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IMPAIRED INSULIN RESPONSE AFTER ORAL BUT NOT INTRAVENOUS GLUCOSE IN HEART- AND LIVER-TRANSPLANT RECIPIENTS

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Background. **The prevalence of diabetes is high after transplantation. We hypothesized that liver transplantation induces additional alterations of glucose homeostasis because of liver denervation.**

Methods. **Nondiabetic patients with a heart (n=9) or liver (n=9) transplant and healthy subjects (n=8) were assessed using a two-step hyperglycemic clamp (7.5 and 10 mmol/L). Thereafter, an oral glucose load (0.65 g/kg fat free mass) was administered while glucose was clamped at 10 mmol/L. Glucose appearance from the gut was calculated as the difference between glucose appearance (6,6 $^2H_2$ glucose) and exogenous glucose infusion. Plasma insulin, glucagon-like peptide (GLP)-1 and gastric inhibitory polypeptide (GIP) concentrations were compared after intravenous and oral glucose.**

Results. **After oral glucose, the glucose appearance from the gut was increased 52% and 81% in liver- and heart-transplant recipients ($P<0.05$). First-pass splanchnic glucose uptake was reduced by 39% in liver-transplant and 64% in heart-transplant patients ($P<0.05$). After oral but not intravenous glucose, there was an impairment of insulin secretion in both transplant groups relative to the controls. Plasma concentrations of GIP and GLP-1 increased similarly in all three groups after oral glucose.**

Conclusions. **First-pass hepatic glucose extraction is decreased after heart and liver transplant. Insulin secretion elicited by oral, but not intravenous glucose, is significantly reduced in both groups of patients. There was no difference between liver- and heart-transplant recipients, indicating that hepatic denervation was not involved. These data suggest an impairment in the β-cell response to neural factors or incretin hormones secondary to immunosuppressive treatment.**

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Diabetes mellitus is frequently encountered in patients having received a solid-organ transplant, with an estimated prevalence of 10% to 30% (*1*). Because rates of posttransplantation diabetes mellitus are similar among liver-, heart-, and kidney-transplant recipients, it has been suggested that they share a common etiology. Thus, the possibility that posttransplantation diabetes may be secondary to an impaired insulin secretion or a decreased insulin sensitivity induced by drugs administered to prevent graft rejection has been raised by several authors (*1*, *2*). In particular, glucocorticoid hormones are well known to decrease insulin secretion and to

produce insulin resistance, and it has been observed that early steroid withdrawal markedly reduced the incidence of posttransplantation diabetes mellitus (*3–5*). Although less well studied, the calcineurin inhibitors cyclosporin and tacrolimus have been shown to decrease insulin synthesis or secretion by islet cells in several studies (*6*, *7*). Insulin resistance has also been described in patients receiving these drugs, but the mechanisms involved remain unknown (*1*).

Although diabetes induced by immunosuppressive drugs would affect most solid–organ-transplant recipients equally, it is possible that glucose metabolism may be altered by factors specific to the type of transplant. Studies performed in dogs have shown that portal versus intravenous (IV) glucose delivery stimulates hepatic glucose uptake and insulin secretion by pancreatic beta cells (*8*). These effects involve activation of portal glucose sensors and neural signaling pathways. It has therefore been suggested that hepatic denervation after liver transplant may lead to alterations of hepatic glycoregulatory functions (*9*). Studies performed on liver-transplant recipients, however, reported only a modest decrease (*10*) or no alteration (*11*) of fasting endogenous glucose production, a normal postprandial hepatic glycogen synthesis (*12*) and a normal stimulation of glucose production during exercise (*13*). These observations together suggest that hepatic glycoregulatory function remains nearly normal after liver transplantation. It remains possible, however, that alterations of liver-borne neural signals interfere with insulin secretion after liver transplantation. It is also possible that defective hepatic glucose uptake in liver-transplant patients was missed because the patients studied had marked postprandial hyperglycemia.

Insulin secretion in nondiabetic transplant recipients has not been well characterized. There is evidence from one study that insulin secretion in response to IV glucose is relatively normal in liver-transplant recipients (*10*). However, in another study, it appeared that insulin secretion in response to a glucose meal was blunted in patients with liver transplants (*11*). The insulin response to oral glucose is augmented beyond the stimulation of hyperglycemia by neural signals and the actions of gastrointestinal hormones such as glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic polypeptide (GIP) (*14*). Whether this enhancement of insulin secretion, termed the incretin effect, is impaired in patients following organ transplantation has not been investigated. We therefore assessed whole-body glucose use, splanchnic glucose uptake, and insulin secretion during IV glucose infusion and after oral glucose at comparable levels of hyperglycemia in groups of liver- and heart-transplant patients and healthy control subjects.

METHODS

Nine patients having had a liver transplantation, and eight patients having had a heart transplant in the previous 1 to 3 years were recruited for this study. Their anthropometric characteristics and current drug regimens are shown in Table 1. All patients were rejection free and had a fasting plasma glucose concentration less than 6.0 mmol/L. Liver transplant patients had all received an oral glucose tolerance test, and three of nine had impaired glucose tolerance. Immunosuppressive treatment and clinical data are shown in Tables 1 and 2. Kidney function was modestly impaired in both groups of patients, but liver-function tests did not indicate significant hepatic abnormalities. Ciclosporin concentrations in blood were similar in heart-transplant patients and in liver-transplant patients receiving this drug. Three liver-transplant recipients and four heart-transplant recipients were treated with antihypertensive drugs at the time of the study. Eight healthy subjects, with nondiabetic glucose tolerance, were recruited as a control group.

Experimental Protocol

All participants were studied on a single occasion during a 300 minute two-step hyperglycemic clamp with an oral glucose challenge. Subjects presented to the metabolic investigation laboratory in the morning after an overnight fast and signed an informed consent document approved by the institutional review board. After measurement of height and weight, a venous cannula was inserted into an antecubital vein of one arm and used for dextrose infusion. A second cannula was inserted into a wrist vein of the other arm for the periodic collection of blood samples. This hand was placed in a thermostabilized box heated to 50°C to achieve partial arterialization of venous blood.

After 1 hour of rest to obtain stable metabolic conditions, a basal blood sample was obtained (time 0 minutes), and a variable infusion of 20% dextrose, labeled with 1.5% 6,6 $^2H_2$ glucose (MassTrace, Worcester, MA), was started. Plasma glucose was clamped at 7.5 mmol/L for 1 hour (time 0–60 minutes) and increased to 10 mmol/L for a second hour (time 60–120 minutes). Blood samples were obtained every 2 minutes during the initial 10 minutes of glucose infusion and subsequently at 15 to 30 minute intervals (*15*). At time 120 minutes, an oral glucose load (0.65 g/kg fat free mass, dissolved in 250 mL lemon-flavored water) was administered. For the next 3 hours, plasma glucose concentrations were maintained at 10 mmol/L by variable infusion of glucose.

Analytical Procedures

Plasma glucose concentrations were measured by the glucose oxidase method using a Beckman glucose analyzer II (Beckman Instruments, Brea, CA). Plasma insulin, glucagon, and C-peptide concentrations were measured by radioimmunoassay (RIA), using kits from Linco (St. Charles, MO). GLP-1-ir was measured by RIA using antiserum 89390 (kindly provided by Dr. Jens Holst, Paanum Institute, Copenhagen, Denmark) using plasma extracted with 70% ethanol (*16*). The antiserum was diluted 1:20,000, and 50 μL was added to each assay tube. Synthetic GLP-1[7–36] amide (Pensinsula Laboratories, San Carlos, CA) was used for standards and iodinated for use as a tracer, and a double antibody technique was used to separate bound from free peptide. The recovery of standard peptide added to plasma and extracted in ethanol was greater than 80%, the intra- and interassay coefficients of variation for this RIA were 6% and 8%, respectively, and the minimum detectable concentration of GLP-1[7–36] NH_2 was 1.17 pM. GIP was determined by RIA of unextracted

TABLE 1. Subjects characteristics

	Sex	Age (yrs)	Weight (kg)	Body mass index (kg/m^2)	Prednisone (mg/day) (no. of patient treated)	Ciclosporin (mg/day) (no. of patients treated)	Tacrolimus (mg/day) (no. of patients treated)
Liver transplant recipients	3F/6M	41.6±4.8	72±3	24.2±1.4	6.2±0.8 (5)	208±22 (6)	4.7±0.4 (3)
Heart transplant recipients	1F/7M	40.9±6.3	79.5±8	25.0±1.2	5 (1)	147±21 (8)	
Healthy controls	3F/5M	38.2±7.1	74±15	25.9±2.4			

TABLE 2. Clinical data [a]

	Blood ciclosporin (μg/L)	Blood tacrolimus (μg/L)	Creatinine (μmol/min) (normal range 44–80)	ASAT (mU/L) (normal range 9–32)	ALAT (mU/L) (normal range 9–36)
Liver transplant recipients	177 (96–248)	7 (3–14)	152 (94–256)	32 (17–55)	41 (17–78)
Heart transplant recipients	152 (78–212)		128 (80–204)	36 (22–56)	46 (28–68)

[a] Data are expressed as mean (range).

plasma (*17*). Synthetic GIP was used for standards and iodinated as a tracer, and a double antibody separation was used. Inter- and intra-assay coefficient of variation (CV) was 10% and 4% for this assay. Plasma 6,6 $^2H_2$ glucose was analyzed by gas-chromatography–mass spectrometry as previously described (*18*).

Calculations

Whole-body glucose appearance and disappearance were calculated from plasma 6,6 $^2H_2$ glucose enrichment using hot infusate equations (*19*). The difference between whole-body glucose appearance and glucose infusion may theoretically represent either endogenous glucose production or the systemic appearance of glucose from the gut. Because rates of whole-body glucose appearance was not different from the glucose infusion rate during the initial 2 hours of hyperglycemic clamp alone, we assumed that endogenous glucose production was completely suppressed after oral glucose. This assumption is supported by hyperinsulinemic-clamp studies showing complete inhibition of glucose output at insulin concentrations lower than those observed in this study (*10*). Therefore, we inferred that after glucose ingestion, the difference between whole-body glucose appearance and the amount of glucose infused was equal to the systemic appearance of glucose absorbed from the gut. First-pass splanchnic glucose uptake was then calculated by subtracting gut glucose appearance from the glucose load consumed, assuming near complete absorption of the load after 3 hours (*20*). The first-phase insulin secretion was calculated as the incremental area under the insulin curve over the 10 minutes after the initiation of the glucose infusion. Second-phase insulin secretion and the insulin response to oral glucose were also calculated as the areas under the curve from 10 to 120 and 120 to 300 minutes, respectively. To obtain an index of the incretin effect (e.g., the effect of nonglycemic factors to augment insulin secretion after glucose ingestion), the following calculation was performed: [(mean insulin 120–130 min)−(mean insulin 105–120 min)×100]/(mean insulin 120–130).

Statistical Analysis

Results are presented as the mean±SEM. Comparison between groups (i.e., liver vs. heart transplant and transplant patients with steroids vs. transplant patients without steroids vs. healthy subjects) were done by two-way analysis of variance and paired *t* tests with Bonferroni's adjustment.

RESULTS

Characteristics of the research subjects are shown in Table 1. Only 5 of the 17 transplant recipients were taking glucocorticoids as part of their immunosuppressive regimen.

Plasma glucose concentrations were similar in the liver- and heart-transplant recipients and control subjects both in the fasting state and during the administration of IV and oral glucose (Fig. 1) (Table 2). The rate of whole-body glucose disappearance and glucose infusions tended to be lower in both groups of transplant patients during the IV infusion of glucose, but this difference was not statistically significant. After oral glucose, these parameters increased markedly in all three groups of subjects, but glucose disappearance and the glucose infusion rate were significantly lower in both liver- and heart-transplant recipients compared with healthy controls ($P<0.05$ for liver-transplant patients, $P<0.01$ for heart-transplant patients) (Fig. 1). The systemic appearance of oral glucose is shown in Figure 2. The oral glucose loads amounted to 468±10, 475±21, and 467±5 mg/kg in liver- and heart-transplant recipients and healthy controls, respectively. Cumulative systemic appearance of oral glucose over 3 hours was 311±27 and 371±99 mg/kg per 3 hours in liver- and heart-transplant recipients versus 204±40 mg/kg per 3 hours ($P<0.05$) in healthy controls. First-pass splanchnic glucose uptakes over the same period were 164±18, 96±96, and 264±35 mg/kg per 3 hours, which corresponded to 41%, 39%, and 60% of the load in liver, heart transplant, and healthy controls, respectively ($P<0.05$ liver and heart transplant vs. healthy controls).

The plasma insulin and C-peptide concentrations observed during the initial 2 hours of the two-step hyperglycemic clamp are shown in Figure 3 and Table 2. Compared with healthy subjects, transplant patients had relatively normal insulin secretion in response to IV glucose, with an intact first-phase response, and second-phase insulin secretion that increased with increasing glycemia (Table 3). In both subgroups of transplant patients, fasting plasma glucagon concentrations were increased, but the difference reached statistical significance only during the second plateau of glycemia. After oral glucose administration, plasma insulin concentrations increased markedly in healthy subjects even though plasma glucose concentrations were maintained at a nearly constant glycemia of 10 mmol/L (Fig. 4). The incretin effect amounted to 175±26%. In comparison, the incretin effect after oral glucose was blunted in both the hepatic (93±16%) and cardiac (72±10%) transplant patients ($P<0.05$ in both cases) (Table 4). When postprandial insulin concentrations in the transplant recipients taking glucocorticoids were compared with those in patients on steroid-free regimens, there was no significant difference. Plasma C-peptide concentrations tended to be higher in basal conditions and during IV glucose administration in the transplant subjects. After glucose ingestion, plasma C-peptide increased in all three groups of subjects, but its peak tended to be delayed in liver- and heart-transplant patients. In both subgroups of transplant patients, fasting plasma glucagon concentrations were not significantly increased relative to the controls. Glucagon levels decreased in all three groups of subjects but remained higher in the transplant patients during the second plateau of glycemia (70±6, 79±14, and 48±5 ng/L for the liver-transplant, heart-transplant, and control subjects, respectively).

Figure 5 shows plasma GLP-1 and GIP concentrations during IV glucose and after oral glucose when glycemia was maintained constant at 10 mmol/L by exogenous insulin infusion. Basal plasma GLP-1 concentrations were higher in transplant patients compared with controls, but the differ-

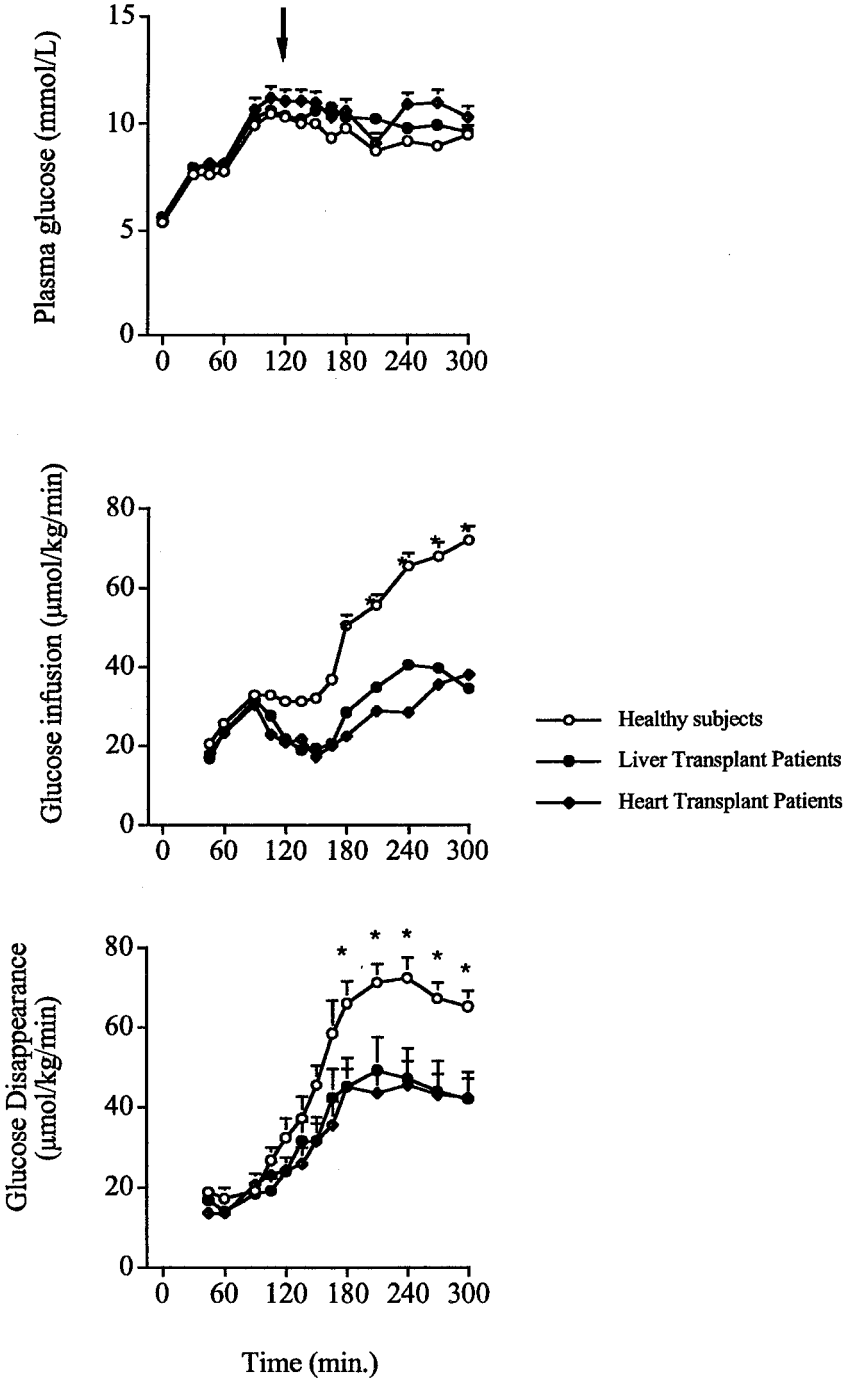

FIGURE 1. Plasma glucose concentrations, exogenous glucose infusion rate, and whole-body glucose disappearance during a two-step hyperglycemic clamp (time 0–120 minutes) and oral glucose infusion with plasma glucose clamped at about 10 mmol/L (time 120–300 minutes). (*arrow*) oral glucose administration. *$P<0.05$ or less, healthy subjects vs. heart and liver transplant patients.

ence did not reach statistical significance (44.1±7.8, 44.8±9.5, and 25.5±3.6 pmol/L for liver-transplant, heart-transplant, and controls, respectively; not significant). Plasma GLP-1 increased in all three groups following glucose ingestion, and there was no difference among the responses on the basis of comparison of the area under the curve. Similarly, plasma GIP levels were comparable before glucose ingestion (101.4±12.7, and 116.6±32.6, 89.3±18.8 pmol/L for liver transplant, heart transplant, and controls, respectively), and the postprandial responses were similar in all three groups of subjects (Fig. 5).

DISCUSSION

Both hepatic glucose metabolism and insulin secretion may be disturbed as a result of liver denervation after liver transplantation. We therefore compared first-pass glucose uptake and insulin secretion after oral glucose in groups of liver- and heart-transplant recipients. Because these two processes are highly dependent on ambient glucose concentration, they were assessed while glycemia was maintained constant by exogenous glucose infusion. Patients with heart transplant and healthy age, sex, and weight-matched sub-

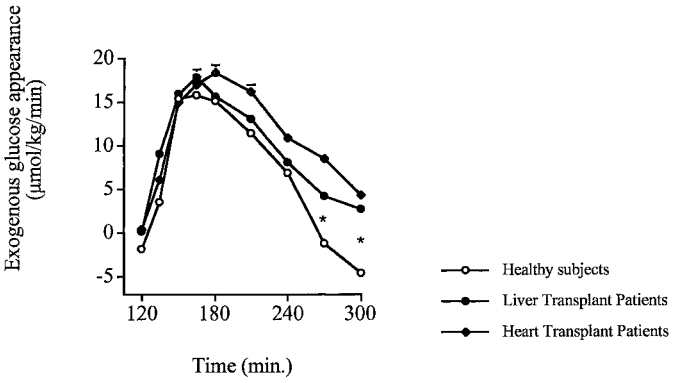

FIGURE 2. Exogenous glucose appearance from the gut after oral glucose ingestion.

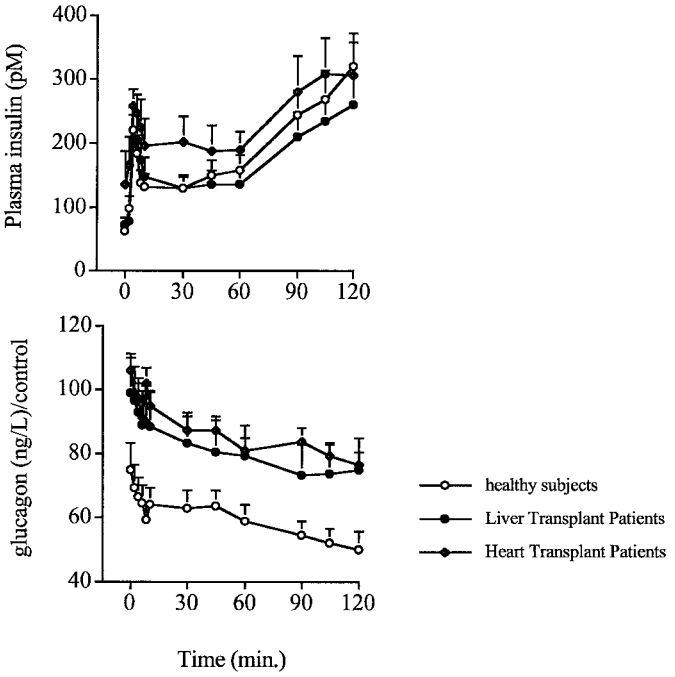

FIGURE 3. Plasma insulin and glucagon concentrations during the two-step hyperglycemic clamp without oral glucose (initial 2 hours of the experimental protocol).

jects were also studied to evaluate the effects of drugs administered to prevent graft rejection. The major finding from this study was that cardiac- and liver-transplant patients with normal glucose tolerance had normal insulin responses to IV glucose but a significant impairment in the augmentation of insulin secretion in response to oral glucose compared with the controls. This impairment was similar in both groups of transplant recipients and suggests a common etiology rather than a cause specific to hepatic denervation.

The transplant patients had been receiving the same treatment for at least 3 months, and all were taking calcineurin inhibitors (ciclosporin in all 8 heart-transplant recipients and in 6 liver transplant recipients, tacrolimus in 3 liver-transplant recipients). Separate analyses showed comparable insulin responses, both to IV and oral glucose, in patients receiving these two drugs. Five patients received glucocorticoid treatment in addition to calcineurin inhibitors. This subgroup of patients did not otherwise differ from the other study patients. In particular, they had similar body mass index (23.6 ± 1.2 kg/m^2) as other patients. Because it has been suggested that glucocorticoids may be primarily involved in the metabolic complications occurring after transplantation, this subgroup of patients was also analyzed separately. The acute insulin response to IV glucose and the insulin concentrations attained during the two-step hyperglycemic clamp were comparable in patients receiving steroids and healthy control subjects.

In contrast with this nearly normal insulin response to IV glucose, the response elicited by oral glucose was unambiguously decreased in transplant patients. A very similar pattern was observed in both liver- and heart-transplant recipients and was not affected by the presence of glucocorticoids in the drug regimen. The mechanisms responsible for this decrease in oral–glucose-induced insulin secretion remain open to discussion. Our first hypothesis was that calcineurin inhibitors may have impaired the synthesis or release of glucoincretin hormones from the gut. However, measurement of plasma GLP-1 and GIP, the two major incretins presently known, showed that the plasma concentrations of these two hormones were not depressed after transplantation. GLP-1 levels were even somewhat increased in transplant patients. Furthermore, both hormones rose appropriately after glucose ingestion.

Because we did not observe impaired gut hormone secretion in the transplant subjects nor elicited a clear reason for these patients to have uniformly impaired neurally mediated insulin secretion, we sought other common features of both transplant groups. All of these patients were treated with calcineurin inhibitors, which are known to affect pancreatic endocrine cells and so are implicated in the abnormal insulin response to oral glucose. This inference is supported by a previous observation made in transplant patients (i.e., that cyclosporin did not inhibit the release of insulin in response to IV glucose but did decrease insulin secretion in response to arginine), indicating that this medication differentially affects the response to various secretagogues (*21*). It is therefore plausible that drugs of this class also specifically affect the intracellular signaling activated by GLP-1 and GIP, pathways that are thought to be primarily dependent on the generation of cyclic adenosine monophosphate and stimulation of protein kinase A (*22*). Alternatively, it is known that enteral feeding elicits activation of neural pathways, which in turn modulate insulin secretion (*23*). Activation of the parasympathetic limb of the autonomic nervous system is recognized to be specifically involved in a postprandial potentiation of insulin secretion (*23*), and it may be that calcineurin inhibitors attenuate this process. Although we cannot distinguish between these possibilities through this study, it will be important to pursue the specific in vivo effects of calcineurin inhibitors on insulin secretion because our data indicate that even patients with normal glucose tolerance tests may be affected. This suggests that the effects of immunosuppressive drugs may be one of the proximal events in the development of posttransplant diabetes.

We considered the possibility that increased hepatic insulin extraction contributed to lower postprandial insulin concentrations in liver- and cardiac-transplant recipients. Although insulin clearance can be calculated using plasma

TABLE 3. Plasma glucose and insulin concentrations during the hyperglycemic clamp without oral glucose

	Healthy subjects	Liver-transplant recipients	Heart-transplant recipients
Fasting plasma insulin (pmol/L)	61±8	71±12	134±53
Fasting plasma glucose (mmol/L)	5.3±0.1	5.5±0.1	5.6±0.3
Acute insulin response over the initial 10 min (pmol/L) 10 min	942±193	882±198	894±176
Plasma insulin at time 45–60 min	152±24	135±21	187±32
Plasma glucose at time 45–60 min	7.7±0.2	8.1±0.1	7.7±0.2
Plasma insulin at time 105–120 min	293±48	246±42	306±54
Plasma glucose at time 105–120 min	10.3±0.3	11.0±0.5	10.2±0.2

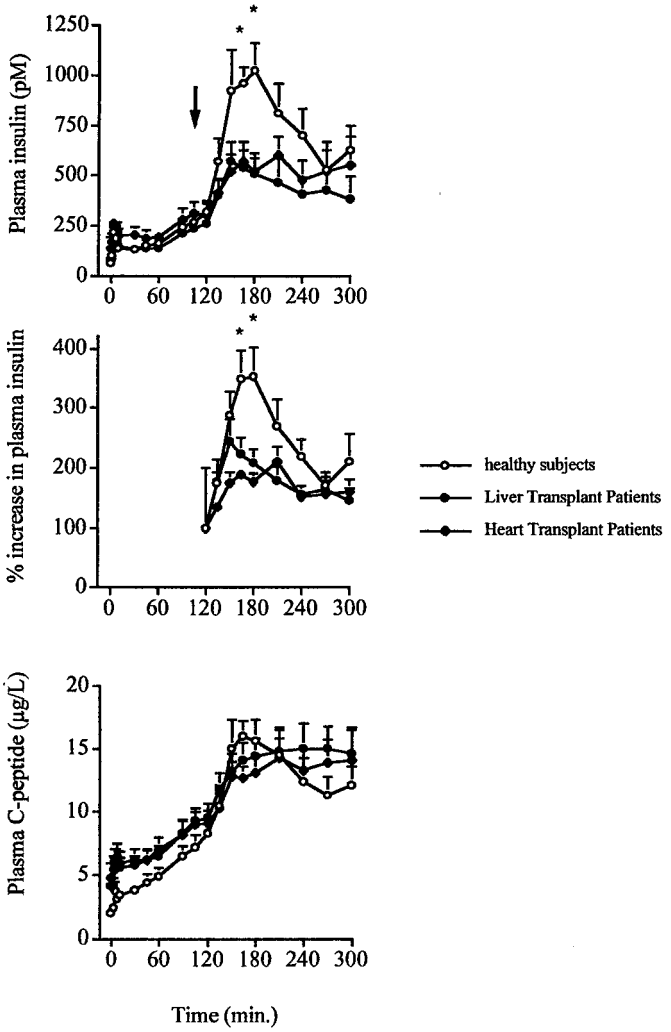

FIGURE 4. Plasma insulin concentrations observed during intravenous glucose (time 0–120 minutes) and after oral glucose with plasma glucose concentration clamped at 10 mmol/L (time 120–300 minutes). (top) absolute insulin concentrations; (middle) insulin concentration expressed as a percent of the values measured at 120 minutes; (bottom) plasma C-peptide concentrations. (*arrow*) oral glucose administration.

TABLE 4. Insulin secretion elicited by oral glucose

	Plasma glucose concentration (mmol/L)	Insulin area under the curve (nmol/L)-3h
Healthy subjects	9.4±0.3	132±21
Liver-transplant recipients	10.4±0.5	80±17[a]
Heart-transplant recipients	10.1±0.2	94±18[a]

[a] *P* vs. healthy subjects.

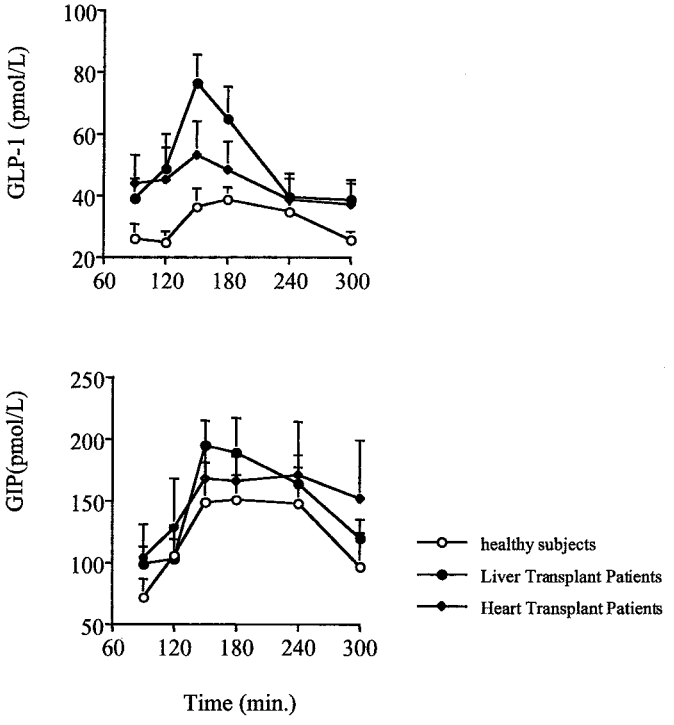

FIGURE 5. Plasma glucagon-like-peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) after oral glucose administration.

concentrations of C-peptide, in the case of the current study, this is problematic. The pattern of C-peptide release over time, with increased basal concentrations, and a delayed widened peak after oral glucose suggest that plasma C-peptide kinetics were altered in transplant patients, likely because of mild impairment of renal function secondary to immunosuppressant drugs. Christiansen and colleagues (*24*) have previously shown that calculation of insulin secretion rates and insulin clearance in patients following solid-organ transplant requires knowledge of individual C-peptide kinetics, and we did not obtain these measures in this group of subjects. Although we cannot exclude the possibility that changes in clearance contributed to some of the alterations in insulin levels observed in transplant patients, our observation of a delayed and lower C-peptide peak is consistent with

the conclusion that insulin secretion was indeed reduced after transplantation.

Finally, we cannot discard the possibility that calcineurin inhibitors more broadly impair β-cell insulin synthesis or secretion and that these effects were not apparent during the relatively modest challenge provided by IV hyperglycemia alone. It is possible that the ingestion of oral glucose during the hyperglycemic clamp, when the combined effects of neural, incretin and glycemic stimuli act on the β cell, unmasks a more general defect in insulin secretion in the transplant patients. However, this does not seem likely to us because other sensitive markers of β-cell function, such as first-phase insulin release to IV glucose, were not impaired in the transplant subjects. Therefore, we think our data are best explained by a specific defect in insulin secretion in response to ingested glucose in recipients of solid-organ transplant.

To evaluate first-pass hepatic glucose uptake, the systemic appearance of oral glucose was calculated by subtracting IV glucose infusion rates from whole-body glucose appearance. This procedure has been used previously in healthy and insulin-resistant humans during clamp studies and has been adequately validated (*25*, *26*). Oral glucose appearance showed a similar overall pattern in both groups of transplant patients and in healthy controls, suggesting that neither liver denervation nor drugs markedly altered gastric motility and glucose absorption rates. The total amount of oral glucose that reached the systemic circulation was significantly lower in heart- and liver-transplant patients compared with healthy controls. Consequently, the calculated first-pass glucose extraction was decreased in transplant patients. This decrease could not be ascribed to liver denervation, however, because no difference was observed between liver- and heart-transplant recipients. This inhibition of first-pass glucose uptake is probably best explained by the decreased postprandial insulin release occurring as a result of administration of calcineurin inhibitors.

In summary, these results indicate that the regulation of postprandial insulin secretion is abnormal in glucose-tolerant patients following heart or liver transplant. Our hypothesis is that this effect can be attributed to calcineurin inhibitors that may impair the incretin effect either by actions exerted at the level of pancreatic β cells or through effects exerted at the level of the autonomic nervous system. Further studies will be required to delineate the mechanisms involved. These results were obtained in nondiabetic patients, and the sample size was too small to evaluate whether these alterations of postprandial insulin secretion are directly related to glucose tolerance or family history of diabetes. At this point, we can only speculate on the clinical implications of the present observations. We propose that in solid–organ- transplant recipients, impaired secretion of insulin after oral glucose is not sufficient to lead to the development of hyperglycemia by itself but may represent an additional risk factor for diabetes in transplant patients. Calcineurin inhibitors appear involved in these defects, and, therefore, it will be of importance to evaluate whether the use of other classes of drugs such as mycophenolate mofetil would be advantageous in this regard. Future studies will also be required to evaluate the potential use of GLP-1 agonists to stimulate postprandial insulin secretion in post-transplant diabetes mellitus.

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