

Exercise Induces Hypoglycemia in Rats With Islet Transplantation

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Recently, islet transplantation in patients with type 1 diabetes has had greater success than in the past, but the important question of whether the kinetics of islet secretion are able to accommodate the metabolic demands of special conditions such as exercise remains unanswered. Syngeneic rat islets (4,000 islet equivalents/rat) were transplanted into the liver, kidney, and peritoneal cavity (encapsulated or nonencapsulated) of rats with streptozocin-induced diabetes. Normoglycemic transplanted rats and age-matched controls were subjected to 30 min of moderate exercise on a treadmill 5 weeks after transplantation. Although control rats maintained near normoglycemia during and after exercise, the rats with islet transplants had significantly lower blood glucose levels. For the rats with islets in the liver, increased C-peptide levels were found at 30 min (790 ± 125 and $1,450 \pm 250$ pmol/l at 0 and 30 min, respectively; $P < 0.01$), whereas a decrease was found in controls and in rats with islets transplanted into the peritoneal cavity or under the kidney capsule. Moreover, increased glucagon levels were found after exercise in the rats with islets transplanted into the liver (62 ± 6 , 165 ± 29 , 155 ± 27 , and 97 ± 13 pg/ml at 0, 30, 60, and 90 min, respectively; $P < 0.05$), whereas no changes in glucagon levels were observed in controls. In conclusion, moderate exercise caused hypoglycemia in rats with islet transplants in different sites including liver, kidney, and peritoneal cavity. C-peptide and glucagon responses to exercise were very different in rats with transplanted islets compared with controls. This islet dysfunction led to exercise-induced hypoglycemia. *Diabetes* 53:360–365, 2004

Major improvements in islet isolation techniques, and the implementation of steroid-free immunosuppressive regimens, can maintain insulin independence in the majority of type 1 diabetic patients for at least 1 year after transplantation (1). Although fasting and nonfasting blood glucose levels of transplanted patients are in the normal or near normal range, it has been shown that insulin responses of the

transplanted islets to glucose and arginine are much less than those from the normal pancreas. One major finding has been that the number and severity of episodes of hypoglycemia decrease impressively after transplantation (2); however, glucagon secretion has been found to be defective in response to insulin-induced hypoglycemia in patients who have received islet transplants into the liver (3,4). Aside from these studies, little is known about the kinetics of insulin and glucagon secretion from transplanted islets and the functional status of counterregulatory mechanisms during different situations such as exercise. In this study, regulation of glucose metabolism during moderate exercise was investigated in rats receiving islets transplanted into the liver via the portal vein, peritoneal cavity, or subcapsular site of the kidney.

RESEARCH DESIGN AND METHODS

Male Lewis rats weighing 200–240 g (Harlan Sprague Dawley; Harlan, Indianapolis, IN) were used as donors and recipients in this study. Diabetes was induced with an intraperitoneal injection of streptozocin (Sigma, St Louis, MO) at a dosage of 90 mg/kg for 10–14 days before transplantation of islets. Rats who had morning fed blood glucose levels >330 mg/dl on two separate occasions were used as recipients. Animals were kept in a conventional animal facility with ad libitum access to food and water.

Isolation of islets. Islets were isolated from the pancreas by distending the pancreatic duct with a defined mixture of collagenase and proteases (Liberase RI; Roche, Indianapolis, IN). After being digested at 37°C, the islets were separated on a discontinuous histopaque density gradient (Histopaque 1077; Sigma) and further purified by handpicking, as previously described (5). The islets were cultured overnight in 80-cm², nontreated Falcon culture flasks (Becton Dickinson, Franklin Lakes, NJ) containing RPMI 1640 medium supplemented with 11.2 mmol/l glucose (Life Technologies, Rockville, MD), 10% neonatal calf serum (Mediatech, Herndon, VA), glutamine (2 mmol/l; Life Technologies), and penicillin and streptomycin (100 IU/ml and 100 µg/ml, respectively) at 37°C in a humidified air atmosphere containing 5% CO₂.

Microencapsulation. Rat islets were encapsulated in 3.3% highly purified alginate (61% mannuronic acid and 39% guluronic acid; Pharmacia, Peapack, NJ) the day after being isolated. The islets were mixed with the alginate solution (3,000 islets/ml) and microcapsules were made by extruding the alginate-islet suspension through an air-driven droplet generator into a 20-mmol/l BaCl₂ solution. The microcapsules were 800–1,000 µm in diameter and each capsule contained one or two islets, with few of the capsules being empty. Microencapsulated islets were cultured in Dulbecco's modified Eagle's medium/Ham's F12 medium containing 20 mmol/l glucose without serum (Ultradculture; Biowhittaker, Walkersville, MD) overnight before being transplanted.

Transplantation of islets and follow up. Islets were counted and divided into aliquots of 4,000 islet equivalents (IEs) 1 day after being isolated, and then transplanted into the different sites of rats anesthetized with inhaled metoflurane (Metofane; Shering Plough Animal Health, Union, NJ). Body weight, blood glucose (measured with a Precision QID glucometer; Abbott, Bedford, MA), and C-peptide levels (anti-rat C-peptide radioimmunoassay [RIA]; Linco Research, St. Charles, MO) were measured in all groups before and weekly after the transplantation. It should be noted that blood glucose levels are considerably lower than plasma values. We previously determined that blood glucose levels of 50–300 mg/dl are about 65% of plasma values as measured by a Beckman Glucose Analyzer II (Beckman, Palo Alto, CA) (6).

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IE, islet equivalent; IPGTT, intraperitoneal glucose tolerance test; IVGTT, intravenous glucose tolerance test; RIA, radioimmunoassay.

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Transplantation of islets into the liver via the portal vein. Islets suspended in 0.2–0.5 ml of RPMI medium and collected in a 1-ml syringe were injected through a 27-gauge needle into the portal vein exposed with an abdominal incision. After islets were infused, the portal vein was compressed manually with absorbable gelatin (GEL FOAM; Pharmacia Upjohn, Kalamazoo, MI) to minimize the risk of bleeding.

Transplantation of islets into the peritoneal cavity. Islets alone or contained in microcapsules were injected into the abdominal cavity through a sterile squeezable plastic transfer pipette (Fisher Scientific, Chicago, IL). In some animals transplanted with islets alone, a second transplantation with 2,000 IEs of islets was required because of only partial improvement in the blood glucose levels after the first transplantation.

Transplantation of islets under the kidney capsule. Islets were transplanted under the capsule of the left kidney using PE-50 tubing (Becton Dickinson) connected to a 1-ml Hamilton syringe, as previously described (7).

Exercise. Transplanted and control rats were subjected to moderate exercise 4–6 weeks after transplantation. Before the formal study, rats were subjected to a 10-min warm-up exercise session at a speed of 12 m/min on 2 consecutive days. During the study, rats were exercised over a 30-min period at a speed of 24 m/min up a 5% grade using a treadmill (Model 42–15; Quinton Instruments, Seattle, WA). Blood glucose levels were measured with a portable glucometer (Precision QID) from snipped tails before, during (10 and 20 min), at the end of (30 min), and after (60 and 90 min) exercise. Samples for C-peptide and glucagon measurements were taken at the same time points with the exception of the 10- and 20-min points. Blood samples for the glucagon assay were added to tubes containing 10 units of Trasylol/100 μ l of blood (Serologicals Proteins, Kankakee, IL). Plasma samples were obtained by immediate centrifugation and stored at -80°C until the measurements were carried out using a RIA kit (Linco Research).

Intravenous glucose tolerance tests. Intravenous glucose tolerance tests (IVGTTs) were performed 3–5 weeks after transplantation (1 week before the exercise experiment) in rats with transplants and nontransplanted age-matched controls. After rats were fasted for 12–14 h, glucose (1g/kg body weight as 45% solution) was injected into the orbital sinus over a period of 1 min into anesthetized rats. Blood glucose levels were determined from snipped tails at 0, 2, 5, 10, 15, 30, 60, 90, 120, and 180 min after glucose injection. Plasma C-peptide samples were taken at the same time points, with the exception of 2, 5, and 15 min.

Intraperitoneal glucose tolerance tests. Intraperitoneal glucose tolerance tests (IPGTTs) were performed in nonanesthetized rats 2 weeks after exercise. After a 12–14 h fast, glucose (1g/kg body weight as 45% solution) was injected into the peritoneal cavity through a 23-gauge needle. Blood glucose and plasma C-peptide samples were taken at the same time points as for the IVGTTs.

Statistical analysis. Results are expressed as means \pm SE. Student's *t* tests for paired and unpaired data were used to compare values for exercise, IVGTT, and IPGTT.

RESULTS

Hyperglycemia was normalized in rats receiving islet transplants. Rats receiving islet transplants into the liver or kidney or encapsulated islets into the peritoneal cavity normalized blood glucose levels in <7 days; however, in $n = 2$ of 4 rats receiving nonencapsulated islet transplants into the peritoneal cavity, blood glucose levels sometimes fell only into the 200–300 mg/dl range, thus requiring a second transplantation 1 week after the first transplantation (Fig. 1A). Plasma C-peptide levels sharply increased 1 week after the transplantations and decreased to a constant level (Fig. 1B). It is notable that the C-peptide levels in rats with either encapsulated or nonencapsulated islets in the peritoneal cavity were lower than in normal rats or rats with islets transplanted into the liver, with this difference being most obvious during the 21- to 70-day period after the transplants.

Moderate exercise caused a significant decrease in blood glucose in rats with islet transplants. When subjected to treadmill exercise, control rats had a modest rise in glucose levels; 30 min after the cessation of exercise, glucose fell to levels comparable with the starting point (106 ± 4 mg/dl at baseline vs. 101 ± 8 mg/dl at 30

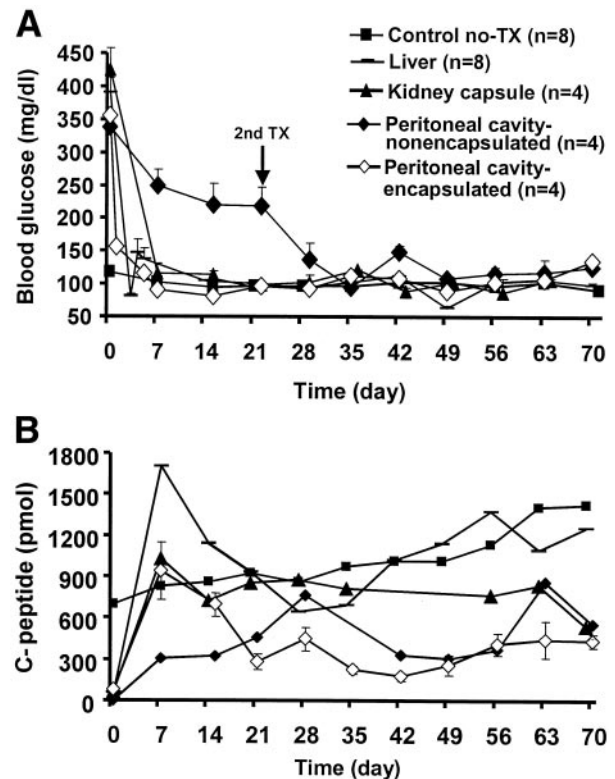


FIG. 1. Normalization of blood glucose levels by islet transplantation. Fed glucose levels (A) were normalized and C-peptide levels (B) increased in rats that received islet transplants into the peritoneal cavity, liver, and kidney capsule (4,000 IEs of islets transplanted for each rat). In two rats receiving nonencapsulated islet transplants into the peritoneal cavity, a second transplantation was necessary to induce and maintain normoglycemia.

min after exercise cessation [60 min from initiation]). In contrast, for the transplanted rats, the fall in glucose values was prominent at 30 min after cessation of exercise (-35 ± 9 mg/dl for the liver, -33 ± 6 mg/dl for the kidney, and -46 ± 7 and -46 ± 6 mg/dl for peritoneal cavity [encapsulated and nonencapsulated, respectively]) (Fig. 2A). It should be noted that the rats with nonencapsulated islets in the peritoneal cavity had higher blood glucose levels during exercise than the other transplant groups and controls; however, the fall in the blood glucose levels was similar when the difference in blood glucose levels between 0 min and later time points (Δ glucose) was calculated (Figs. 2B). Furthermore, the decrease (Δ) in the blood glucose at the 60-min time point was same in the two groups with islets in the peritoneal cavity, both those with and without capsules.

Rats transplanted with islets in the liver and with encapsulated islets in the peritoneal cavity had increased C-peptide levels at the end of the exercise. As shown in Fig. 3A, in control and experimental rats with transplanted islets under the kidney capsule and nonencapsulated islets in the peritoneal cavity, the C-peptide levels fell after 30 min of exercise and even further at 60 min (30 min after the cessation of exercise). It was surprising that increases in C-peptide levels were seen after 30 min of exercise in rats with islets transplanted into the liver and encapsulated islets in the peritoneal cavity.

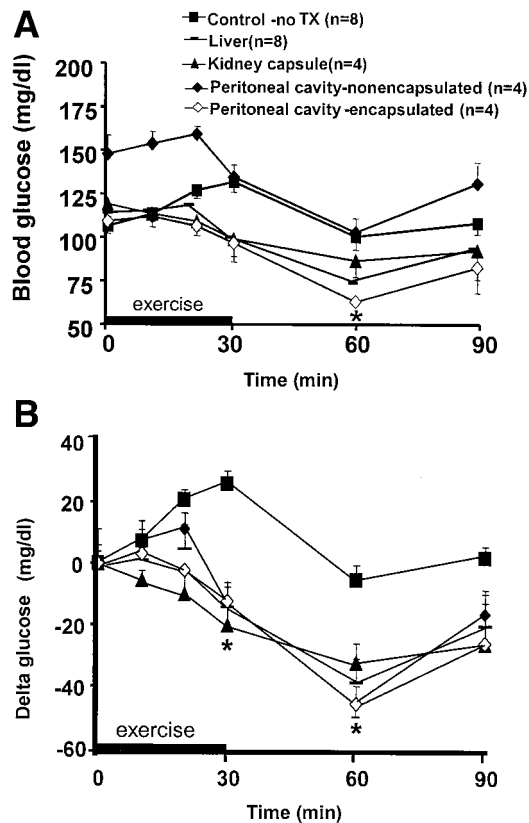


FIG. 2. Blood glucose levels decreased during and after exercise in all of the rats with islet transplants compared with normal controls. The fall in the blood glucose was most prominent at 60 min (30 min after the completion of the exercise; $*P < 0.01$ control vs. transplanted rats at 30 and 60 min, and 60 vs. 0 min within the groups with transplants). Rats with nonencapsulated islets transplanted into the peritoneal cavity had higher blood glucose levels (A) than the controls and other transplant groups; however, the difference of blood glucose (Δ glucose) between later time points with 0 min were very similar to those in other transplanted groups (B).

Rats with islet transplants in the liver and peritoneal cavity (either nonencapsulated or encapsulated islets) had increased glucagon levels after exercise. Control rats had a modest fall in plasma glucagon levels during and after exercise. In contrast, after 30 min of exercise, at the time when glucose levels had fallen, plasma glucagon rose, most notably in the group with islets in the liver and the group with nonencapsulated islets or encapsulated islets in the peritoneal cavity (Fig. 3B). For the group with islets in the liver, glucagon values rose from basal levels of 62 ± 6 to 165 ± 29 pg/ml after 30 min of exercise, whereas in the group with nonencapsulated islets in the peritoneal cavity, the increase was from basal values of 50 ± 5 to 133 ± 26 pg/ml, and in the group with encapsulated islets, the increase was from basal values of 61 ± 7 to 105 ± 11 pg/ml. For the group with islets under the kidney, the increase in the glucagon levels was not significant.

Rats with islet transplants in the peritoneal cavity had late increases in C-peptide levels after intravenous or intraperitoneal glucose injections. We assessed the effect of the transplantation site on the blood glucose and C-peptide changes using IVGTT (Fig. 4) and IPGTT (Fig. 5). Blood glucose levels of transplanted rats increased to higher peak levels than those of the control

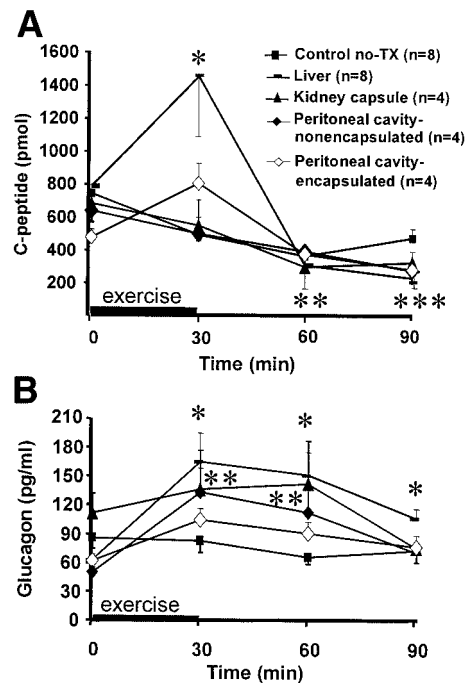


FIG. 3. Rats with islet transplants in the liver and peritoneal cavity (encapsulated islets) had elevated plasma C-peptide and glucagon levels during and after exercise. C-peptide levels in the liver and encapsulated groups were increased at the 30-min time point ($n = 4$; $1,450 \pm 250$ pmol/l and 805 ± 125 pmol/l, respectively, vs. 496 ± 103 pmol/l for the control rats; $*P < 0.01$ for liver and encapsulated vs. control or 0 min), then decreased at 60 and 90 min for encapsulated group ($**P < 0.05$ at 60 min and $***P < 0.01$ at 90 min vs. 0 min for both groups). Rats with nonencapsulated islets in the peritoneal cavity or kidney and controls showed progressive decrease in the C-peptide levels at the end of and after exercise compared with pre-exercise levels (A). Rats with islets in the liver and peritoneal cavity had increases in plasma glucagon levels at the end of and after exercise ($*P < 0.05$ at 30, 60, and 90 min vs. 0 min for liver and encapsulated groups and vs. control rat at 60 min for the transplants in the liver; $**P < 0.05$ at 30 and 60 min vs. 0 min for the nonencapsulated islet transplants in the peritoneal cavity).

group 5 min after intravenous glucose injections, and decreased more slowly at later time points (Fig. 4A). Plasma C-peptide levels of the control rats were highest, whereas those of rats with islets in liver and kidney were lower. The rats with nonencapsulated islets in the peritoneal cavity showed increases in C-peptide levels only at 90 min and later time points, whereas the rats with encapsulated islet transplants had no increase in plasma C-peptide levels (Fig. 4B).

After intraperitoneal glucose injection, peak blood glucose values were observed between 10 and 30 min except for the encapsulated transplant group, in which the peak blood glucose was observed at 60 min. The peak glucose values were generally higher in the transplanted rats than in controls. Among the transplanted groups, the rats with transplanted islets in the peritoneal cavity (encapsulated or nonencapsulated islets) had the highest glucose levels and slowest glucose clearance (Fig. 5A). Plasma C-peptide levels after the intraperitoneal glucose injection had a peak level at 10 min in the control group, followed by a gradual decrease at later time points (Fig. 5B). Rats with transplants in the liver ($n = 4$) showed the same pattern as controls except with C-peptide values. In contrast, rats with nonencapsulated islets in the peritoneal cavity had increased C-peptide levels only at time points after 60 min,

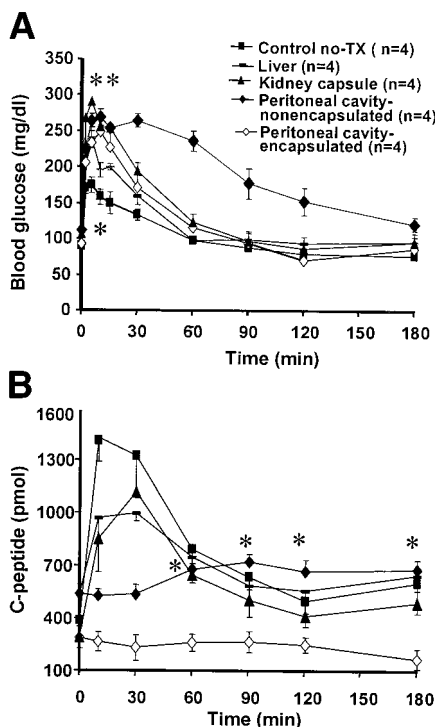


FIG. 4. Intravenous glucose tolerance tests. Higher blood glucose (*A*) and delayed plasma C-peptide (*B*) changes were observed during IVGTT in rats transplanted with islets into the peritoneal cavity. Blood glucose levels were elevated to higher peak levels in the transplanted islets rats than in the control group 5 min after glucose injection ($*P < 0.01$ for control vs. liver or encapsulated islets, $**P < 0.001$ vs. nonencapsulated islets or kidney) and decreased more slowly at further time points (*A*). Plasma C-peptide levels of the control rats paralleled the changes of blood glucose ($n = 4$) (*B*). Plasma C-peptide levels in the liver and kidney group ($n = 4$) (*B*) were similar to those in the control rats, whereas the nonencapsulated group of rats showed increased C-peptide levels only at later time points ($*P < 0.02$ at 90, 120, and 180 min vs. 0 min) (*B*). Plasma C-peptide levels of rats with encapsulated islet transplants did not increase at all.

as was observed in the IVGTT ($n = 4$) (Fig. 5*B*). Rats with encapsulated islets had even more delayed C-peptide secretion than the nonencapsulated islets in the peritoneal cavity.

DISCUSSION

In this study, rats with islet transplants developed hypoglycemia during moderate exercise, whereas normal control rats maintained normoglycemia. This fall in glucose was seen in all transplant groups, which included islets transplanted into the liver, kidney, and peritoneal cavity, even with alginate-encapsulated islets in the peritoneal cavity. It appears that somehow these islets did not appropriately suppress insulin secretion, as indicated by C-peptide levels, for the challenge of exercise, raising important questions about the function of transplanted islets in this and other settings. During acute and even prolonged exercise, blood glucose levels are maintained, which allows continuing cognitive function. To compensate for increased muscle consumption of glucose, hepatic glucose output is increased, mainly because of a decrease in the insulin/glucagon ratio activation of the local intranslet (8) and systemic sympathetic nervous system (9). The sympathetic nervous system makes direct contributions to the inhibition of insulin secretion by increasing glucagon

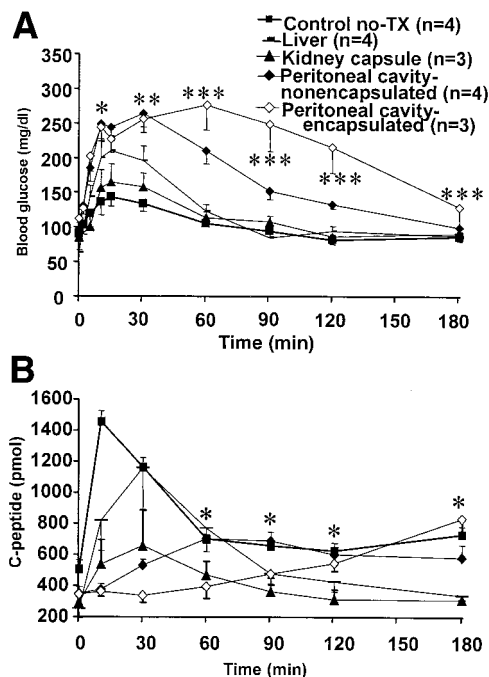


FIG. 5. Intraperitoneal glucose tolerance tests. Higher blood glucose (*A*) and delayed plasma C-peptide (*B*) changes were observed during IPGTT in the rats transplanted with islets into the peritoneal cavity. The peak glucose values were higher in the transplanted rats (except the kidney group) than in controls. Among the transplanted groups, the rats with transplanted islets in the peritoneal cavity (encapsulated or nonencapsulated islets) had the highest glucose values and slowest glucose clearance (peritoneal cavity vs. controls: $*P < 0.01$ at 15 min and later time points, $**P < 0.001$ at 30 min; vs. other transplant groups: $***P < 0.05$ for 60, 90, 120, and 180 min) (*A*). Blood glucose clearance in the other transplant groups (liver and kidney) was similar to that of the control group. Plasma C-peptide levels of control group peaked at 10 min and then gradually decreased at later time points (*B*). Rats in the liver group ($n = 4$) showed a parallel pattern to that observed in controls with lower C-peptide values after intraperitoneal glucose injections. In contrast, rats with nonencapsulated islets in the peritoneal cavity had increased C-peptide levels only at time points 60 min and later ($*P < 0.02$ at 60 min and later time points vs. 0 min) (*B*). Rats with encapsulated islets had even more delayed C-peptide secretion with increases being seen only at 90 min and later.

release, which leads to increase in hepatic glucose output. Even in the case of normoglycemia during exercise, β -cells are in part suppressed by adrenergic activation. We know that β -cells are normally sensitive to falling glucose levels, showing reduced insulin secretion even as glucose levels drop from 5.8 mmol/l (104 mg/dl) to 4.4 mmol/l (78 mg/dl) in one study in dogs (10).

Because transplanted islets are in an unnatural site compared with their normal home in the pancreas, there is concern about whether their function is truly normal. For example, although current islet transplantations in humans usually use islets from two or even more cadaver pancreases, the islets typically do not normalize glucose levels, but instead result in a state of impaired glucose tolerance (1). Because glucose tolerance is usually unequivocally normal with whole organ pancreas transplants, this glucose intolerance found with islet transplants has been blamed on insufficient islet mass. Now it is becoming clear that, in addition to the islet mass issue, there likely are complex abnormalities in islet function that may be subtle enough to allow most normal activities, such as eating, fasting, and most types of exercise. Although virtually nothing has been published about exer-

cise in transplant recipients, we know from anecdotal clinical experience that some patients partake in moderate exercise without obvious hypoglycemia. Perhaps these individuals are protected by having glucose intolerance and/or taking enough food before exercise to prevent symptomatic hypoglycemia.

Further considering the secretory dysfunction of islet transplants in humans, insulin responses to acute stimulation with intravenous glucose have been shown to be $21 \pm 5\%$ of controls, whereas those to arginine were $56 \pm 11\%$ (1). Interpretation of these results is complicated by the inclusion of subjects with varying degrees of glucose intolerance, meaning that some subjects probably had a loss in response because of glucotoxicity. It is known that first-phase insulin responses start to fall when fasting glucose levels rise to >100 mg/dl and disappear with levels of only 115 mg/dl (11). In contrast, insulin responses to arginine are maintained as glucose levels increase (12). Thus the insulin secretion results seen in current recipients of human islets are probably caused by a combination of reduced β -cell mass, glucotoxicity, toxic effects of immunosuppressive agents, and other as-yet-unidentified factors. It is also known that recipients of islet transplants have mostly intact counterregulatory responses to insulin-induced hypoglycemia, except for a striking lack of a glucagon response (3), in contrast to the normal glucagon responses found with pancreas transplants (13).

Differences in vascularization and innervation of transplanted islets could also lead to abnormal function. Anatomic and physiological studies have shown that arterial blood flow is directed from the core of the islet to the mantle, thus from β -cells to α -cells (14,15). These findings fit the concept that intra-islet insulin exerts an important influence on glucagon secretion, the concept being that hypoglycemia lowers local insulin secretion, thereby enhancing glucagon secretion (16,17). Debate continues about the relative contributions of local insulin secretion and sympathetic activation to explaining the increases of glucagon secretion seen with hypoglycemia (18,19). Nonetheless, it is clear is that clumps of transplanted islets undergo substantial structural remodeling in the graft site (20) so that intra-islet interactions may be disrupted. Perhaps some of the transplanted β -cells are downstream from glucagon-producing α -cells and therefore are subjected to the potent secretory influences of glucagon.

Considering other ways in which the functioning of transplanted islets may differ from that of islets in their natural pancreatic milieu, it has recently been shown that the oxygen tension of islet grafts is lower than that of islets in the pancreas (21) and that their vascular pattern is different than that of normal islets (22). Despite this seemingly adverse environment, small numbers of islets, containing $<20\%$ of pancreatic β -cell mass, can normalize glucose levels in diabetic mice (23). In the current study, the similar fed blood glucose levels and lower plasma C-peptide levels observed in transplanted rats suggest that the functional islet mass in the transplanted animals was not higher than that in controls. Furthermore, the higher peak glucose levels and delayed glucose clearance accompanied by lower C-peptide levels in the transplanted rats during IVGTT suggest that functional islet mass after transplantation may actually be less than that of controls.

It should be noted that the functional islet mass necessary to maintain normoglycemia might be variable at different transplantation sites. Future experiments with marginal islet masses transplanted into different sites may eventually provide answers to these questions.

In the present study, the pathophysiology responsible for the fall in glucose levels with exercise appeared to differ among the different transplant situations, with none being easy to explain. The group with islets in the liver had increases of C-peptide and glucagon levels with exercise. Increased insulin secretion seems to be a logical explanation for the fall in glucose levels, with the glucagon levels being insufficient to counteract the insulin effect. It is possible that sympathetic activation with exercise led to increased glucagon release from the pancreas, which reached the β -cells in the liver via the portal vein at a high enough concentration to stimulate insulin secretion. In addition, it is possible that glucagon within the graft was secreted downstream upon β -cells. The rats with islets in the kidney had a similar fall in glucose levels with exercise, but a very different pattern of hormone secretion, with C-peptide levels dropping and a modest increase in glucagon being seen. Perhaps insulin secretion did not fall enough to limit the decline in glucose, again raising questions about possible incorrect regulation of insulin secretion in the graft.

The group with encapsulated islets in the peritoneal cavity had a clear increase in C-peptide and glucagon levels at 30 min. This increase in C-peptide may not have been caused by increased secretion, but rather by release of insulin from the capsule dead space caused by agitation of fluid compartments in the peritoneal cavity by the motion of exercise. If there really was increased insulin secretion from the β -cells in the capsules, higher C-peptide levels might have been expected at the 60-min time point. It is known that release of secreted C-peptide from alginate capsules in response to a glucose challenge is greatly slowed by diffusion of the peptide (24). In parallel, the delayed glucose clearance and poor C-peptide secretion in response to intravenous or intraperitoneal glucose stimulation observed in our study (Figs. 4 and 5) suggest that the peritoneal cavity has substantial weaknesses as a site for islet transplantation.

In conclusion, rats with islet transplants in several sites developed hypoglycemia when exposed to moderate exercise. As islet transplantation becomes more effective and available to adults and children, the question of vulnerability to hypoglycemia may become an important issue that will need to be better understood and require special treatment strategies.

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