Glucose, Insulin, and Glucagon Levels During Exercise in Pancreas Transplant Recipients

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OBJECTIVE — To determine whether pancreas transplant recipients maintain normal blood glucose levels during physical exercise.

RESEARCH DESIGN AND METHODS — We measured serum glucose, insulin, C-peptide, and plasma glucagon levels in six pancreas transplant recipients and six healthy control subjects matched for age, sex, body size, and level of conditioning during 1 h of bicycle exercise at a workload set to achieve 40% of each individual's previously determined peak oxygen consumption ($\dot{V}O_2$).

RESULTS — Serum glucose values were not different between control subjects and transplant recipients before the start of exercise (5.0 ± 0.1 and 4.9 ± 0.1 mmol/l, respectively). Serum glucose levels fell slightly but significantly in both recipients and control subjects during exercise. There were no significant differences in glucose levels between the two groups at any time point during exercise, although mean nadir glucose during exercise was slightly lower in transplant recipients compared with control subjects (4.4 ± 0.1 vs.4.8 ± 0.1 mmol/l, $P = 0.04$). In control subjects, insulin and C-peptide levels fell significantly within 15–30 min of exercise and glucagon levels rose significantly after 60 min of exercise. In transplant recipients, there was a trend for insulin and C-peptide levels to fall and glucagon levels to rise during exercise, although these changes were delayed and were not statistically significant.

CONCLUSIONS — No significant abnormalities in blood glucose were detected in pancreas transplant recipients during bicycle exercise at 40% of peak $\dot{V}O_2$ for 1 h. Compared with levels in control subjects, subtle alterations in insulin and glucagon levels may occur in transplant recipients during exercise. However, these alterations do not appear to result in either hyperglycemia or hypoglycemia during light exercise for up to 1 h.

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ANOVA, analysis of variance; BMI, body mass index.

Recipients of functioning pancreas transplants maintain normal blood glucose levels during both fasting and unstressed daily activities (1–7). The ability of the transplanted pancreas to maintain glucose homeostasis in other physiological settings, which may be encountered by transplant recipients, has not, to our knowledge, been studied. In particular, it is unknown whether pancreas transplant recipients can maintain normal blood glucose levels during physical exercise.

During exercise, insulin levels must fall and glucagon levels must rise if normoglycemia is to be maintained (8–10). Sympathetic nerves to the pancreas may be important mediators of these hormonal changes (11–15). Thus, it is possible that patients with a denervated transplanted pancreas will lack the normal neural signals to the islets during exercise, thereby limiting the expected decrease in insulin and increase in glucagon. This in turn might lead to a decline in blood glucose during exercise. On the other hand, it has been proposed from animal studies that denervated islets may demonstrate the phenomenon of denervation supersensitivity during physical stress (16). This would cause abnormal sensitivity to circulating catecholamines during stress, resulting in impaired insulin secretion and hyperglycemia (16). Thus, one can propose opposing hypotheses, i.e., in response to a physical stress such as exercise, humans with a denervated pancreas transplant may demonstrate abnormal glucose regulation—either hypoglycemia due to absence of normal neural pathways or hyperglycemia due to denervation supersensitivity.

The purpose of this study was to test whether human recipients of a denervated pancreas can maintain normal glucose levels during physical exercise. We measured metabolic and hormonal responses of transplant recipients and control subjects during 1 h of light bicycle exercise maintained at 40% of peak oxygen consumption ($\dot{V}O_2$).

Table 1—*Characteristics of pancreas transplant recipients and control subjects participating in exercise studies*

	Recipients	Control subjects	*P* value
n (W/M)	6 (2/4)	6 (2/4)	—
Age (years)	34.0 ± 2.0	32.0 ± 1.0	0.39
BMI (kg/m^2)	22.1 ± 0.7	24.2 ± 1.3	0.18
Peak $\dot{V}O_2$ (ml · kg^{-1} · min^{-1})	24.9 ± 3.7	30.7 ± 2.5	0.22
Workload to achieve 40% peak $\dot{V}O_2$ (W)	33.0 ± 8.0	48.0 ± 6.0	0.16
Duration of diabetes prior to transplant (years)	22.0 ± 1.0	—	—
Time since transplant (years)	2.0 ± 0.4	—	—

Data are means ± SE.

RESEARCH DESIGN AND METHODS

Exercise protocol

Six pancreas transplant recipients (2 women and 4 men) with functioning allografts and six healthy nondiabetic control subjects matched for age, sex, body mass index (BMI), and exercise conditioning status (trained or untrained) were studied (Table 1). All recipients had systemic venous drainage of the allograft; all were receiving prednisone, cyclosporin, and azathioprine; and none was receiving insulin, oral hypoglycemic medications, β-adrenergic receptor agonists or antagonists, or α-adrenergic receptor antagonists. One recipient was receiving verapamil and clonidine, one was receiving verapamil only, and two were receiving furosemide.

All subjects underwent a symptom-limited maximal bicycle test to determine peak $\dot{V}O_2$ (17). Subjects exercised on a cycle ergometer in the fasted state, and oxygen consumption (l/min), carbon dioxide production (l/min), and minute ventilation (l/min) were measured using breath-by-breath analysis (Medical Graphics 2000) and recorded as an average over the preceding 30 s. Exercise was initiated at 20 W and increased by 30-W increments every 2 min until symptomatic maximum. Peak $\dot{V}O_2$ (ml · kg^{-1} · min^{-1}) was recorded as the highest value over any 30-s period and in all cases corresponded to the value in the terminal portion of exercise.

On a separate day at least 2 days after the maximal test, fasted subjects returned for the constant-level submaximal exercise test. An intravenous catheter was inserted in an antecubital vein for blood sampling, after which subjects sat quietly for 60 min. Subjects moved to the cycle ergometer 20 min before starting to exercise ($t = -20$ min). At $t = -15$ min, subjects began breathing through the analyzer mouthpiece. Resting blood samples were drawn at $t = -10$, -5, and 0 min. Subjects then began pedaling at a workload set to achieve 40% of each subject's previously determined peak $\dot{V}O_2$. Blood samples were drawn at $t = 5$, 15, 30, 45, and 60 min after commencement of exercise. $\dot{V}O_2$ was measured continuously during the initial 15 min of exercise, and the cycle workload was adjusted if necessary during this period to achieve the desired $\dot{V}O_2$. Exercise was terminated after 60 min, and additional blood samples were drawn during recovery at $t =$ 15, 30, and 45 min postexercise. Pulse rate was monitored continuously by electrocardiogram, and blood pressure was measured by an automated cuff sphygmomanometer. All protocols were approved by the University of Minnesota Human Subjects Committee, and all subjects gave written consent.

Assays

Blood samples for measurement of serum glucose and plasma glucagon were kept on ice and centrifuged, and the serum or plasma was separated and frozen at −20°C. Glucagon samples were collected in prechilled tubes containing aprotinin and EDTA. Blood samples for measurement of serum insulin and C-peptide were allowed to clot briefly at room temperature and centrifuged, and the serum was frozen at −20°C. Insulin, C-peptide, and glucagon were measured by radioimmunoassay (18–20). Serum glucose was measured by the glucose oxidase method.

Statistical analysis

Pre-exercise parameters were compared between groups by nonpaired Student's *t* test. Within each group, the effect of exercise on metabolic and hormonal parameters was analyzed by one-factor analysis of variance (ANOVA) for repeated measures followed by Fisher's protected least-squares difference for individual comparisons (21). When comparisons were made between groups, Student's *t* test for unpaired data was used. *P* values < 0.05 were considered statistically significant.

RESULTS — Baseline parameters before exercise for the pancreas transplant recipients and control subjects are shown in Table 1. There was no significant difference between the two groups in age, BMI, peak $\dot{V}O_2$, or workload to achieve 40% peak $\dot{V}O_2$. Resting heart rate, fasting serum glucose and C-peptide, and fasting plasma glucagon levels were not different before exercise (Figs. 1–4). Serum insulin levels were significantly higher in transplant recipients compared with control subjects (125 ± 12 vs. 61 ± 11 pmol/l, $P < 0.003$) as previously reported (2–6).

Heart rate increased significantly in both groups at the onset of exercise (Fig. 1). Mean heart rate during exercise was slightly higher in control subjects compared with recipients (116 ± 4 vs. 104 ± 4 beats/min, respectively, $P = 0.06$). Mean arterial pressure at rest was 96 ± 6 and 90 ± 2 mmHg in recipients

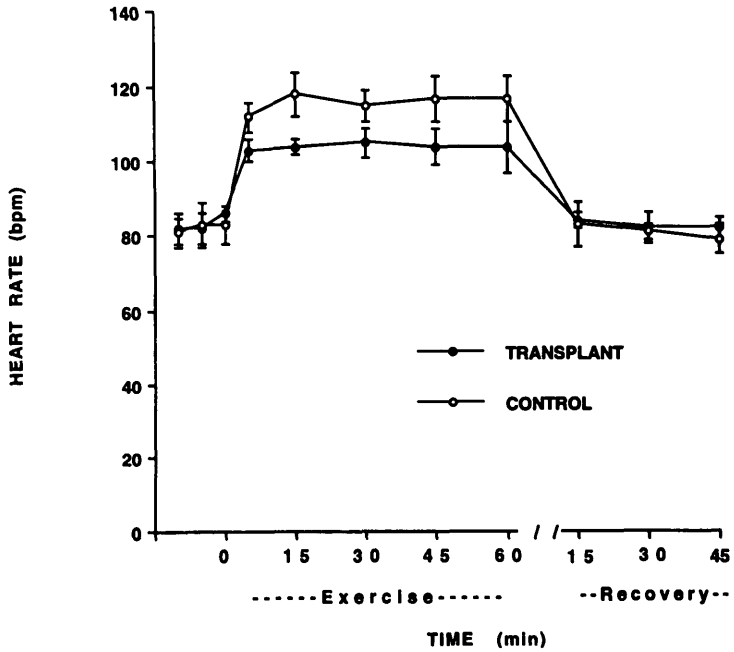

Figure 1—*Heart rate during exercise for 1 h at 40% peak $\dot{V}O_2$ and during recovery in pancreas transplant recipients (●) and control subjects (○). Data are means ± SE.*

and control subjects, respectively, and did not change significantly during exercise in either group.

Serum glucose levels declined slightly, but significantly, in both groups during exercise (Fig. 2). In transplant recipients, serum glucose was significantly lower than resting value at 30 min of exercise (4.5 ± 0.2 vs. 4.9 ± 0.2 mmol/l) and remained slightly lower for the remainder of the exercise period (P = 0.008, ANOVA; Fig. 2). Control subjects also had a significant decline in glucose with exercise (P = 0.005, ANOVA), although the decrease in serum glucose was smaller and was significant only at the 45-min time point (Fig. 2). When compared between groups, there was no significant difference in serum glucose levels at any time point during exercise or recovery (P > 0.05, Student's t test for unpaired data). Mean nadir glucose during the 60-min exercise period was slightly lower in transplant recipients compared with control subjects (4.4 ± 0.1 vs.4.8 ± 0.1 mmol/l, respectively, P = 0.04). Serum glucose levels after 45 min of recovery in recipients and control subjects were 4.7 ± 0.2 and 4.8 ± 0.1 mmol/l, respectively (Fig. 2).

Serum insulin levels fell significantly during exercise in control subjects (P = 0.03, ANOVA), and were 70 ± 9% of pre-exercise levels within 15 min of exercise (46 ± 12 vs. 61 ± 11 pmol/l) (Fig. 3). Similarly, C-peptide levels fell with the onset of exercise in control subjects and were significantly less than pre-exercise levels after 30 min of exercise (Fig. 3). Plasma glucagon levels increased at the onset of exercise in control subjects and were significantly higher than resting levels after 60 min of exercise (173 ± 53 vs. 123 ± 25 ng/l, P < 0.05) (Fig. 4). Serum insulin and C-peptide levels tended to fall and plasma glucagon levels tended to rise in transplant recipients during exercise, although these changes tended to occur later in the exercise period and did not reach statistical significance (Figs. 3 and 4).

CONCLUSIONS— These studies were conducted to determine whether human pancreas transplant recipients maintain normal blood glucose levels during exercise. We hypothesized that lack of neural input to the transplanted

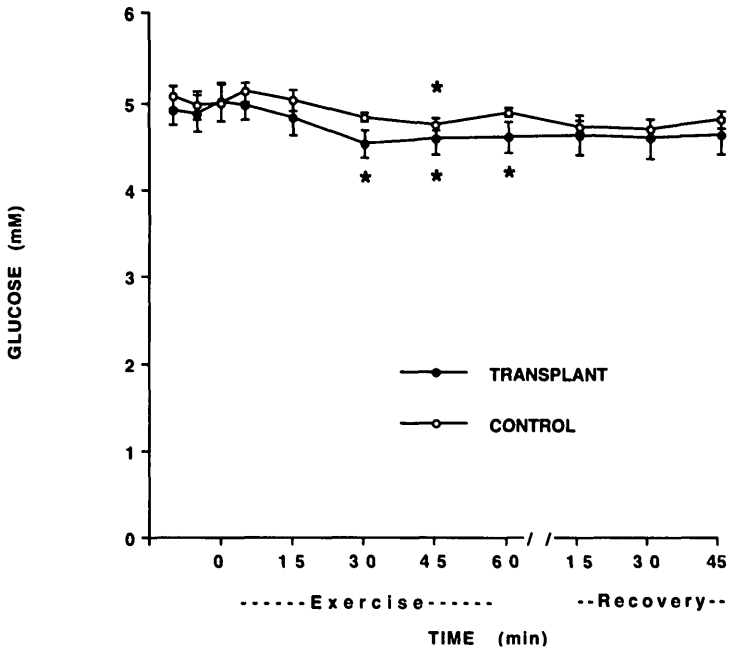

Figure 2—*Serum glucose at rest, during exercise for 1 h at 40% peak $\dot{V}O_2$, and during recovery in pancreas transplant recipients (●) and control subjects (○). Data are means ± SE. *P < 0.05 vs. resting value for corresponding group.*

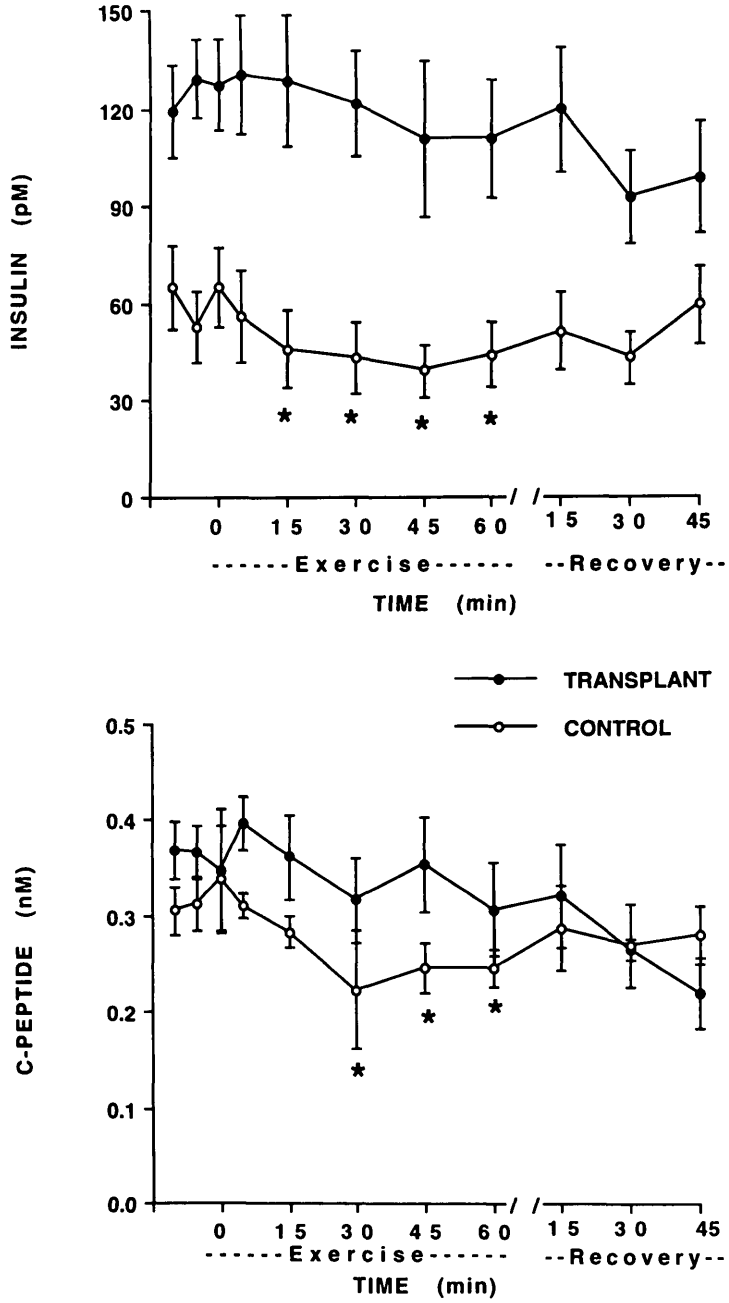

Figure 3—*Serum insulin and C-peptide at rest, during exercise for 1 h at 40% peak $\dot{V}O_2$, and during recovery in pancreas transplant recipients (●) and control subjects (○). Data are means ± SE. *P < 0.05 vs. resting value for corresponding group.*

pancreas might result in either *1*) an abnormal fall in glucose during exercise due to absence of adrenergic neural signals to the islet to inhibit insulin release and stimulate glucagon release or 2) an abnormal rise in serum glucose during exercise due to supersensitivity of the denervated β-cell to inhibition of insulin release by circulating catecholamines. We found no significant difference in serum glucose levels between recipients and control subjects during exercise and subsequent recovery. Glucose levels did decline earlier after the onset of exercise in transplant recipients and remained slightly, although not significantly, lower during the exercise period. As expected, insulin and C-peptide levels fell significantly and glucagon levels rose significantly in control subjects during exercise (8). Similar trends in insulin and glucagon levels were seen in transplant recipients, although the changes were somewhat delayed and not statistically significant.

Despite a large body of literature characterizing the effects of autonomic neural input on islet hormone secretion, the physiological role and importance of neural regulation of the endocrine pancreas remain unknown (22–24). Studies in both canine models and humans have investigated the effects of denervation of the pancreas on islet function in the fasting state or during glucose stimulation in an unstressed setting. Results from these studies are inconclusive, suggesting that denervation of the pancreas has no effect on islet function (25–27) or results in increased basal or stimulated insulin levels because of the loss of tonic neural inhibition (28–31). The cumulative experience from studies of human pancreas transplant recipients suggests that blood glucose levels during fasting, during unstressed daily activities, or in response to oral or intravenous glucose are indistinguishable from those in nondiabetic control subjects (1–7). Thus, pancreatic neural innervation does not appear to be essential for maintenance of glucose homeostasis in humans in these settings. To our knowledge, the present data are the first investigations in human pancreas transplant recipients that have assessed the ability of the transplanted pancreas to maintain normal glucose levels during a physiological stress. This is an important question because previous studies in both animals and humans suggest that sympathetic nerves to the endocrine pancreas may regulate islet hormone secretion during stress states such as exercise, hypoxia, or neuroglycopenia (12–15, 32–35).

Our results suggest that after pan-

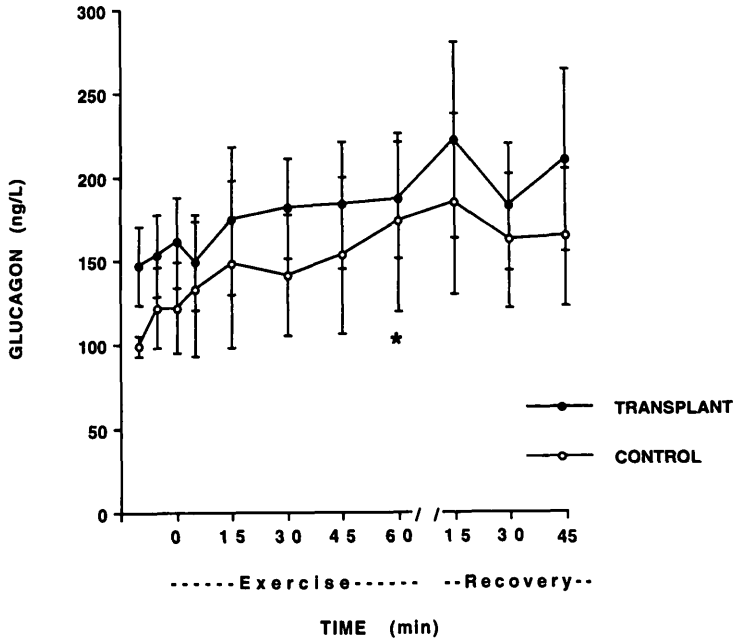

Figure 4—*Plasma glucagon at rest, during exercise for 1 h at 40% peak $\dot{V}O_2$, and during recovery in pancreas transplant recipients (●) and control subjects (○). Data are means ± SE. *P < 0.05 vs. resting value for corresponding group.*

creas transplantation there may be subtle alterations in insulin and glucagon secretion during exercise. These alterations do not result in blood glucose levels that are likely to be clinically significant. None of the transplant recipients had difficulty completing the exercise protocol or had symptoms suggestive of a fall in blood glucose. Serum insulin levels did decline in transplant recipients, although this decline did not occur until glucose levels had already fallen. Similarly, glucagon levels tended to rise in recipients as exercise progressed although the changes were not significant. Thus, it appears likely that despite the lack of neural innervation to the pancreas, other mechanisms serve to regulate insulin and glucagon secretion and prevent hypoglycemia. Such mechanisms could include direct effects of falling blood glucose in decreasing insulin secretion, inhibition of insulin and stimulation of glucagon release by circulating catecholamines, and increased glucagon release in response to falling insulin levels (9,10).

We cannot exclude the possibility that more profound changes in blood glucose could be detected with a more strenuous or longer exercise protocol. We chose an exercise intensity of 40% of peak $\dot{V}O_2$ for 1 h as this is an exercise level representative of daily activities in transplant recipients and normal subjects. Furthermore, this level of exercise has been used in other studies and is known to result in reciprocal changes in insulin and glucagon levels in normal individuals (8). Anecdotally, one of the transplant recipients in our study participated in marathon races without symptoms or evidence of hypoglycemia.

In addition to abolishing normal signal pathways from the central nervous system, it has been suggested that denervation of the islet may lead to the phenomenon of denervation supersensitivity (16). It has been shown in a variety of tissues and organs that denervation renders the tissue or organ more sensitive to the effects of the neurotransmitter that would normally be released (36). Studies in diabetic rats after intraportal islet transplantation demonstrated hyperglycemia after either exogenous epinephrine infusion or the physical stress of supine restraint (16). Denervation supersensitivity of the β-cell to the inhibitory effects of catecholamines on insulin secretion was proposed as the explanation for these findings. In human pancreas transplant recipients, however, insulin levels did not fall and hyperglycemia did not occur during exercise. Thus, we found no evidence that denervation was likely to result in hyperglycemia during stress in pancreas transplant recipients.

In summary, we have compared metabolic and hormonal responses of pancreas transplant recipients and normal control subjects during exercise to determine whether transplant recipients maintain normal blood glucose levels during physical stress. While insulin and glucagon levels failed to change significantly during exercise, glucose levels in transplant recipients were not significantly different from those of control subjects. Thus, despite the lack of neural innervation to the transplanted pancreas, pancreas transplant recipients are able to maintain normal blood glucose levels during light exercise for 1 h.

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