Fasting significantly decreased blood glucose levels in all groups. In P group, insulin pellets were still in, so animals had mild hypoglycemia after 48h fast. ITT were done under anesthesia. Blood glucose increased after anesthesia in all experimental animals. Rats in H group had jugular vein permanent cannulation, while rats in K, O and P group underwent surgery to cannulate jugular vein before ITT, so blood glucose post-surgery in K and O groups were significantly higher than that in H group due to surgical stimulation. Fasting significantly decreased basal blood glucose levels post-anesthesia. After insulin infusion, all animals achieved hypoglycemia (<60mg/dl). There was no significant intragroup difference in glucose nadir reached among fed, 16h-, and 48h- fast conditions in H, K, O and P groups respectively. The mean glucose nadirs in the H group were significantly lower than those observed in the K, O and P groups.

Although H, K and O groups maintained euglycemia throughout the experiment, both fed and fasting C-peptide levels in H group were higher than those in K and O groups. C-peptide levels in K and O groups may be elevated because of surgical stimulation of glucose levels during cannulation of jugular veins. C-peptide levels also showed a decline after fasting in K and O groups. Animals in P group failed to achieve euglycemia and their C-peptide levels decreased to immeasurable levels in all groups.

No significant differences in basal plasma glucagon levels were observed among different transplantation groups. Basal glucagon levels were not elevated after fasting for 16h or 48h in all groups. Glucagon responses to hypoglycemia were observed in all transplantation groups. Unlike the H group, there were no intragroup differences in K, O and P groups when comparing glucagon responses to hypoglycemia among fed, 16h fasting, and 48h fasting conditions.

DISCUSSION

The purpose of this study was to determine whether defective glucagon secretion from intrahepatically transplanted islets during hypoglycemia in Lewis rats is related to intrahepatic glycogen stores. Glycogen predictably undergoes glycogenolysis and increases free glucose levels in the liver during hypoglycemia. The study was designed to examine glucagon
responses to hypoglycemia caused by intravenous insulin administration. Comparisons were made among results from hepatic and non-hepatic transplantation sites, as well as non-transplanted normal animals and STZ-treated diabetic animals. The results demonstrated diminished glucagon responses to hypoglycemia from islets transplanted into the hepatic site. Fasting for 16 and 48 hours depleted glycogen from the liver and improved glucagon responses from intrahepatic islets but did not change the magnitude of the responses from islets in the non-hepatic sites. These data suggest that the defective glucagon response from the intrahepatic islets is due to ongoing glycogenolysis and increased glucose flux within the microenvironment of alpha cells in the liver.

This conclusion is consistent with what is known about the blood circulation of intrahepatic islets. It is known that systemically circulating blood reaches intrahepatically transplanted islets via distribution of arteriolar flow from the central hepatic artery to the core of the islet that contains beta cells (9). This accounts for the decrease in C-peptide levels observed during hypoglycemia in heptically transplanted animals. Thereafter, blood flow via the periportal islet circulation goes from beta cells to alpha and delta cells on the periphery of the islet. In the situation of the intrahepatic islet, this means that its alpha cells are in intimate contact with hepatocytes, from which glucose is released during glycogenolysis. We suggest that passive diffusion of glucose from these neighboring hepatocytes provides a dominant signal of high glucose concentration in the microenvironment of the alpha cell, so that is overwhelms a weaker hypoglycemic signal reaching the alpha cells from hepatic arterioles. Consequently, glucagon secretion is relatively suppressed rather than fully stimulated. Full restoration of glucagon response by fasting and liver glycogen depletion is consistent with this formulation.

In the current study, we observed a significant albeit much attenuated glucagon response to hypoglycemia in fed hepatic transplanted rats, a phenomenon which was not seen in human diabetic recipients of islet allografts (1,2,4,5). One explanation may be that the experimental period we used lasted 5 months after STZ administration; thus beta cell regeneration in native pancreas may have occurred. If so, this would have allowed a return to normal intraislet regulation of alpha cells by upstream beta cells such that a cessation of beta cell secretion during hypoglycemia would have triggered glucagon release during hypoglycemia (13,14).

It is notable that glucagon responses to hypoglycemia in the fasting state, compared to the normal control rats, were reduced in the animals with islets transplanted under the kidney capsule, in the omentum and in the peritoneum as well as in the intrahepatic site (Table 1), and therefore one might question whether location of the islets next to glycogen is necessary to explain the diminished function of intrahepatic islets during hypoglycemia. However, it is almost certain that given the number of islets transplanted (1800-2200) in the various sites, and the likely demise of some of the transplanted islets, that the intact pancreas in the normal rats contained many more alpha cells, and thus had larger glucagon responses to hypoglycemia. The important comparisons are within transplant groups, i.e. as shown on Table 1, fasting and glycogen depletion improved glucagon responses to hypoglycemia only in the intrahepatic transplant group. We have normalized the glucagon responses in each group by dividing the increase in glucagon levels during hypoglycemia by the basal C-peptide level (data not shown), making the assumption that C-peptide might reflect beta cell, and therefore by implication, alpha cell mass.
When we do so, the defective glucagon response before fasting in the intrahepatic transplanted animal is even more evident, whereas there are no differences in glucagon responses among all sites, including normal rat pancreases, in the fasted state. Thus, we conclude that the glucagon responses from all transplanted sites are smaller because of less alpha cell mass than that found in normal pancreas, and that the defective glucagon response to hypoglycemia is only present in the intrahepatic site, which can be corrected by fasting and glycogen depletion. The only caveat is that the defective alpha cell responses in the STZ animals are due to beta cell death and not to decreased alpha cell mass, i.e. absent beta cells in STZ animals render the insulin switch-off signal during hypoglycemia to alpha cells unavailable, so the glucagon response to hypoglycemia is defective.

We also observed a higher glucagon response in H group compared to O, P, and K groups. Although H, O and K groups achieved euglycemia in recipients, liver transplantation probably results in a higher number of engrafted islets compared to the other transplantation sites, which was reflected by the higher basal fed C-peptide levels. It worth noting that O, P and K groups did not have permanent jugular vein cannulation. Thus, the three separate jugular vein surgeries just before the insulin infusion might have stimulated higher fed glucose and therefore C-peptide levels in those groups. Also due to post-surgical higher basal blood glucose, it was much more difficult to achieve hypoglycemia in those groups compared to H group. The glucose nadirs were not as low in the non-H transplantation groups, which may not have fully activated the transplanted alpha cells. ITTs were performed one week apart in H group but one month apart in other transplantation groups, which makes animals in other transplantation groups 1-2 months older than H group. This age issue might also have influenced the glucagon response. Nonetheless, the central experiment in this study was to examine glucagon responses to hypoglycemia after fasting. Differences were found only in the hepatic group, and not in the groups using other transplantation sites.

These observations are clearly clinically relevant. If islet transplantation normalized glucose levels, and if patients no longer needed to use exogenous insulin therapy, then no danger of hypoglycemia would exist, and intrahepatic alpha cell responses to hypoglycemia would not be needed. However, currently published information indicates that many if not most human diabetic recipients of alloislet transplantation return to insulin therapy within several years post-transplant and therefore are once again at risk for hypoglycemia. This argues for the importance of finding an alternate non-hepatic site that will not interfere with full glucagon responses to hypoglycemia. Recently, additional metabolic defects in intrahepatically transplanted islets involving insulin synthesis, insulin secretion and glucose oxidation have been reported (15). One study reported that alpha cell responses to mild non-insulin-induced hypoglycemia (80 +/- 3 mg/dl) in dogs were subnormal (16).

In conclusion, these studies strongly suggest that the mechanism of defective glucagon responses to hypoglycemia in recipients of intrahepatic islet transplantation involves the intimate relationship between the alpha cells in the islets and the hepatocytes in the liver that undergo glycogenolysis during hypoglycemia. Alternate non-hepatic sites for islet transplantation will circumvent this loss of full alpha cell function, which is an important consideration in view of the likelihood that many recipients of intrahepatic islets will return to exogenous insulin treatment.
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**TABLE 1.** Levels of glucose, C-peptide and glucagon in H, K, O, P, C and S groups.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>n</th>
<th>Blood Glucose Pre-surg. (mg/dl)</th>
<th>*Blood Glucose Post-surg. (mg/dl)</th>
<th>Blood Glucose ITT nadir (mg/dl)</th>
<th>Plasma C-peptide Post-surg. (pM)</th>
<th>Plasma C-peptide ITT nadir (pM)</th>
<th>Plasma Glucagon Post-surg. (pg/ml)</th>
<th>Plasma Glucagon ITT Post-surg. AUC(pg/ml/90min)</th>
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<tbody>
<tr>
<td>Hepatic</td>
<td>10</td>
<td>102 ± 3</td>
<td>170 ± 11</td>
<td>22 ± 3</td>
<td>681 ± 77</td>
<td>ND</td>
<td>38 ± 4</td>
<td>8,839 ± 1,988 **</td>
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<td>Fed</td>
<td>10</td>
<td>97 ± 2</td>
<td>110 ± 6</td>
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<td>388 ± 80</td>
<td>ND</td>
<td>32 ± 3</td>
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<td>16h fast</td>
<td>10</td>
<td>81 ± 2</td>
<td>96 ± 4</td>
<td>17 ± 3</td>
<td>294 ± 50</td>
<td>ND</td>
<td>30 ± 4</td>
<td>29,639 ± 4,550</td>
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<td>Kidney</td>
<td>4</td>
<td>132 ± 32</td>
<td>280 ± 59</td>
<td>44 ± 4</td>
<td>555 ± 171</td>
<td>ND</td>
<td>50 ± 2</td>
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<tr>
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<td>101 ± 8</td>
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<td>102 ± 4</td>
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<td>93 ± 34</td>
<td>ND</td>
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<td>Omental</td>
<td>3</td>
<td>113 ± 20</td>
<td>361 ± 60</td>
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<td>Peritoneal</td>
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<td>195 ± 65</td>
<td>209 ± 98</td>
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<tr>
<td>C group</td>
<td>5</td>
<td>92 ± 2</td>
<td>132 ± 8</td>
<td>49 ± 3</td>
<td>1,101 ± 223</td>
<td>ND</td>
<td>62 ± 10</td>
<td>40,777± 8,192</td>
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<tr>
<td>Fed</td>
<td>7</td>
<td>69 ± 7</td>
<td>86 ± 3</td>
<td>40 ± 3</td>
<td>457 ± 76</td>
<td>ND</td>
<td>35 ± 4</td>
<td>48,810± 13,785</td>
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<tr>
<td>16h fast</td>
<td>7</td>
<td>62 ± 5</td>
<td>86 ± 4</td>
<td>32 ± 4</td>
<td>303 ± 75</td>
<td>ND</td>
<td>27 ± 4</td>
<td>37,632± 5,335</td>
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<tr>
<td>S Group</td>
<td>4</td>
<td>232 ± 52</td>
<td>262 ± 53</td>
<td>43 ± 6</td>
<td>ND</td>
<td>ND</td>
<td>43 ± 11</td>
<td>385± 773</td>
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<tr>
<td>Fed</td>
<td>6</td>
<td>203 ± 67</td>
<td>181 ± 53</td>
<td>36 ± 6</td>
<td>ND</td>
<td>ND</td>
<td>29 ± 4</td>
<td>340± 433</td>
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<tr>
<td>16h fast</td>
<td>8</td>
<td>68 ± 15</td>
<td>81 ± 11</td>
<td>29 ± 5</td>
<td>ND</td>
<td>ND</td>
<td>21 ± 4</td>
<td>916± 329</td>
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* H and C groups had permanent jugular vein cannulation, so they had no intrajugular vein surgery at the time of the studies. Post-surgical blood glucose levels were drawn under anesthesia, whereas pre-surgical glucose levels were drawn with no anesthesia. **p = 0.05-0.01, fed vs. fasting.
Figure 1. Blood glucose levels post-transplantation. 1800-2200 islets from inbred mates were transplanted to diabetic rats at day 0. Islets transplanted into the liver, kidney capsule and omentum maintained euglycemia in recipients, while islets transplanted into the peritoneal cavity did not.
Figure 2. Liver glycogen measurement in hepatic islets transplanted rats and normal control rats under fed, 16h- and 48h- fasting.
**Figure 3.** Sections of rat liver with the Periodic Acid Schiff reaction and counterstained with hematoxylin. Glycogen is abundant in the liver of fed animals (A). Cellular stores glycogen are partially depleted after overnight fasting (B, D). Hepatocytes are almost entirely devoid of glycogen after 48hr fasting (C). One transplanted islet is shown in contact with glycogen-containing hepatocytes (D, arrows) after 16-hr fast. x40.
**Figure 4.** Glucose, C-peptide and glucagon responses to insulin-induced hypoglycemia in hepatic transplanted rats. Rats had permanent jugular vein cannulations. Intravenous insulin tolerance tests were performed on each rat three times under fed, fasting 16h and fasting 48h conditions with one week apart. Four rats had insulin tolerance test again on the fourth week after returning to the fed condition.
Figure 5. Glucose, C-peptide and glucagon responses to insulin-induced hypoglycemia in non-streptozotocin (STZ), non-transplanted normal control rats. Intravenous insulin tolerance tests were performed three times one week apart in each rat under fed, 16h fasting, and 48h fasting conditions. Panel D shows comparison of area under the curve glucagon responses to insulin-induced hypoglycemia in normal control rats and STZ- treated diabetic rats.
Figure 6. Comparison of glucagon responses (calculated as area under the curve) to insulin-induced hypoglycemia in normal control rats and intrahepatically transplanted rats under fed, 16h- and 48h- fasting conditions. Compared to control group, glucagon response to hypoglycemia in H group were impaired in fed state. Depletion of liver glycogen by fasting was accompanied by restored glucagon responses to hypoglycemia in H group. * = p<0.05-0.01.