Hepatic Insulin Resistance After Pancreas Transplantation in Type I Diabetes

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Hyperinsulinemia and peripheral insulin resistance caused by systemic insulin delivery and prednisone therapy are recognized consequences of pancreas transplantation. However, there is little information about insulin action on the liver. To investigate hepatic insulin sensitivity in recipients of pancreas transplants, we devised a staged euglycemic hyperinsulinemic clamp to measure hepatic glucose production (HGP) in 10 type I diabetic pancreas transplant recipients, 10 pair-matched healthy control subjects, and 6 nondiabetic kidney transplant recipients. Clamps were performed in two sequential stages. In stage 1, a 2-h low-dose insulin infusion (0.4 $mU \cdot kg^{-1} \cdot min^{-1}$) was used to partially suppress HGP. In stage 2, insulin-mediated suppression of HGP was challenged by a 1.5-h glucagon infusion (0.8 $ng \cdot kg^{-1} \cdot min^{-1}$), while continuing the hyperinsulinemic euglycemic–clamp conditions. During both stages, somatostatin (250 μg/h) was infused to suppress endogenous insulin secretion. All subjects underwent stage 1, and all except one pancreas recipient and a respective matched healthy control subject completed stage 2. Fasting HGP was greater in pancreas recipients than in healthy control subjects (15.1 ± 0.7 vs. 12.0 ± 0.4 $\mu mol \cdot l^{-1} \cdot kg^{-1} \cdot min^{-1}$, $P < 0.005$) but similar in healthy control subjects and in kidney recipients. During stage 2, both total (706 ± 28 vs. 469 ± 31 $\mu mol \cdot l^{-1} \cdot kg^{-1}$, $P < 0.005$) and incremental (62 ± 20 vs. −21 ± 16 $\mu mol \cdot l^{-1} \cdot kg^{-1}$, $P < 0.005$) HGP responses to glucagon infusion were significantly greater in pancreas recipients than in healthy control subjects. Changes in HGP in kidney recipients during stage 2 were not significantly different from those in healthy control subjects. In conclusion, fasting HGP is increased in pancreas transplant recipients. Furthermore, recipients have hepatic insulin resistance as demonstrated by an enhanced stimulatory effect of glucagon on HGP during insulin-mediated HGP suppression. Because the magnitude of hepatic insulin resistance was a significant ($P < 0.01$) predictor of HbA_{1c} level, we suggest that variable hepatic insulin resistance may be responsible for some of the variance observed in glycemic levels after successful pancreas transplantation. *Diabetes* 45:134–138, 1996

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CV, coefficient of variation; HGP, hepatic glucose production.

The success of pancreas allografts in normalizing plasma glucose levels in type I diabetes has increased both the demand for transplants and the number performed in recent years (1,2). However, although HbA_{1c} values are frequently normal (3), studies using the hyperinsulinemic euglycemic glucose clamp (4,5) as well as Bergman's Minimal Model technique (6) indicate that insulin-mediated glucose disposal is impaired in pancreas transplant recipients. This peripheral insulin resistance is probably a consequence of downregulation of tissue insulin receptors caused by sustained hyperinsulinemia (7), which is primarily a result of systemic rather than portal vein insulin delivery (8) as well as antagonism of glucose utilization caused by the use of prednisone for immunosuppression.

Chronic glucocorticoid administration also antagonizes insulin action in the liver by stimulating gluconeogenesis and glycogenolysis and increasing hepatic glucose production rates (9). The use of prednisone in pancreas transplant recipients therefore raises the possibility of not only peripheral but also hepatic insulin resistance. On the other hand, Homan et al. (10) have suggested that absence of the normal portal-peripheral insulin gradient after pancreas transplantation may result in exposure of the liver to lower-than-normal insulin concentrations and subsequently enhance hepatic insulin action by upregulation of insulin sensitivity. The question of whether hepatic insulin resistance occurs after pancreas transplantation is of more than academic concern. In normal health, only a modest rise in the plasma insulin concentration is required to significantly suppress fasting hepatic glucose production (HGP) (11), whereas in type II diabetes, the failure of insulin to appropriately suppress HGP is an important cause of fasting (12) and postprandial (13) hyperglycemia. However, little information currently exists about hepatic insulin action in humans after pancreas transplantation.

To test the hypothesis that pancreas transplantation in patients with type I diabetes is associated with hepatic insulin resistance, we used a two-staged euglycemic hyperinsulinemic glucose clamp. In the first stage, HGP was partially suppressed by an insulin infusion. Then, insulin-mediated suppression of HGP was challenged by infusing glucagon during the second stage of the clamp.

RESEARCH DESIGN AND METHODS

Patients. Ten type I diabetic recipients of pancreas allograft transplants, either solitary ($n = 2$) or combined with a kidney allograft ($n = 8$), from the University of Minnesota Diabetes Center were studied (Table 1). All pancreas transplant recipients were normoglycemic, not

TABLE 1
Patient profile

	Pancreas recipients	Kidney recipients	Healthy control subjects
Age (years)	40 ± 2	36 ± 3	39 ± 2
BMI (kg/m^2)	24 ± 1	27 ± 1	25 ± 1
Sex (M/F)	5/5	3/3	5/5
HbA_{1c} (%)	5.4 ± 0.2	5.3 ± 0.1	5.1 ± 0.1
Creatinine (μmol/l)	152 ± 14	141 ± 18	88 ± 4
Diabetes duration (years)	23 ± 2	—	—
Time since transplant (months)	61 ± 7	57 ± 2	—
Immunosuppression therapy			
Prednisone (mg/day)	10 ± 1	11 ± 1	—
Cyclosporin (mg/day)	303 ± 30	264 ± 24	—
Azathioprine (mg/day)	128 ± 11	88 ± 21	—

Data are means ± SE. There was no significant difference in azathioprine dose between pancreas and kidney recipients.

receiving exogenous insulin or oral hypoglycemic agents, and immunosuppressed with a triple drug regime of cyclosporin, prednisone, and azathioprine. The control groups were 10 healthy nondiabetic subjects and 6 nondiabetic kidney transplant recipients receiving the same immunosuppressive drugs, selected to match the pancreas transplant recipients with respect to age, sex, and BMI. The study protocol was approved by the University of Minnesota Committee on the Use of Human Subjects in Research, and all participants provided written consent.

Experimental procedure. Subjects were admitted to the University of Minnesota Clinical Research Center the evening before the study. To ensure that the pancreas and nondiabetic kidney transplant recipients were not glucocorticoid-deficient during the experiment, the timing of their once-daily prednisone dose was changed from A.M. to P.M. 5 days previously so that the last dose of prednisone was taken 12 h before the start of the glucose clamp. The timing of other medications was not changed. All medications were omitted on the morning of the clamp and taken after completion of the study. At 0630, after an overnight fast, a primed (20 μCi) continuous (0.20 μCi/min) intravenous infusion of [3-^{3}H]glucose (Du Pont-NEN, North Billerica, MA) was begun. After a 2-h isotope equilibration, a euglycemic hyperinsulinemic glucose clamp was performed in two sequential stages. During stage 1 (2-h duration), insulin (Humulin R, Eli Lilly, Indianapolis, IN) was infused at 0.4 $mU \cdot kg^{-1} \cdot min^{-1}$, and the plasma glucose concentration was maintained at a target concentration of 5.4 mmol/l by a variable 20% glucose infusion labeled with [3-^{3}H]glucose to replicate the plasma glucose specific activity after the isotope equilibration period (basal specific activity). The formula used to predict basal-specific activity has been described previously (14). Arterialized blood samples were obtained at 5-min intervals for glucose estimation from a cannula, inserted retrogradely in a dorsal hand vein placed in a heated (55°C) box. During stage 2 (1.5-h duration), the insulin infusion was continued and plasma glucose was again maintained at 5.4 mmol/l, while glucagon (Eli Lilly) was simultaneously infused at a rate of 0.8 $ng \cdot kg^{-1} \cdot min^{-1}$. Somatostatin (Bachem, Torrance, CA) was infused constantly at 250 mcg/h during both stages of the clamp experiment to suppress endogenous insulin release. All subjects underwent stage 1, and all but one pancreas transplant recipient and respective matched healthy control subject completed stage 2.

Analytical methods. Samples for plasma glucose were centrifuged immediately at the bedside and analyzed using a glucose oxidase method (Beckman Glucose Analyzer 2, Fullerton, CA). Samples for determination of glucose-specific activity were taken at 10-min intervals from −30 to 0 min before the start of insulin infusion and every 15 min thereafter. Plasma for glucose-specific activity was deproteinized using $Ba(OH)_2$ and $ZnSO_4$ by the method of Somogyi (15) and air dried, and the radioactivity was counted in a liquid scintillation counter (interassay coefficient of variation [CV], 5.4%). Serum insulin (interassay CV, 6.5%), C-peptide (interassay CV, 5.0%), and plasma glucagon (interassay CV, 8.0%) levels were measured as described previously (16). All samples were stored at −20°C until analysis.

Calculations. The non–steady-state equations of Steele et al. (17) as modified by De Bodo et al. (18) were used to calculate rates of glucose appearance and disposal. The difference between the rate of glucose appearance determined by [3-^{3}H]glucose and the exogenous glucose infusion rate required to maintain euglycemia was taken to represent the rate of HGP. Fasting (basal) HGP was calculated as the mean of the −30- to 0-min values. The response to glucagon infusion in stage 2 of the clamp experiments was assessed by calculating the total and incremental areas under the HGP versus the time curve and is expressed in $\mu mol \cdot l^{-1} \cdot kg^{-1}$ of glucose.

Statistical analysis. All data in the text and figures are given as mean ± SE. When differences between groups were demonstrated to be normally distributed, analyses were performed by one-tailed Student's *t* tests. Otherwise, differences were evaluated by Wilcoxon's signed-rank (paired data) or Mann-Whitney *U* (unpaired data) tests. A two-way analysis of variance with repetitive measures was used to compare insulin, glucagon, and C-peptide concentrations during the clamp experiments among the subject groups ($P < 0.05$ was considered significant).

RESULTS

Basal conditions. Fasting systemic serum insulin concentrations before the clamp experiments were higher in pancreas transplant recipients ($P < 0.005$, Table 2). However, fasting plasma glucose and glucagon concentrations were not significantly different among the subject groups. Serum C-peptide concentrations were similar in pancreas recipients and nondiabetic kidney recipients and were higher than the concentration in healthy control subjects ($P < 0.01$ for both groups). Fasting HGP was increased in pancreas recipients ($P < 0.005$) but did not differ between kidney recipients and healthy control subjects.

Staged glucose clamp experiments. Throughout the clamp experiments, serum insulin (Fig. 1) and plasma glucagon (Fig. 2) concentrations did not differ significantly among the three subject groups. After insulin and somatostatin infusion, serum C-peptide concentrations quickly decreased (Fig. 1) but remained greater in pancreas and kidney recipients compared with healthy control subjects ($P < 0.01$). There were no significant differences in plasma glucose concentration and glucose-specific activity among the three subject groups during the clamp experiments (Fig. 3).

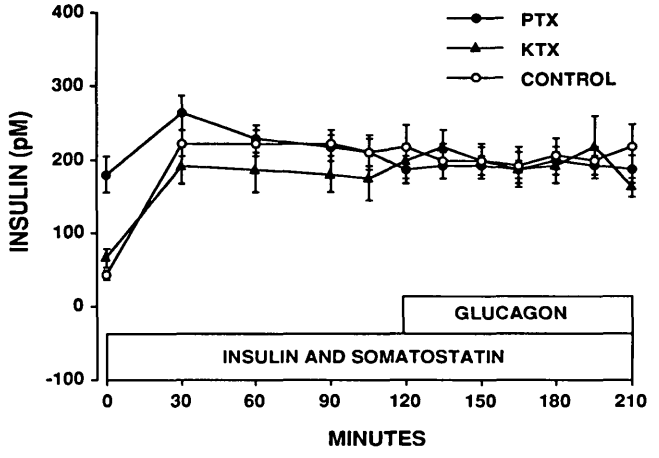
Stage 1. During the euglycemic hyperinsulinemic clamp (stage 1), HGP was suppressed in all subjects. Total glucose disposal and glucose clearance rates calculated using the plasma glucose-specific activity at the end of stage 1 were greater in healthy control subjects (22.5 ± 3.9 $\mu mol \cdot l^{-1} \cdot kg^{-1} \cdot min^{-1}$, 4.3 ± 0.7 $ml \cdot kg^{-1} \cdot min^{-1}$) than in either pancreas recipients (15.1 ± 0.9 $\mu mol \cdot l^{-1} \cdot kg^{-1} \cdot min^{-1}$, 2.8 ± 0.17 $ml \cdot kg^{-1} \cdot min^{-1}$) or kidney recipients (19 ± 1.6 $\mu mol \cdot l^{-1} \cdot kg^{-1} \cdot min^{-1}$, 3.65 ± 0.31 $ml \cdot kg^{-1} \cdot min^{-1}$), but the differences were not statistically significant.

Stage 2. Glucagon challenge during the clamp experiment (stage 2) resulted in similar increments in plasma glucagon

TABLE 2
Fasting values in pancreas transplant recipients, kidney transplant recipients, and healthy control subjects

	Pancreas recipients	Kidney recipients	Healthy control subjects
n	10	6	10
Plasma glucose (mmol/l)	5.8 ± 0.3	5.3 ± 0.2	5.4 ± 0.1
Serum insulin (pmol/l)	186 ± 24*	66 ± 15	43 ± 7
Serum C-peptide (nmol/l)	0.33 ± 0.06*	0.27 ± 0.05	0.17 ± 0.02
Plasma glucagon (ng/l)	204 ± 25	241 ± 55	234 ± 69

Data are means ± SE. *$P < 0.05$ compared with healthy control subjects.



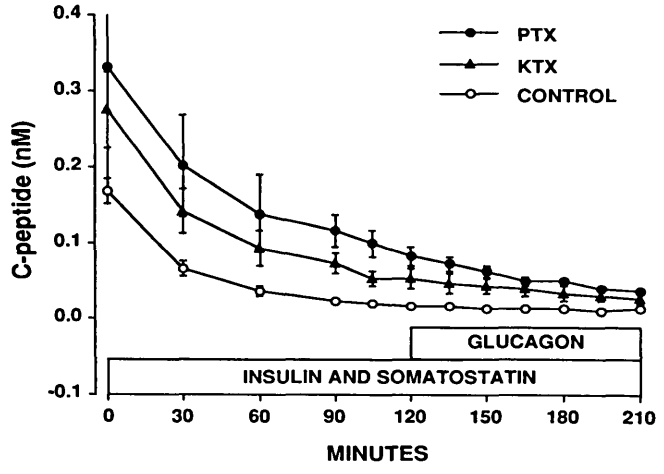
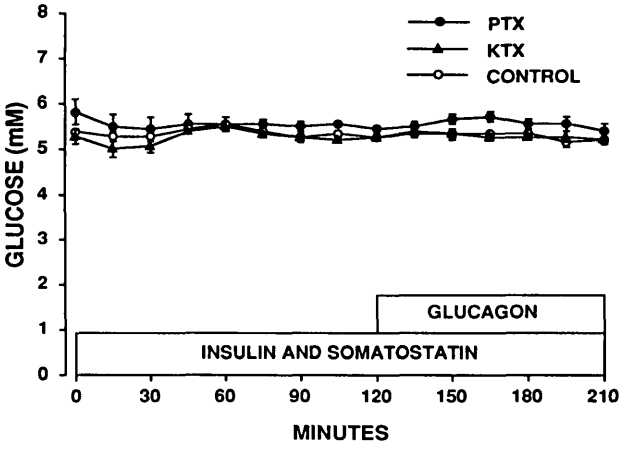


FIG. 1. Serum insulin and C-peptide concentrations during the glucose clamp experiments in pancreas transplant recipients (PTX), healthy control subjects (CONTROL), and kidney transplant recipients (KTX).



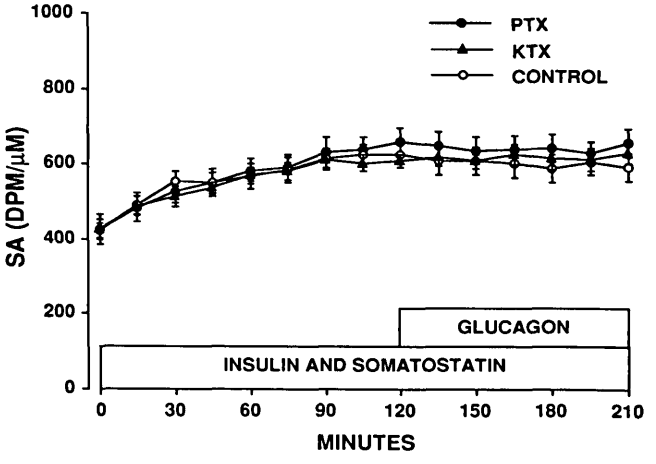


FIG. 3. Plasma glucose concentration and plasma glucose-specific activity (SA) during the clamp experiments in pancreas transplant recipients (PTX), healthy control subjects (CONTROL), and kidney tranplant recipients (KTX).

concentrations in the three subject groups (Fig. 2). In pancreas recipients, glucagon infusion caused a sustained rise in HGP, whereas in healthy control subjects, HGP transiently increased and thereafter was again suppressed. As a result, both the total and incremental areas under the HGP × time curve were significantly greater in pancreas recipients ($P < 0.005$, Fig. 5). The HGP response during glucagon infusion was not significantly different in healthy control subjects and kidney recipients. Total glucose disposal and glucose clearance rates during the last 15 min of stage 2 were significantly less in pancreas recipients (13.8 ± 1.0 $\mu mol \cdot l^{-1} \cdot kg^{-1} \cdot min^{-1}$, 2.56 ± 0.19 $ml \cdot kg^{-1} \cdot min^{-1}$) compared with healthy control subjects (25.6 ± 5 $\mu mol \cdot l^{-1} \cdot kg^{-1} \cdot min^{-1}$, 4.92 ± 0.96 $ml \cdot kg^{-1} \cdot min^{-1}$, $P < 0.01$) and with kidney recipients (21.5 ± 2.2 $\mu mol \cdot l^{-1} \cdot kg^{-1} \cdot min^{-1}$, 4.13 ± 0.42 $ml \cdot kg^{-1} \cdot min^{-1}$, $P < 0.01$).

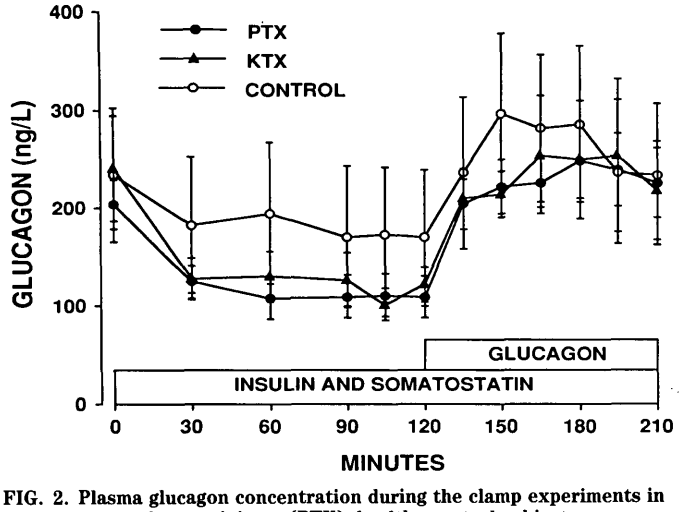


FIG. 2. Plasma glucagon concentration during the clamp experiments in pancreas transplant recipients (PTX), healthy control subjects (CONTROL), and kidney transplant recipients (KTX).

In pancreas recipients, there was only a weak correlation between fasting HGP and plasma glucose concentration (r = 0.34, P = NS). However, the HbA_{1c} level was positively correlated with the total area under the HGP against time curve calculated during stage 2 (r = 0.88, $P < 0.01$, Fig. 6).

DISCUSSION

The results of this study indicate that there is hepatic insulin resistance after pancreas transplantation in type I diabetes. In comparison with healthy control subjects, fasting HGP was greater in pancreas recipients despite a higher calculated portal vein insulin concentration. During stage 2 of the clamp experiments, glucagon's ability to counteract the insulin effect and stimulate incremental HGP was greater in pancreas transplant recipients. The latter finding indicates hepatic insulin resistance in pancreas recipients and not simply increased glucagon effectiveness from glucocorticoid synergism (19) because the hepatic response to glucagon

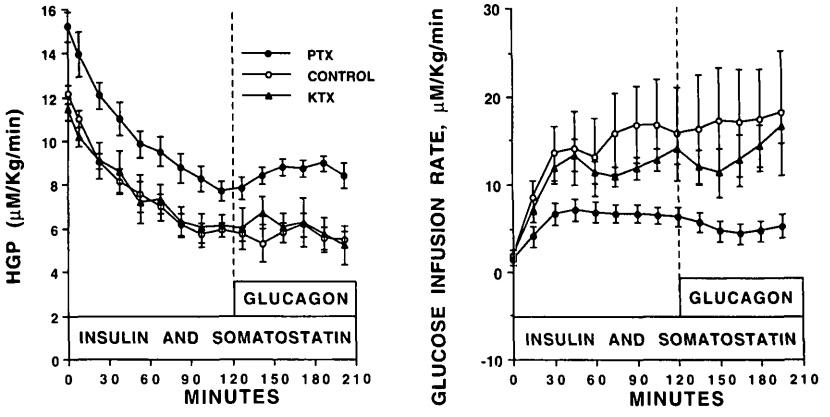


FIG. 4. HGP and exogenous glucose infusion rates during stage 1 (insulin infusion) and stage 2 (combined insulin/glucagon infusion) of the glucose clamp experiments in pancreas transplant recipients (PTX), healthy control subjects (CONTROL), and kidney transplant recipients (KTX).

during stage 2 was similar in healthy control subjects and nondiabetic kidney transplant recipients who were also receiving glucocorticoids.

These findings do not concur with the suggestion by Homan et al. (10) that anastomoses of the pancreas graft to the systemic rather than portal venous circulation leads to increased hepatic insulin sensitivity in pancreas transplant recipients. This hypothesis assumes a portal vein insulinopenia in pancreas transplant recipients under physiological conditions. The mean fasting systemic insulin concentration in our patients, which must approximate their fasting portal vein insulin concentration under steady-state conditions, was 186 pmol/l compared with 43 pmol/l in healthy control subjects. The liver is considered to extract from 50–75% of insulin secreted in the fasted state (20). Therefore, the mean corresponding portal vein insulin concentration in control subjects can be calculated to be no greater than 172 pmol/l and thus probably less than in pancreas transplant recipients.

Although the hyperglycemic effects of glucocorticoids are well described, the absence of any significant differences in our measures of hepatic insulin sensitivity in nondiabetic kidney recipients and healthy control subjects suggests that standard long-term prednisone administration in pancreas transplant recipients is not by itself an important contributory factor to hepatic insulin resistance. Bell et al. (21), in a study using low- and high-dose insulin infusion rates, assessed hepatic insulin sensitivity and found that many type I diabetes patients with acceptable glucose control have hepatic insulin resistance (21). Moreover, insulin resistance is particularly marked in uremic diabetic patients awaiting pancreas transplantation (4). It seems possible that this preoperative metabolic abnormality in patients with type I diabetes selected for pancreas transplantation may render them more susceptible to hepatic insulin resistance postoperatively despite good graft function. Alternatively, there may be hepatic consequences of chronic systemic hyperinsulinemia. It has been reported that downregulation of peripheral insulin receptors in pancreas recipients impairs not only insulin-mediated glucose disposal but also the antilipolytic action of insulin so that free fatty acid levels are higher during insulin infusion (7). As free fatty acids promote gluconeogenesis (22), failure to suppress their provision during hyperinsulinemia could delay suppression of hepatic glucose production.

Fasting HGP rates were increased in our pancreas transplant recipients. In contrast, it has previously been reported that fasting HGP in pancreas recipients with similar HbA_{1c} levels is not significantly different than in healthy control subjects (4,23). However, in at least one study (4), the usual once-daily morning dose of prednisone was not administered until after completion of the experimental protocol. Thus, during these experiments, the plasma glucocorticoid concentration was probably reduced below the usual mean daily concentration because of pituitary-adrenal suppression caused by long-term prednisone therapy. To avoid the potential problem of steroid insufficiency in fasted patients taking A.M. corticoid therapy, we changed the timing of prednisone administration in all our pancreas transplant and nondiabetic kidney transplant recipients to 8 P.M. each evening for 5 days before the clamp experiment.

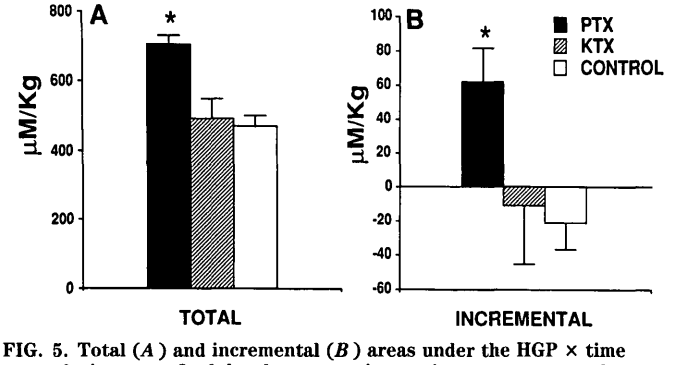


FIG. 5. Total (*A*) and incremental (*B*) areas under the HGP × time curve during stage 2 of the clamp experiments in pancreas transplant recipients (PTX), kidney transplant recipients (KTX), and healthy control subjects (CONTROL).

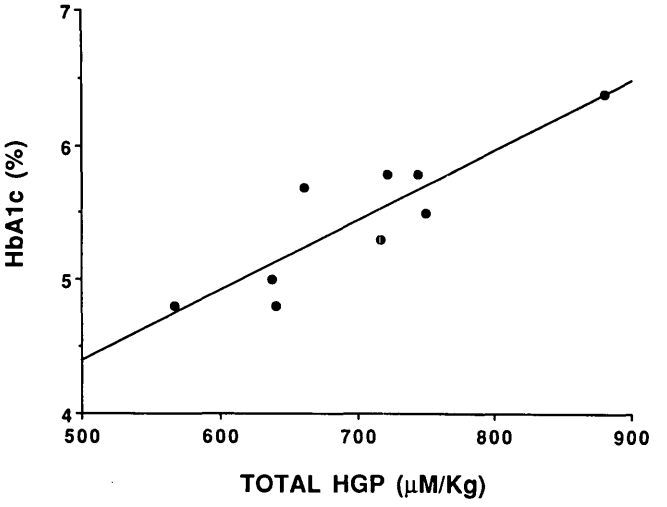


FIG. 6. Relationship between total HGP calculated during stage 2 of the clamp experiments and HbA_{1c} level in pancreas transplant recipients. r = 0.88, $P < 0.01$.

The HbA_{1c} level in our pancreas transplant recipients was positively correlated with the total HGP response during glucagon infusion in stage 2. Because this provides a measure of hepatic insulin sensitivity, this correlation suggests that the variation in HbA_{1c} values observed in pancreas recipients with apparently adequate insulin secretory function of the allograft may be a function of overall insulin resistance.

A greater fasting HGP in our pancreas transplant recipients did not predict a higher fasting plasma glucose concentration. This apparent contradiction may be explained by fasting systemic hyperinsulinemia. In healthy humans, the fasting systemic insulin concentration is low, and most glucose disposal is non–insulin mediated (24). However, the peripheral tissues in our fasted pancreas recipients were exposed to ~4 times as much insulin as the peripheral tissues in their healthy matched control subjects. Hence, despite peripheral insulin resistance, fasting insulin-mediated glucose disposal in some pancreas recipients may be increased and able to compensate for overproduction of glucose by the liver, with the net result of fasting plasma glucose levels within the normal range. It is even possible that a sufficiently large increase in fasting peripheral insulin-mediated glucose disposal could promote HGP by excessively lowering plasma glucose and thereby reducing the stimulation for graft insulin secretion.

In conclusion, despite normalized glucose levels, hepatic insulin sensitivity is diminished and fasting HGP is increased in type I diabetes after successful pancreas transplantation. A strong correlation exists between the total HGP response during glucagon infusion and the HbA_{1c} level in pancreas recipients with good graft function. Because the HGP response to glucagon in this experiment is probably inversely related to hepatic insulin resistance, our findings emphasize that exacerbation of insulin resistance in pancreas transplant recipients must especially be avoided. Otherwise, although graft function may remain satisfactory, independence from exogenous insulin therapy may be compromised.

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REFERENCES

1. Sutherland DER: Pancreas transplantation: state of the art. *Trans Proc* 24:762–766, 1992
2. Robertson RP: Pancreas and islet transplantation for diabetes: cures or curiosities? *N Engl J Med* 327:1861–1868, 1992
3. Robertson RP, Diem P, Sutherland DER: Time-related, cross-sectional and prospective follow-up of pancreatic endocrine function after pancreas allograft transplantation in type 1 insulin-dependent diabetic patients. *Diabetologia* 34 (Suppl. 1):S57–S60, 1991
4. Luzi L, Secchi A, Facchini F, Battezatti A, Staudacher C, Spotti D, Castoldi R, Ferrari G, Carlo VD, Pozza G: Reduction of insulin resistance by combined kidney-pancreas transplantation in type 1 (insulin-dependent) diabetic patients. *Diabetologia* 33:549–556, 1990
5. Saudek F, Pelkanova T, Bartos V, Reneltova I, Kazdova L, Kovar J, Karasova L: Insulin action and insulin binding following pancreas transplantation. *Diabetologia* 34 (Suppl. 1):S71–S75, 1991
6. Osei K, Cottrell DA, Henry ML, Tesi RJ, Ferguson RM, O'Dorisio TM: Insulin insensitivity and glucose effectiveness in type 1 diabetic allograft pancreas recipients. *Trans Proc* 24:828–830, 1992
7. Boden G, Chen X, Ruiz J, Heifets M, Morris M, Badosa F: Insulin receptor down-regulation and impaired antilipolytic action of insulin in diabetic patients after pancreas/kidney transplantation. *J Clin Endocrinol Metab* 78:657–663, 1994
8. Diem P, Abid M, Redmon JB, Sutherland DER, Robertson RP: Systemic venous drainage of pancreas allografts as independent cause of hyperinsulinemia in type 1 diabetic recipients. *Diabetes* 39:534–540, 1990
9. McMahon M, Gerich J, Rizza R: Effects of glucocorticoids on carbohydrate metabolism. *Diabetes Metab Rev* 4:17–30, 1988
10. Homan M, Nicholson CP, Katz H, Perkins J, Haymond M, Jensen M, Butler P, Rizza R: Effects of chronic systemic insulin delivery on insulin action in dogs. *Diabetologia* 34:702–708, 1991
11. Rizza RA, Mandarino LJ, Gerich JE: Dose-response characteristics for effects of insulin on production and utilization of glucose in man. *Am J Physiol* 240:E630–E639, 1981
12. Consoli A, Nurjhan N, Capani F, Gerich J: Predominant role of gluconeogenesis in increased hepatic glucose production in NIDDM. *Diabetes* 38:550–557, 1989
13. Firth RG, Bell PM, Marsh HM, Hansen I, Rizza RA: Postprandial hyperglycemia in patients with non-insulin-dependent diabetes mellitus: role of hepatic and extra hepatic tissues. *J Clin Invest* 77:1525–1532, 1986
14. Seaquist ER, Pyzdrowski K, Moran A, Teuscher AU, Robertson RP: Insulin-mediated and glucose-mediated glucose uptake following hemipancreatectomy in healthy human donors. *Diabetologia* 37:1036–1043, 1994
15. Somogyi M: Determination of blood sugar. *J Biol Chem* 160:69–73, 1945
16. Teuscher AU, Seaquist ER, Robertson RP: Diminished insulin secretory reserve in diabetic pancreas transplant and nondiabetic kidney transplant recipients. *Diabetes* 43:593–598, 1994
17. Steele R, Wall JS, De Bodo RC, Altszuler N: Measurement of size and turnover rate of body glucose pool by the isotope dilution method. *Am J Physiol* 187:15–25, 1956
18. De Bodo RC, Steele R, Altszuler N, Dunn A, Bishop JS: On the hormonal regulation of carbohydrate metabolism: studies with C^{14} glucose. *Rec Prog Horm Res* 19:445–448, 1963
19. Eigler N, Sacca L, Sherwin RS: Synergistic interactions of physiologic increments of glucagon, epinephrine, and cortisol in the dog. *J Clin Invest* 63:114–123, 1979
20. Horwitz DL, Starr JI, Mako ME, Blackard WG, Rubenstein AH: Proinsulin, insulin, and C-peptide concentrations in human portal and peripheral blood. *J Clin Invest* 55:1278–1283, 1975
21. Bell PM, Firth RG, Rizza RA: Assessment of insulin action in insulin-dependent diabetes mellitus using [6^{14}C]glucose, [3^{3}H]glucose and [2^{3}H]glucose. *J Clin Invest* 78:1479–1486, 1986
22. Williamson JR, Kreisberg RA, Felts PW: Mechanism for the stimulation of gluconeogenesis by fatty acids in perfused rat liver. *Proc Natl Acad Sci USA* 56:247–254, 1966
23. Katz H, Homan M, Velosa J, Robertson RP, Rizza R: Effects of pancreas transplantation on postprandial glucose metabolism. *N Engl J Med* 325:1278–1283, 1991
24. Baron AD, Kolterman OG, Bell J, Mandarino LJ, Olefsky JM: Rates of non-insulin-mediated glucose uptake are elevated in type II diabetic subjects. *J Clin Invest* 76:1782–1788, 1985