Glucose Metabolism After Pancreas Autotransplantation

The Effect of Open Duct Versus *Urinary Bladder Drainage Technique*

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Glucose metabolism and insulin secretion after pancreas transplantation may be affected by the technique used for ductal drainage. We evaluated peripheral glucose and insulin levels after oral (oral glucose tolerance test [OGTT]) and sustained stable hyperglycemic challenge (clamp) in dogs who had undergone pancreas autotransplantation with intraperitoneal drainage (PAT) or with urinary bladder to pancreatic duct anastomosis (PAT/B). Both groups had basal glucose values comparable to normal controls; PAT/B animals had fasting hyperinsulinemia. Pancreas autotransplantation animals had an increased integrated glucose response to OGTT and blunted insulin response to hyperglycemic clamp. Urinary bladder to pancreatic duct anastomosis animals had a significantly decreased integrated glucose response to OGTT compared to PAT and an exaggerated insulin response to hyperglycemic challenge, which approximated normal control values by the last 30-minute period of the clamp. Interestingly M values, which approximate glucose metabolized during the hyperglycemic challenge, were depressed in both surgical groups. It is concluded that the technique of bladder drainage allows a 'normalization' of peripheral levels of insulin that is associated with amelioration of an altered glucose response after oral challenge. However the clamp studies show that, despite the improvement in insulin response, an insensitivity may exist to a wide range of endogenous levels after pancreas transplantation.

CLINICAL PANCREATIC ALLOTRANSPLANTATION has been used with increasing frequency for treatment of patients with type I diabetes.[1,2] The anatomic changes that accompany surgical manipulation of the pancreas graft, which subsequently is revascularized, as opposed to islet cell harvesting, might be predicted to have important consequences on postoperative glucose metabolism. Three important surgical alterations include (1) reduction of beta cell mass by use of segmental transplantation or due to preservation techniques, (2) systemic drainage of pancreatic venous effluent, and (3) denervation.

Each of these anatomic effects has been investigated as a possible contributor to alterations in glucose metabolism after pancreas transplantation.[3–5] We documented changes in glucose metabolism after pancreatic autotransplantation in which pancreatic ductal drainage is intraperitoneal. This circumstance is associated with marked exocrine atrophy and, perhaps, chronic pancreatitis.[4,5] Sollinger[6] and others[7,8] introduced an important technique in pancreas transplantation, using exocrine duct drainage into the urinary bladder. The advantage of this technique appears to be markedly decreased mortality and morbidity rates in clinical and animal models, but its effect on postoperative glucose metabolism has not been documented.

We sought to evaluate the effect of this important technique on glucose metabolism and compare it to a technique widely used in canine freely drained intraperitoneal segmental pancreas transplantation, which has been used as a model for human transplantation. This is an important consideration because many studies using the canine model have used the intraperitoneal technique because of its simplicity and the fact that it is well tolerated by the animals. We hypothesized that the bladder-drained technique might result in improvement in glucose metabolism, thereby increasing its attraction as an important technique for clinical pancreas transplantation. Therefore the bladder-drained animal model would be more appropriate to evaluate carbohydrate metabolism after pancreatic transplantation.

Materials and Methods

All experiments were begun by 8:30 A.M. on overnight fasted, conscious female mongrel dogs (body weight, 20 to 24 kg) who had been trained to stand quietly in harnesses. The animals were kept on a weight-maintaining diet (Pro Pet dog food, Newark, DE) with exocrine pan-

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creatic supplement (10 g/day; Viokase, A.H. Robins, Richmond, VA). All provocative studies and surgical procedures were carried out in accordance with established federal and University of Virginia Vivarium guidelines (UVA protocol number 1126-06-85). At the time of study, each animal was clinically healthy and had maintained or regained its preoperative body weight. Four animals in each surgical group were allowed to recuperate from surgery for at least 6 weeks and then studied at least in duplicate.

Provocative Studies

Oral glucose tolerance tests (OGTT) were performed on each animal. A bolus of 20% glucose (2 g/kg body weight; Travenol, Deerfield IL) was ingested by each animal within 5 to 10 minutes of delivery. A 16-gauge intravenous saphenous vein catheter was used for sample collections. After two separate basal samples were taken for measurement of glucose and insulin, sequential samples were drawn at 15, 30, 45, 60, 90, 120, 150, 180, and 240 minutes. Serum glucose values were determined immediately by the glucose oxidase method on the Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). The remainder was separated into prechilled test tubes for subsequent insulin assay. All determinations were performed in duplicate. Plasma insulin levels were determined with a double-antibody radioimmunoassay by the University of Virginia Diabetes Core Laboratory.

For the hyperglycemic clamp studies, two 16-gauge catheters were placed in the saphenous vein of each hind limb. A central line was advanced 30 cm for glucose infusion. Sample collections were obtained from the peripheral line, eliminating any possibility of mixing. Fifteen minutes after catheter insertion, basal samples were obtained, after which a square wave of hyperglycemia (150 mg/dL above basal) was created rapidly and maintained for 120 minutes.[7,8] This was accomplished by a priming infusion of glucose followed by a computer-adjusted variable infusion of 20% glucose solution. The infusion rate adjustments were based on 5-minute plasma glucose determinations fed into a servo-controlled negative feedback formula coupled to a variable-speed infusion pump (Harvard Apparatus, Natick, MA). Samples were obtained every 5 minutes for determination of plasma glucose levels and every 10 minutes for determination of insulin levels.

Surgical Procedures

Pancreas autotransplant—intraperitoneal drainage (Fig. 1). Female mongrel dogs were prepared for operation with overnight fasting. Each animal was anesthetized with sodium pentobarbitol (25 to 30 mg/kg), intubated with an endotracheal tube, placed on a volume respirator, and

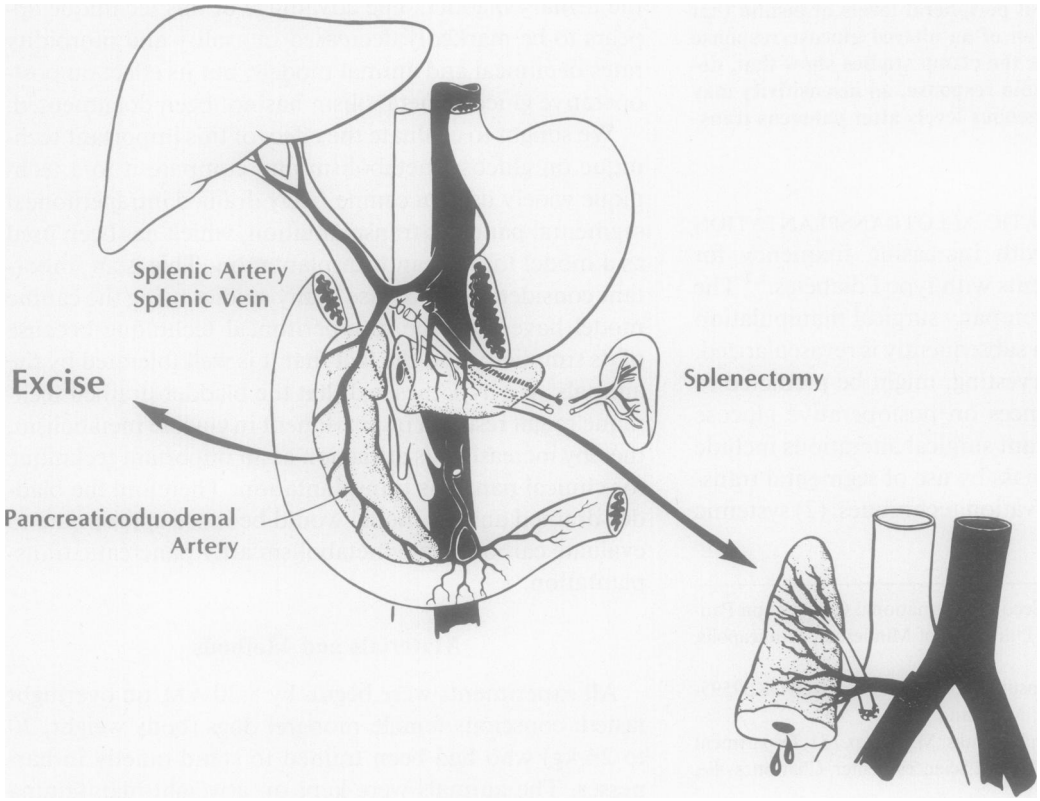

FIG. 1. The technique of intraperitoneal drainage (PAT). The details of the surgical procedure are outlined in the text.

given halothane anesthesia (2%). The abdomen was entered through a midline incision. Proximal pancreatectomy was performed, removing the duodenal lobe of the pancreas, preserving the vascular supply to the intact duodenum and leaving the splenic lobe of the pancreas intact. The splenic artery was identified as it left the celiac axis. The splenic vein was likewise identified. Hypothermic preservation by perfusion of chilled normal saline (containing 500 units heparin per liter) was performed in a retrograde fashion by cannulating the distal splenic artery using an 18-gauge catheter. The venous anastomosis then was accomplished in an end-to-side fashion into the iliac vein. An end-to-side arterial anastomosis was completed between the splenic and the external iliac artery. The pancreatic duct was allowed to drain into the abdominal cavity. We reported long-term survival of these animals, documenting clinical health, normal eating patterns, and return to preoperative body weight.[4,5] Postoperative animals received pancreatic exocrine supplementation as mentioned above.

Pancreas autotransplant—urinary bladder drainage (PAT/B) (Fig. 2). Laparotomy, proximal pancreatectomy, and autotransplantation of the splenic lobe were performed in an identical fashion as the PAT animals, as described above. On completion of this part of the procedure, an incision of approximately 1 cm in length was made in the external muscle layer of the bladder and carried to the mucosa. A 2-mm incision then was made in the bladder mucosa and this was reapproximated using interrupted sutures (6-0 absorbable) to the pancreatic duct over an 18-gauge polyethylene stent (2 cm long). The pancreatic capsule anteriorly and posteriorly was then approximated to the incised edge of the bladder musculature with 5-0 nonabsorbable sutures.

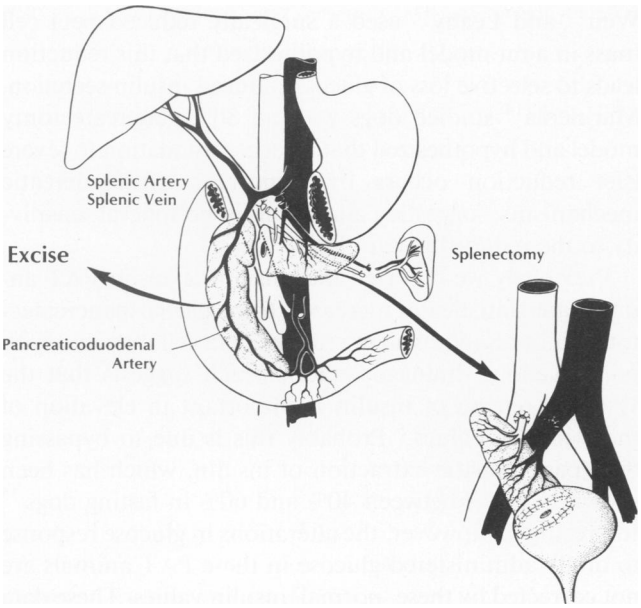

FIG. 2. The technique of urinary-bladder drainage (PAT/B). The details of the surgical procedure are outlined in the text.

Calculation and Statistical Analysis

The mean concentrations of glucose and insulin were calculated for each time point for both clamp and oral challenge studies according to treatment protocol. Basal adjusted integrated responses for the oral glucose tolerance test were calculated by the trapezoidal rule. The integrated response then was divided by its time interval, resulting in a mean time-independent concentration. For clamp studies means of individual values were calculated for glucose, insulin, and the estimation of glucose metabolized (M = mg/kg/min) according to treatment. During hyperglycemia, M approximates the glucose infusion necessary to maintain the hyperglycemic plateau after corrections are made for volumes and kinetics of glucose distribution.[9,10] Mean values for the last 60-minute period of the clamp (60 to 120 minutes) then were analyzed. Means for all groups were simultaneously compared with each other using the general linear modeling analysis of variance, and significance was determined by the least squares means comparison method.[11]

Results

Data for the oral glucose tolerance test, clamp studies, and basal values for glucose and insulin for the control and the two postoperative groups are presented in Table 1 and Figures 3 and 4.

Basal Values

Basal (fasting) values for glucose and insulin in the three groups are presented in Table 1. The normal control value for glucose of 92 ± 4 mg/dL is not statistically different from either of the two postoperative groups. Normal insulin values of 12 ± 2 μU/mL are not statistically different than the PAT animals. The PAT/B animals had a fasting hyperinsulinemia of 22 ± 2 μU/mL.

Oral Glucose Challenge (OGTT)

These data are presented in Figure 3 and Table 1. Pancreas autotransplantation animals have a statistically elevated integrated glucose response compared to normal controls (215 ± 12 mg/dL compared to 103 ± 11 mg/dL, respectively). The technique of bladder drainage results in a decrement in the integrated glucose response that is statistically less than PAT animals but still increased from normal controls. Interestingly the integrated insulin response after PAT is elevated. In Figure 3 this is apparent

TABLE 1. *Insulin Response to Oral (OGTT) and Intravenous Hyperglycemic (Clamp) Challenge*

	Basal		OGTT		Clamp (60–120 min)			
	G	INS	G INT	INS INT	G	INS	M	M/INS
Control	92 ± 4	12 ± 2	103 ± 11	32 ± 5	240 ± 3	74 ± 7	26 ± 2	.38 ± .1
PAT	96 ± 3	16 ± 3	215 ± 12*	50 ± 6*	244 ± 3	54 ± 7*	16 ± 2*	.35 ± .1
PAT/B	99 ± 2	22 ± 2†	138 ± 6†‡	36 ± 3	253 ± 4	83 ± 8‡	18 ± 2†	.20 ± .1

* Control *vs.* PAT ($p < 0.05$).
† Control *vs.* PAT/B ($p < 0.05$).
‡ PAT *vs.* PAT/B ($p < 0.05$).
n = 8.

G, glucose (mg/dL); INS, insulin (μU/mL); M, glucose metabolized (mg/kg.min-1); PAT, intraperitoneal duct drainage pancreas autotransplant; PAT/B, bladder-drained pancreas autotransplant; G INT, integrated glucose response; INS INT, integrated insulin response.

by a delayed peak and elevated insulin values from the 90-minute to 240-minute time period. The technique of bladder drainage results in an insulin response that more closely recapitulates normal with peak values at 30 minutes and with an integrated response not statistically different from control (36 ± 3 μU/mL compared to 32 ± 5 μU/mL).

Hyperglycemic Clamp Challenge

These data are presented in Table 1 and Figure 4. All three groups were clamped for a 2-hour period in statistically equivalent hyperglycemic ranges. The insulin response by the normal animals can be seen as abrupt response to hyperglycemia in the 50 to 60 μU/mL range, which increases during the clamp to 74 ± 7 μU/mL during the last 60 minutes. Pancreas autotransplantation animals have an abrupt elevation to levels equivalent to control during the first hour (Fig. 4). Pancreas autotransplantation animals, however, are unable to elevate peripheral insulin levels in response to hyperglycemia and, by the 60-to-120–minute period of the clamp, have an average peripheral insulin level of 54 ± 7 μU/mL, which is statistically lower than control values. Pancreas autotransplantation urinary bladder animals have a hyperinsulinemic response compared to controls with levels immediately approaching 80 to 90 μU/mL. By the 60-to-120–minute interval, they remain statistically equivalent to control animals and significantly elevated compared to PAT.

M values are presented in Table 1 and Figure 4. In control animals the M value elevation during the hyperglycemic challenge reflects endogenous insulin levels. M values increase from approximately 20 mg/kg/min at the beginning of the clamp to reach 26 ± 2 mg/kg/min by the last 60 minutes of the clamp. Both surgical groups have statistically decreased M values over the period of the clamp. Pancreas autotransplantation and PAT/B (16 ± 2, 18 ± 2 mg/kg/min, respectively) remain significantly depressed compared to control animals, despite the obvious differences in endogenous insulin during the same interval. M/INS ratios for controls, PAT, PAT/B are 0.38 ± 0.1, 0.35 ± 0.1, and 0.20 ± 0.1, respectively.

Discussion

Clinical pancreas allotransplantation is enjoying nearly exponential growth worldwide in the last decade. As the understanding for its applicability has become more apparent, increasing questions arise concerning postoperative alterations in carbohydrate metabolism. The recognized surgical alterations that might affect postoperative function of the endocrine pancreas have been studied to delineate which of several might impact on glucoregulation after transplantation. The most important of these surgical alterations are (1) reduction in beta cell mass, (2) systemic drainage of pancreatic venous insulin, and (3) denervation.

Reduction of beta cell mass could be predicted to have definite impact on peripheral insulin levels. Previously we reported decreased insulin levels after intravenous oral challenge using an 80% pancreas resection model and we concur with the findings of others that this results in similar alterations in carbohydrate metabolism.[3,4] Bonner-Weir[12] and Leahy[13] used a surgically reduced beta cell mass in a rat model and hypothesized that this reduction leads to selective loss of glucose-induced insulin secretion. Marincola[14] studied dogs with an 80% pancreatectomy model and hypothesized that an accommodation to severe islet reduction occurs by important extrapancreatic mechanisms, suggesting alterations in peripheral sensitivity to the reduced insulin load.

Previously we reported that insulin levels in PAT animals are statistically increased from partial pancreatectomy animals (equivalent reduced beta cell mass but with portal venous drainage intact), which suggests that the systemic release of insulin is important in elevation of these insulin values.[5] Probably this is due to bypassing 'first pass' hepatic extraction of insulin, which has been shown to range between 40% and 60% in fasting dogs.[15] Interestingly, however, the alterations in glucose response to orally administered glucose in these PAT animals are not corrected by these 'normal' insulin values. These data suggest that perhaps 'insensitivity' exists to the endogenously released insulin, which is systemically released after pancreas transplantation. Analyzing the clamp data (Table

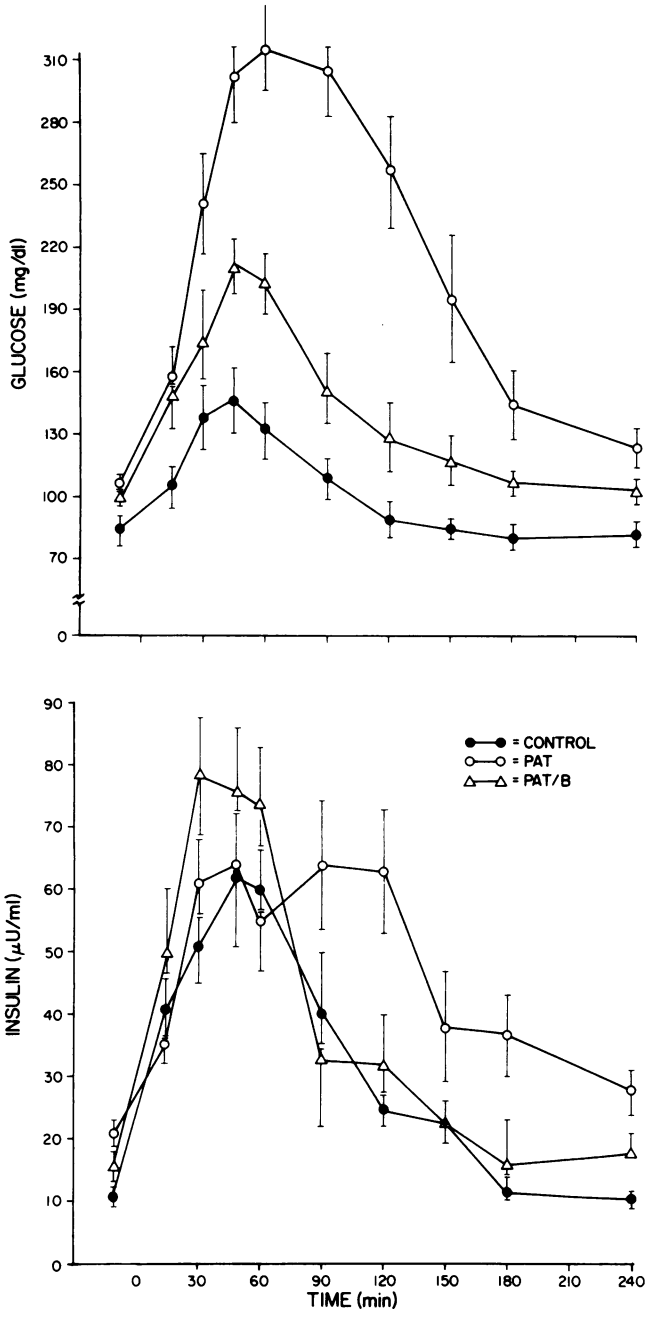

FIG. 3. Oral glucose challenge (OGC). The data for the OGTT are discussed in the text and in Table 1.

1 and Fig. 4) and the OGTT data (Table 1 and Fig. 3) demonstrate these effects. The oral glucose challenge restores peripheral levels of insulin comparable to normal controls, yet the integrated glucose response remains significantly altered. We documented these effects in earlier studies.[4,5] These results correlate with others that demonstrate that systemic drainage is effective in normalizing peripheral insulin levels; however the concomitant effect on glucose metabolism is unclear.[16]

A great deal of literature exists as to the appropriate handling of exocrine pancreatic secretion after pancreas transplantation. The human body clearly does not tolerate a freely drained intraperitoneal duct whereas the canine body can. We have reported severe histologic changes resulting in loss of identifiable exocrine structures after this technique.[4] The intraperitoneal drainage technique or duct ligation is probably associated with a model of pan-

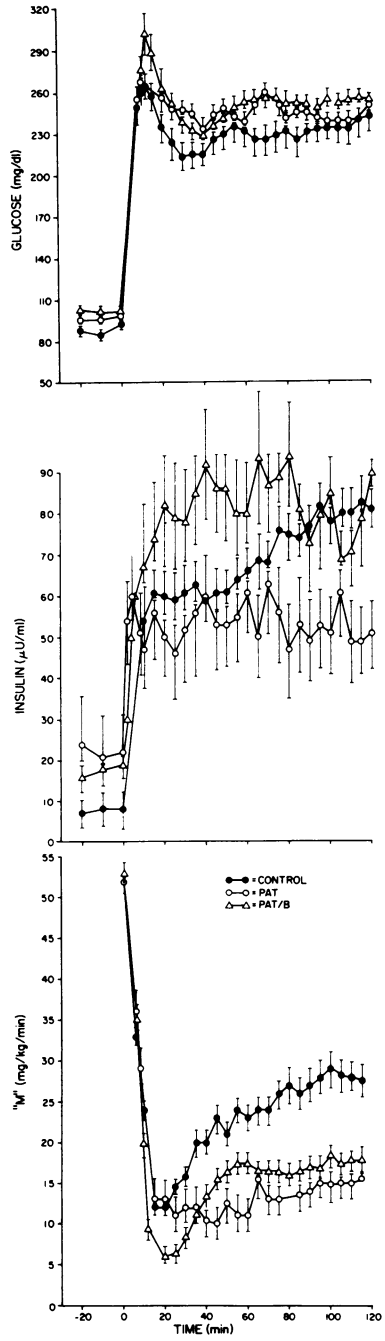

FIG. 4. Hyperglycemic clamp studies. The data for the clamp studies are discussed in the text and in Table 1.

creatitis in the dog that markedly impairs peripheral glucose levels and patterns of insulin secretion after either intravenous or oral glucose challenge.[5,17] In addition others have reported studies using polymer injection techniques, enteric drainage techniques, as well as urinary (ureter and bladder) drainage techniques. The apparent common denominator in all of these techniques is the desire to control digestive enzymes that might disrupt enteric anastomoses or, in addition, be associated with graft pancreatitis. Graft pancreatitis has been hypothesized to alter insulin secretion and consequent carbohydrate metabolism.[18-20] The urinary bladder technique obviates the need for enterotomy and has been associated with increasing clinical success. In addition monitoring of urinary amylase permits early diagnosis of rejection before any alteration in peripheral glucose levels.

In the present study, a distinct difference in insulin secretory patterns was identified comparing the two techniques of exocrine pancreatic drainage. The oral glucose challenge resulted in a distinctly different insulin response for PAT animals compared to PAT/B animals. Despite the fact that the integrated insulin response was less than for the PAT animals, the timing of the initial insulin peak is associated with a decline in the integrated glucose response that approximated the control values. Comparing the results of the two surgical techniques suggests that the initial endogenous insulin peak is an important phenomenon in peripheral glucose disappearance rather than the integrated insulin response *per se*.

The clamp data also are interesting. An entirely different insulin response is demonstrated after the technique of bladder drainage. The PAT animals failed to elevate insulin and have a significantly blunted response, as shown by the peripheral levels in the last 60 minutes of the clamp. The PAT/B animals demonstrate an initial exaggerated insulin response to intravenous challenge that matches the control response by the last hour of glucose infusion. Interestingly, however, both surgical groups have decreased M values that confirm our previous observations about the possibility of insensitivity to these endogenous levels. M/INS ratios demonstrate a decrease from 0.38 $\pm$ 0.1 for controls to 0.20 $\pm$ 0.1 for the PAT/B animals. Therefore these data suggest a possibility of peripheral insensitivity to the endogenous insulin of the PAT/B animals, but further studies would be needed for absolute confirmation. These data appear to correlate with Marincola's data that extrapancreatic mechanisms may be operative after pancreatic transplantation.[14] This finding might be explained more completely by evaluating peripheral insulin receptor status.

The explanation for these effects is unclear. There is an obvious, complex interplay of anatomic and physiologic changes that occurs after transplantation. We used the autotransplant model (devoid of immunosuppression) to try to evaluate these. The two surgical models share in common the three anatomic changes that previously we documented to have important consequences on postoperative metabolism, beta cell reduction, denervation, and systemic drainage of insulin. The only difference between the two groups is the urinary bladder drainage of the exocrine pancreatic duct. In our experience, the latter technique has been associated with increased animal survival (approaching 80% as opposed to approximately 50% previously), and an obvious grossly different pancreatic morphology. The bladder-drained animals have a normal pancreatic remnant identifiable for as long as 3 months after operation. Clearly further histologic and morphologic studies will be needed to confirm this preliminary finding.

One can hypothesize that the important changes that are associated with pancreatitis are minimized by the bladder drainage technique and, therefore, result in significant increases in peripheral insulin levels. The interesting aspect of these observations is the fact that the peripheral levels of insulin *per se* do not correct completely the alterations of carbohydrate metabolism. For example the increased basal insulin levels seen after PAT/B are associated with equivalent glucose levels, suggesting a possible insensitivity in the basal state to increased levels of insulin. In addition increased insulin levels in response to the clamp studies after the PAT/B technique do not result in an amelioration of the M values, again suggesting that glucose insensitivity in the periphery may occur. This unique finding allows a dose-response observation of endogenous insulin levels using the different techniques to demonstrate that postoperative alterations in carbohydrate metabolism may exist independent of the endogenous levels of insulin. The contributions of hepatic glucose output and more complete studies on peripheral sensitivity using exogenous insulin infusions may well be necessary to clarify these observations.

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