Original Article

Intrahepatic Transplanted Islets in Humans Secrete Insulin in a Coordinate Pulsatile Manner Directly Into the Liver

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Intrahepatic islet transplantation is an experimental therapy for type 1 diabetes. In the present studies, we sought to address the following questions: *1*) In humans, do intrahepatic transplanted islets reestablish coordinated puslatile insulin secretion? and *2*) To what extent is insulin secreted by intrahepatic transplanted islets delivered to the hepatic sinusoids (therefore effectively restoring a portal mode of insulin delivery) versus delivered to the hepatic central vein (therefore effectively providing a systemic form of insulin delivery)? To address the first question, we examined insulin concentration profiles in the overnight fasting state and during a hyperglycemic clamp (~150 mg/dl) in 10 recipients of islet transplants and 10 control subjects. To address the second question, we measured first-pass hepatic insulin clearance in two recipients of islet autografts after pancreatectomy for pancreatitis versus five control subjects by direct catheterization of the hepatic vein. We report that coordinate pulsatile insulin secretion is reestablished in islet transplant recipients and that glucose-mediated stimulation of insulin secretion is accomplished by amplification of insulin pulse mass. Direct hepatic catheterization studies revealed that intrahepatic islets in humans do deliver insulin directly to the hepatic sinusoid because ~80% of the insulin is extracted during first pass. In conclusion, intrahepatic islet transplantation effectively restores the liver to pulsatile insulin delivery.
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Type 1 diabetes is characterized by a marked deficit in β-cell mass, causing insufficient insulin secretion (1–3). Conventional insulin therapy with intermittent injections or pump delivery accomplishes imperfect glycemic control with attendant risks of hypoglycemia and microvascular complications as well as considerable personal inconvenience (4). Thus, β-cell replacement is an attractive potential therapy for type 1 diabetes. Pancreas transplantation is frequently performed with kidney transplantation and allows long-standing insulin independence in most patients (5). However, whole-pancreas transplantation carries significant surgical morbidities and finite mortality risks. Intrahepatic islet transplantation via the portal vein is a relatively safe procedure and can accomplish insulin independence for 3–5 years (6,7).

Why intrahepatic islets in people with type 1 diabetes sustain insulin independence for only ~3–5 years remains unknown (7). Proposed hypotheses are that the intrahepatic environment is toxic as a consequence of exposure of islets to high concentrations of immunosuppressive drugs (first pass) and/or relatively low oxygen concentrations (6). Potential advantages of the intrahepatic route of islet transplantation include delivery of insulin directly to the liver. In health, insulin is secreted into the portal-venous circulation to the liver, which extracts ~80% during the first passage. Thus, the physiological balance between hepatic and extrahepatic insulin exposure requires portal delivery of insulin (8–10). Normally, ~70% of insulin secretion arises from distinct secretory bursts occurring at ~4- to 5-min intervals (11,12). Moreover, regulation of insulin secretion is predominantly accomplished by changes in insulin burst mass (12–14). For example, the amplitude of the resulting insulin oscillations in the portal vein is ~1,000 pmol/l in the basal state increasing to ~4,000 pmol/l after meal ingestion (15). The profile of this insulin concentration wavefront dictates the extent of hepatic insulin clearance in as much as the former is more effective than continuous insulin delivery in suppressing hepatic glucose output (10,16,17). Insulin signaling and insulin extraction by the liver may be optimized by a pulsatile mode of delivery.

Synchrony of high-frequency pulsatile insulin secretion is restored in transplanted intrahepatic islets in rats once the islets have been reinnervated (18). This finding is consistent with the hypothesis that although islets may serve as the pacemaker for pulsatile insulin secretion, functional neural networking is required for coordinate pulsatile insulin secretion (19). Whether intrahepatic transplanted islets in humans with type 1 diabetes recover coordinate high-frequency pulsatile insulin secretion is unknown.

Venous drainage of transplanted islets within hepatic lobules is also unknown. If venous drainage were directed into hepatic sinusoid, hepatocytes would be exposed to

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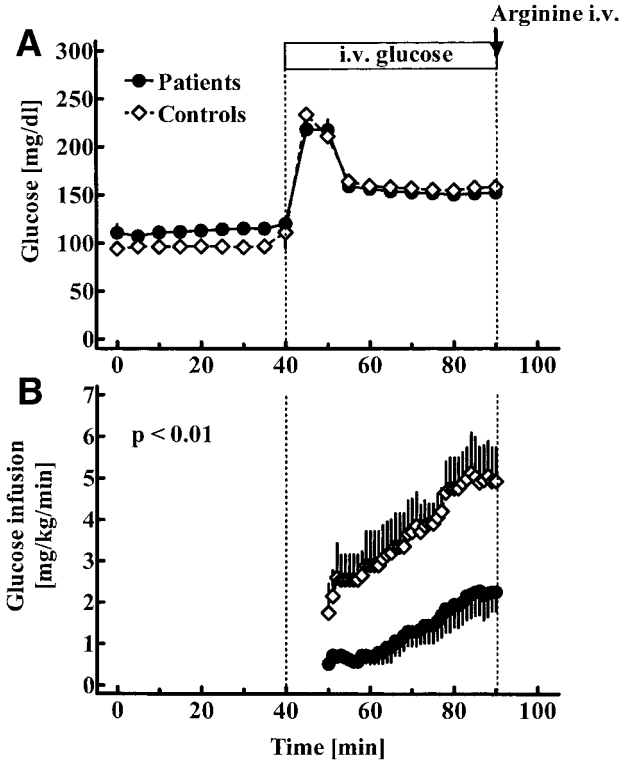

FIG. 1. Plasma concentrations of glucose (*A*) and glucose infusion rates (*B*) during basal conditions (0–40 min) and during a hyperglycemic clamp experiment (50–90 min) in 10 patients after islet transplantation and 10 healthy nondiabetic control subjects. Boluses of intravenous (i.v.) glucose (0.3 g/kg) and arginine (5 g) were administered at $t = 40$ and 90 min, respectively. Data are expressed as means ± SE.

first-pass insulin concentrations reproducing portal insulin delivery. Alternatively, if the venous effluent is via the central vein, insulin secretion by intrahepatic transplanted islets would be systemic rather than portal (20). This distinction may have implications with respect to both optimized hepatic insulin signaling (21) and normal hepatic insulin clearance (8,22,23).

The present studies addressed the following questions: *1*) Do intrahepatic transplanted islets in humans secrete insulin in a coordinate high-frequency pulsatile manner, and if so, is the extent of pulsatile secretion related to the overall insulin secretory capacity? and *2*) Do intrahepatic transplanted islets secrete insulin directly into the portal vein or the systemic circulation?

RESEARCH DESIGN AND METHODS

The studies were approved by the institutional review board at the University of Minnesota (protocol 1) and the Mayo Clinic (protocol 2). Written informed consent was obtained from all participants.

In protocol 1, 10 patients with type 1 diabetes after islet transplantation and 10 healthy nondiabetic control subjects were studied. All participants were studied after an overnight stay in the general clinical research center (GCRC) of the University of Minnesota. Pulsatile insulin secretion was measured in the basal fasting state and during a hyperglycemic clamp followed by an acute intravenous arginine (5-g) bolus. Pulsatile insulin secretion was determined by deconvolution analysis of 1-min insulin concentrations.

In protocol 2, two patients who had recently undergone autologous intrahepatic islet transplantation after total pancreatectomy for painful chronic pancreatitis were studied. Patients were studied over 40 min in the overnight fasting state with blood samples being drawn simultaneously from the hepatic vein and from the arterialized hand vein in 1-min intervals. Splanchnic plasma flow was assessed using the indocyanine green method, and hepatic first-pass insulin clearance was calculated as described previously (9,10). Five healthy nondiabetic individuals were studied as control subjects. Hepatic vein insulin concentration data in those individuals were previously reported (10).

Protocol 1

Ten patients with type 1 diabetes (three men, seven women, age 43.4 ± 4.1 years [means ± SD], BMI 21.9 ± 1.8 kg/m^2, and HbA_{1c} [A1C] 6.2 ± 0.4%) who had received islet transplantation and 10 healthy nondiabetic control subjects (three men, seven women, age 43.4 ± 7.7, and BMI 22.3 ± 1.5 kg/m^2) were studied. One patient had received islets from three organ donors, one from two organ donors, and seven from a single organ donor. The duration between the last islet transplantation and the study was 2.2 ± 1.2 years (range 0.81–4.8), and the mean number of islets transplanted was 484,476 ± 186,965 islet equivalents (range 294,000–867,000). All patients were treated with steroid-free immunosuppressive regimens. Five patients received a combination of tacrolimus, rapamycin, and mycophenolate mofetil; two patients received a combination of tacrolimus and rapamycin; one patient received mycophenolate mofetil and tacrolimus in combination; one patient was treated with everolimus, cyclosporin A, and myfortic in combination; and one patient received a combination of cyclosporin A and myfortic. None of the patients were treated with drugs for glucose control. Four patients required a variable rate intravenous insulin infusion to lower fasting glucose levels into the normal range before the study protocol, the infusion being discontinued at least 15 min before sampling.

Study procedures. The experiments were carried out in the morning after an overnight fast in the University of Minnesota GCRC. Two venous catheters were placed, one in each arm for blood sampling and for glucose and arginine administration, respectively. The sampling protocol was started after a rest period of 45 min. Venous blood samples were obtained at 1-min intervals during basal conditions ($t = 0–40$ min). At $t = 40$ min, an intravenous bolus of glucose (0.3 g/kg body wt) was administered over 1 min, and venous blood samples were collected after 2, 3, 4, 5, 7, and 10 min. At $t = 50$ min, a hyperglycemic clamp aiming at plasma glucose levels at ~150 mg/dl was started by a variable rate glucose infusion (20% glucose), adjusted on the basis of plasma glucose determinations every 5 min. Venous blood samples were collected during the hyperglycemic clamp at 1-min intervals from $t = 50–90$ min. At $t = 90$ min, the glucose infusion was discontinued, and an intravenous bolus of arginine (5 g) was administered. Venous blood samples were collected 2, 3, 4, 5, 6, 7, 8, 9, and 10 min after the arginine bolus.

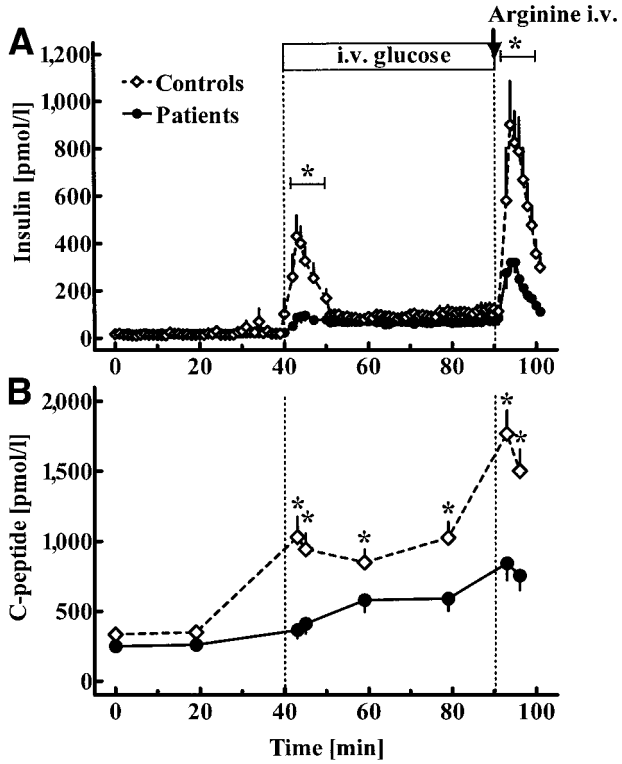

FIG. 2. Plasma concentrations of insulin (*A*) and C-peptide (*B*) during basal conditions (0–40 min) and during a hyperglycemic clamp experiment (50–90 min) in 10 patients after islet transplantation and 10 healthy nondiabetic control subjects. Boluses of intravenous (i.v.) glucose (0.3 g/kg) and arginine (5 g) were administered at $t = 40$ and 90 min, respectively. Data are expressed as means ± SE.

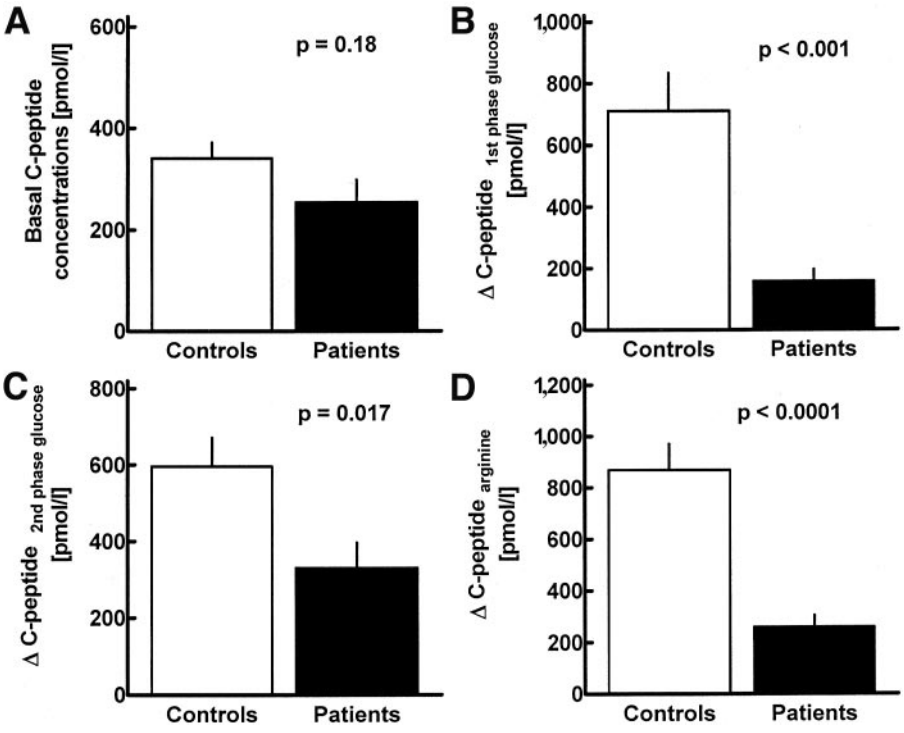

FIG. 3. Mean C-peptide plasma concentrations at baseline (*A*) and first-phase (*B*) and second-phase (*C*) C-peptide responses to intravenous glucose and to arginine (*D*) administration in 10 patients after islet transplantation and 10 healthy nondiabetic control subjects. Data are expressed as means ± SE.

Protocol 2

Two patients (both women), who had recently undergone autologous intrahepatic islet transplantation after total pancreatectomy for painful chronic pancreatitis were studied. One patient had developed diabetes after transplantation, whereas the other one had normal fasting glucose concentrations. The patients were 49 and 27 years old, 164 and 151 cm tall, and weighed 59 and 48 kg, respectively. BMIs were 22.0 and 21.1 kg/m^2, respectively. Fasting glucose concentrations were 95 and 244 mg/dl, and A1C levels were 3.8 and 10.4%, respectively. Five healthy individuals (two men and three women) were studied as control subjects (10). They were 32 ± 6 years old (means ± SD) and 167 ± 5 cm tall. The BMI was 24.9 ± 2.5 kg/m^2. Fasting glucose concentrations were 96 ± 6 mg/dl, and A1C levels were 4.8 ± 0.2%. None of the control

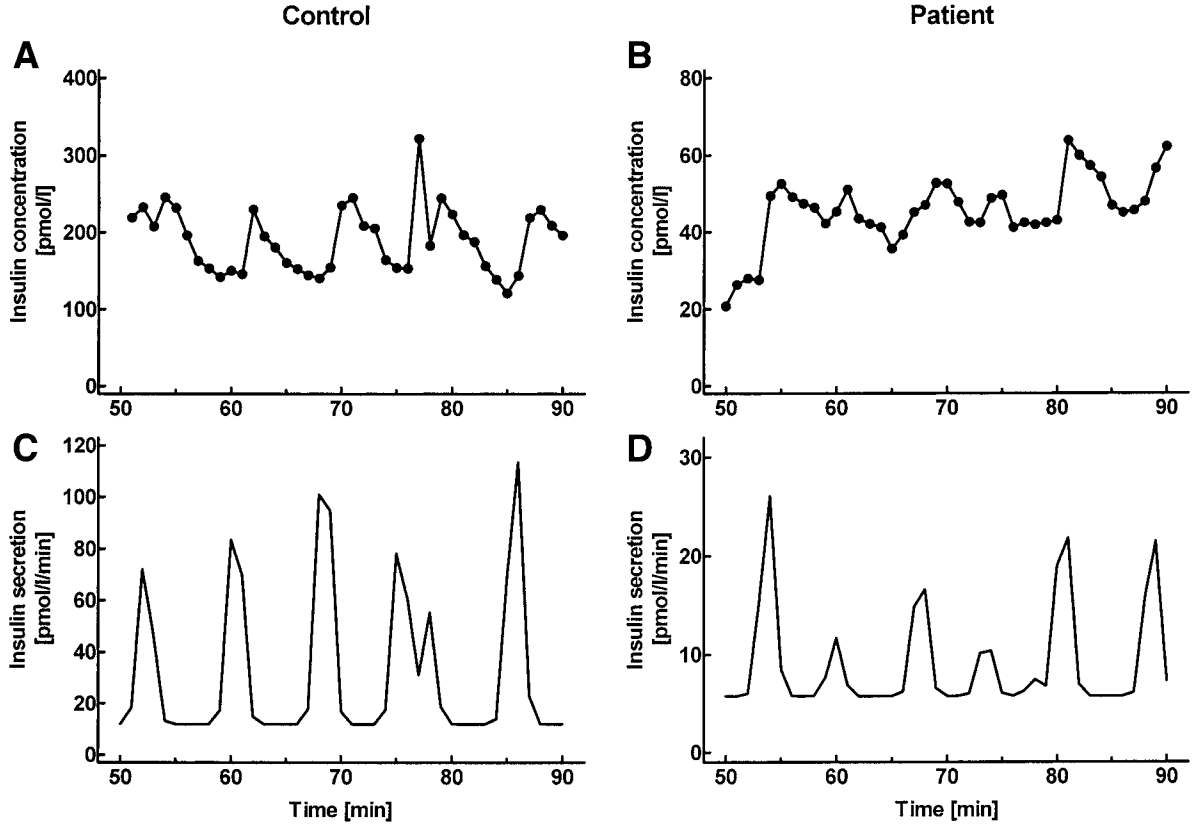

FIG. 4. Plasma concentrations of insulin (*A* and *B*) and corresponding insulin secretion rates (*C* and *D*) during a hyperglycemic clamp experiment in a patient after islet transplantation (*B* and *D*) and a nondiabetic control subject (*A* and *C*).

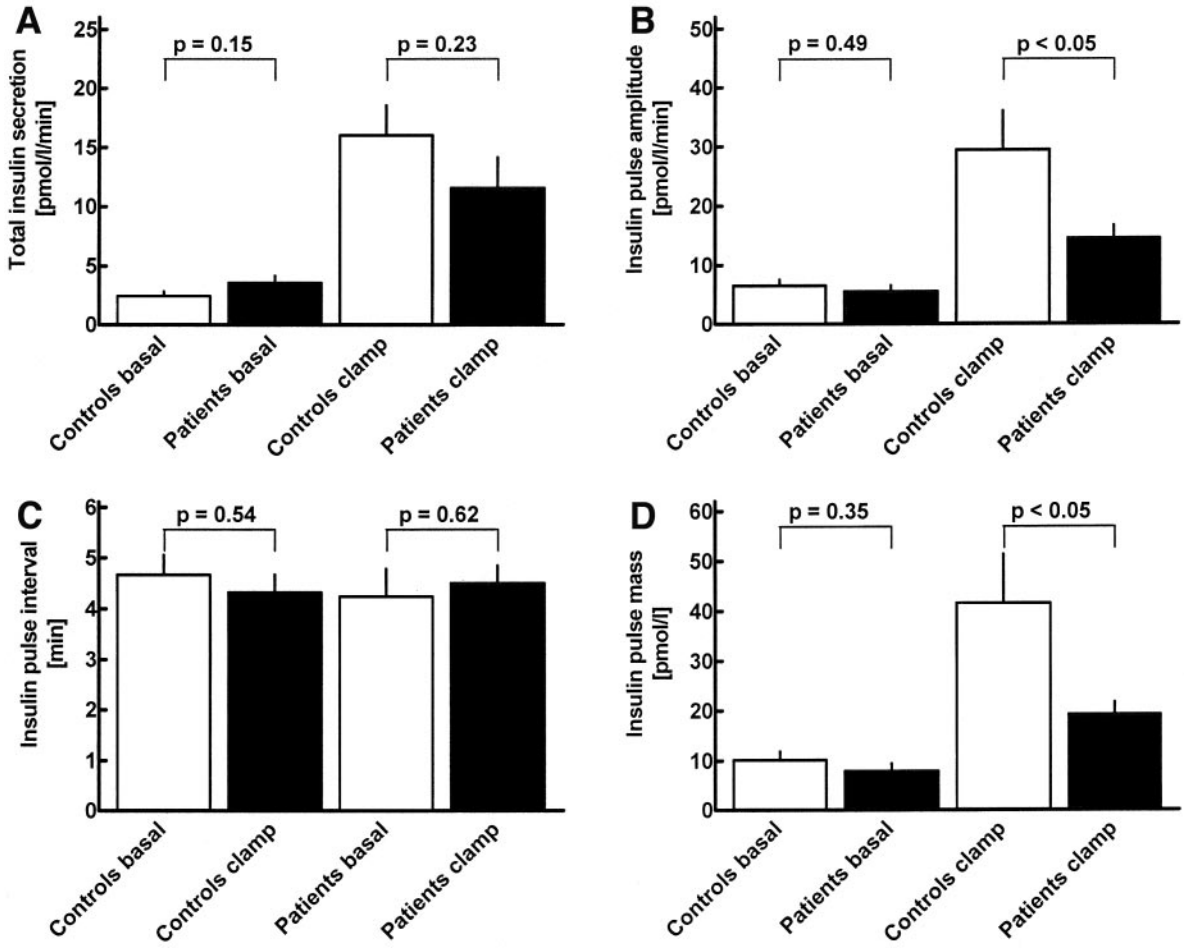

FIG. 5. Total insulin secretion (*A*), pulse amplitude (*B*), pulse interval (*C*), and pulse mass (*D*) in 10 patients after islet transplantation and 10 healthy nondiabetic control subjects studied over 40 min in the basal state and during a hyperglycemic clamp. Data are expressed as means ± SE.

subjects had a history of liver diseases or received any medication with a known influence on glucose homeostasis. The hepatic insulin extraction data in the normal control subjects have been reported previously in a study focused on the role of pulsatile insulin secretion on regulation of hepatic insulin extraction (10).

Study procedures. Study subjects were admitted to the GCRC at Mayo Clinic (Rochester, MN) 1 day before the study. All participants ate a standard meal in the evening and were kept fasting during the night. On the morning of the study, a hepatic vein sampling catheter was placed under local anesthesia using fluoroscopic guidance. After placement of the hepatic vein sampling catheter, the participants were returned to the GCRC, where a second sampling catheter was placed into a retrograde dorsal hand vein. After this, the hand was heated to 55°C in a thermoregulated Perspex hand warmer to achieve arterialization, a previously validated approach (24). A third catheter was placed into the contralateral antecubital vein for the infusion of glucose and indocyanine green (Cardio-Green; Becton Dickinson Microbiology Systems, Cockeysville, MD).

After placement of the catheters, a rest period of 45 min was allowed to reduce the risk of damping of pulsatile insulin secretion by endogenous catecholamines associated with catheter placement. During the following 40 min, plasma was sampled at 1-min intervals from the hepatic vein and arterialized dorsal hand vein.

After the study, all catheters were removed, and appropriate postfemoral catheter care was administered to the femoral vein puncture site. The

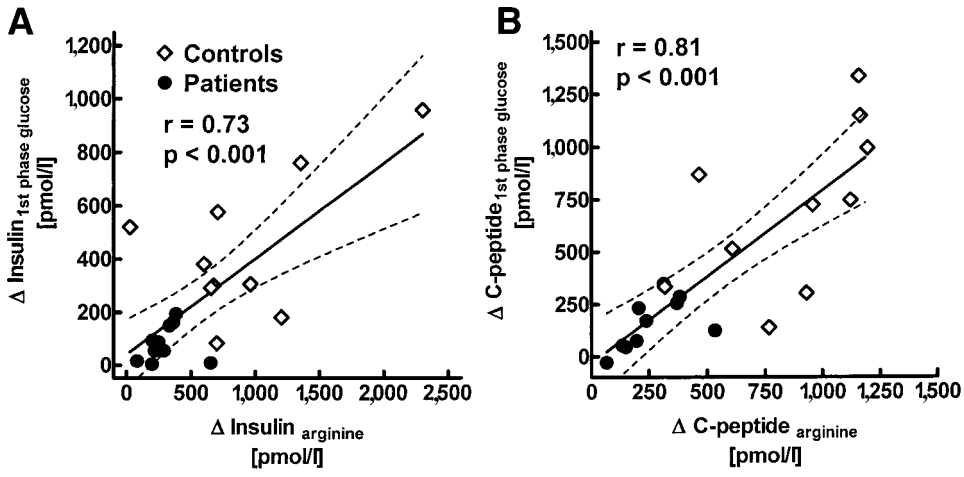

FIG. 6. Linear regression analysis between first-phase insulin (*A*) and C-peptide (*B*) responses to intravenous glucose administration and to intravenous arginine administration in 10 patients after islet transplantation and 10 healthy nondiabetic control subjects. *r*, regression coefficient.

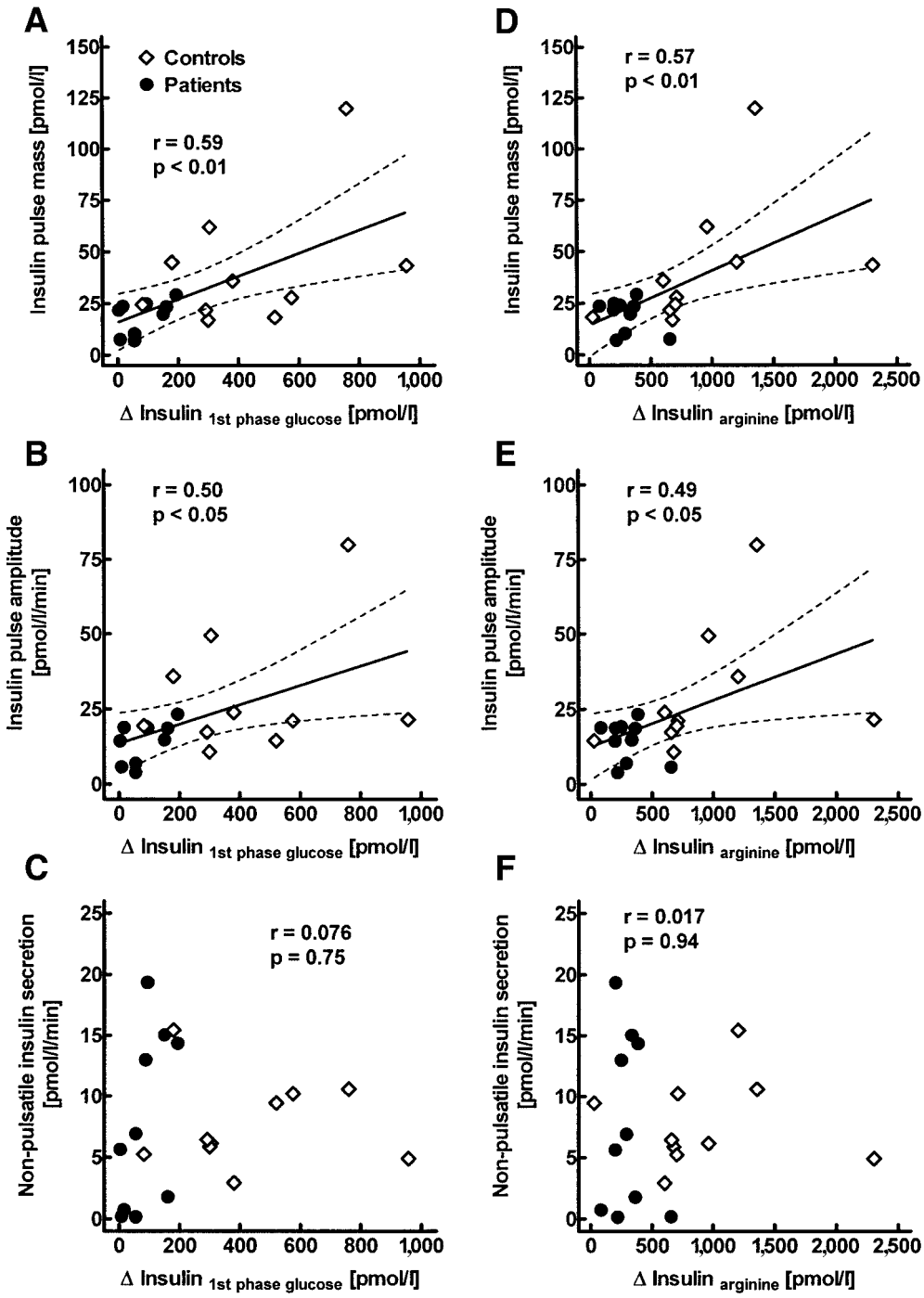

FIG. 7. Linear regression analysis between insulin pulse mass (*A* and *D*), pulse amplitude (*B* and *E*), and pulse interval (*C* and *F*) and the first-phase insulin responses to intravenous glucose (*A–C*) or arginine administration (*D–F*) in 10 patients after islet transplantation and 10 healthy nondiabetic control subjects.

participants remained in bed in the GCRC for the rest of the day and the following night.

Laboratory determinations. Glucose was measured using a glucose oxidase method with a Glucose Analyser 2 (Beckman Coulter, Palo Alto, CA). Insulin was determined in triplicate using two-site immunoenzymatic assays as described previously (25). For protocol 1, C-peptide concentrations were measured using an enzyme-linked immunosorbent assay from Dako (Cambridgeshire, U.K.). Intra-assay coefficients of variation were 3.3–5.7%, and interassay variation was 4.6–5.7%. Human insulin and C-peptide were used as standards.

For protocol 2, C-peptide concentrations were determined using a direct, double antibody sequential radioimmunoassay (Linco Research, St. Charles, MO). Interassay coefficients of variation were 4.9, 4.3, and 8.0% at 0.43, 1.75, and 4.36 nmol/l, respectively. In protocol 2, splanchnic blood flow was estimated by the constant infusion technique using indocyanine green dye as previously described (10).

Data analysis

Calculation of insulin secretory responses (protocol 1). Basal insulin and C-peptide concentrations were calculated as the average of all respective values determined from 0–40 min. First-phase insulin and C-peptide responses to intravenous glucose administration were calculated from the differences (Δ) between the peak insulin and C-peptide concentrations detected 2–10 min after intravenous glucose administration and respective mean basal levels. Second-phase insulin and C-peptide responses to glucose were estimated from the difference (Δ) between mean insulin concentrations determined from $t = 50–90$ min and basal insulin concentrations (0–40 min). Insulin and C-peptide secretory responses to arginine administration were estimated from the difference (Δ) between the respective peak concentrations measured 2–10 min after arginine administration and the mean concentrations from 50–90 min.

Calculation of hepatic insulin clearance (protocol 2). Insulin delivery from the splanchnic bed was calculated from the difference between the plasma insulin levels in the hepatic vein and in the peripheral vein multiplied by the splanchnic plasma flow. The prehepatic C-peptide secretion rate was

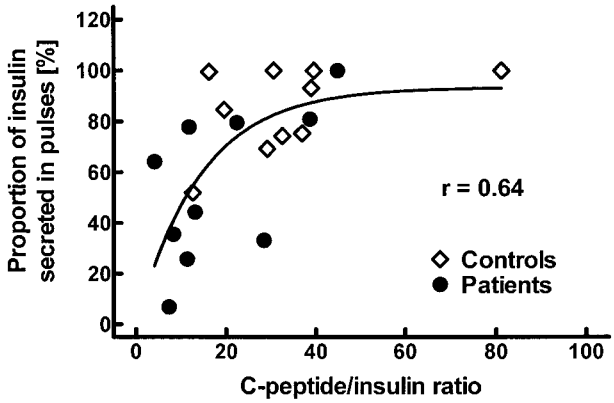

FIG. 8. Relationship between the proportion pulsatile insulin secretion and the C-peptide–to–insulin ratio in 10 patients after islet transplantation and 10 healthy nondiabetic control subjects studied over 40 min the basal state. The best fit curve was calculated according to the equation: $Y = Y_{max} \times [1 - \exp(-K \times X)]$.

calculated from the difference between the plasma C-peptide levels in the hepatic vein and in the peripheral vein multiplied by the splanchnic plasma flow. Fractional hepatic extraction of insulin was calculated from the difference between the C-peptide and the insulin delivery rates as a fraction of the endogenous C-peptide secretion rate (9,10). This calculation of first-pass hepatic insulin extraction requires the assumptions that insulin and C-peptide are secreted in equimolar amounts, that C-peptide is not extracted by the liver, and that gut insulin extraction is negligible.

Deconvolution analysis

The plasma insulin concentration time series were analyzed by deconvolution as previously validated (26) to detect and quantify insulin secretory bursts. Plasma insulin was assumed to result from five determinable and correlated parameters: *1*) a finite number of discrete insulin secretory bursts occurring at specific times and having *2*) individual amplitudes (maximal rate of secretion attained within a burst) and *3*) a common half-duration (duration of an algebraically Gaussian secretory pulse at half-maximal amplitude), which are superimposed on a *4*) basal time-invariant insulin secretory rate and *5*) a biexponential insulin disappearance model in the systemic circulation consisting of estimated half-lives of 2.8 and 5.0 min and a fractional slow compartment of 28%, as previously measured (26). All data analyses were performed in a blinded manner.

Statistical analyses

Results are presented as means ± SE. Statistical analyses were carried out using one-way ANOVA using Statistica (version 6; Statsoft, Tulsa, OK). Linear regression analyses were carried out using GraphPad Prism (version 3.0; GraphPad, San Diego, CA).

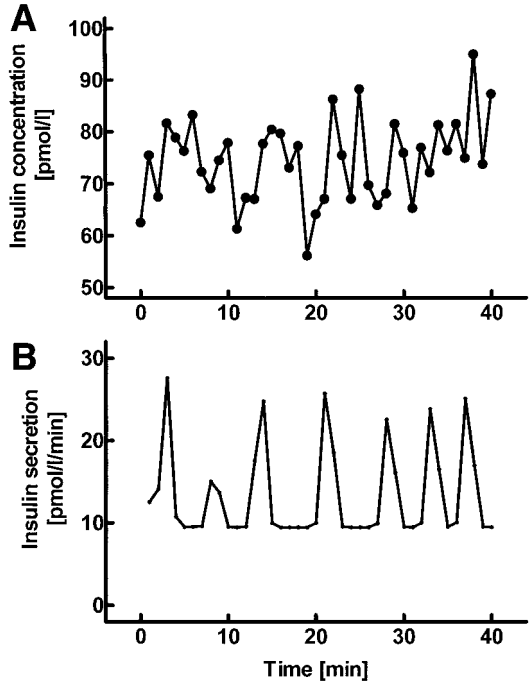

FIG. 9. Plasma concentrations of insulin (*A*) and corresponding insulin secretion rates (*B*) in a patient after islet autotransplantation for painful chronic pancreatitis studied over 40 min in the basal state.

RESULTS

Protocol 1. Fasting glucose concentrations were slightly but not significantly higher at baseline in the islet transplant recipients versus control subjects (117 ± 7 vs. 104 ± 9 mg/dl, respectively; $P = 0.23$) but by design comparable during the hyperglycemic clamp (153 ± 2 vs. 158 ± 1 mg/dl, respectively; $P = 0.08$; Fig. 1). The glucose infusion rates required to maintain hyperglycemia were markedly lower in the islet transplant recipients, implying a substantial defect in glucose-mediated insulin secretion (1.3 ± 0.3 vs. 3.7 ± 0.7 $mg \cdot kg^{-1} \cdot min^{-1}$, respectively; $P < 0.01$; Fig. 1).

At baseline, plasma concentrations of insulin and C-peptide were comparable in islet transplant recipients and control subjects, but given the higher basal glucose concentrations, these data suggest a defect in basal insulin secretion (Fig. 2). Consistent with this, the C-peptide–to–glucose ratio was lower at baseline in patients than in control subjects (2.3 ± 0.4 vs. 3.5 ± 0.4 $pmol \cdot l^{-1} \cdot mg^{-1} \cdot dl^{-1}$, respectively; $P < 0.05$). First-phase insulin secretion in response to intravenous glucose was deficient in islet transplant recipients whether evaluated by insulin or C-peptide or insulin ($P < 0.001$ for Δ $insulin_{1st\ phase}$ and Δ $C\text{-}peptide_{1st\ phase}$; Figs. 2 and 3).

During the hyperglycemic clamp ($t = 50$ to 90 min), C-peptide concentrations were ~30% lower in islet transplant recipients ($P < 0.05$), although insulin concentrations were comparable ($P = 0.18$), implying defective glucose-mediated insulin secretion offset by reduced hepatic insulin clearance. Second-phase insulin responses (calculated over basal concentrations) were reduced in patients compared with control subjects ($P = 0.09$ for Δ $insulin_{2nd\ phase}$ and $P < 0.05$ for Δ $C\text{-}peptide_{2nd\ phase}$; Figs. 2 and 3). The insulin response to acute administration of arginine was also deficient in islet transplant recipients compared with control subjects ($P < 0.01$ for Δ $insulin_{arginine}$ and for Δ $C\text{-}peptide_{arginine}$; Figs. 2 and 3).

Inspection of individual insulin concentration time series revealed insulin oscillations at ~4- to 5-min intervals both in patients and in control subjects (Fig. 4). This impression was confirmed by deconvolution analysis (Fig. 4). Posthepatic insulin delivery in islet transplant recipients (by deconvolution of systemic insulin time series) was comparable in the basal state and lower (but not significantly) during the clamp (Fig. 5). As expected from the insulin concentration profiles, pulsatile insulin secretion was readily detected in all islet transplant recipients and control subjects with a mean interval that was comparable at basal (4.2 ± 0.6 vs. 4.7 ± 0.4 min, respectively; $P = 0.54$) and during the hyperglycemic clamp (4.5 ± 0.4 vs. 4.3 ± 0.4 min, respectively; $P = 0.62$) (Fig. 5). The insulin pulse mass and amplitude were also comparable in the basal state but ~50% deficient during the hyperglycemic clamp in the islet transplant recipients ($P < 0.05$; Fig. 5). Although these data reveal that coordinate pulsatile insulin secretion is restored in patients with islet transplants, the coordination in the basal state was likely less

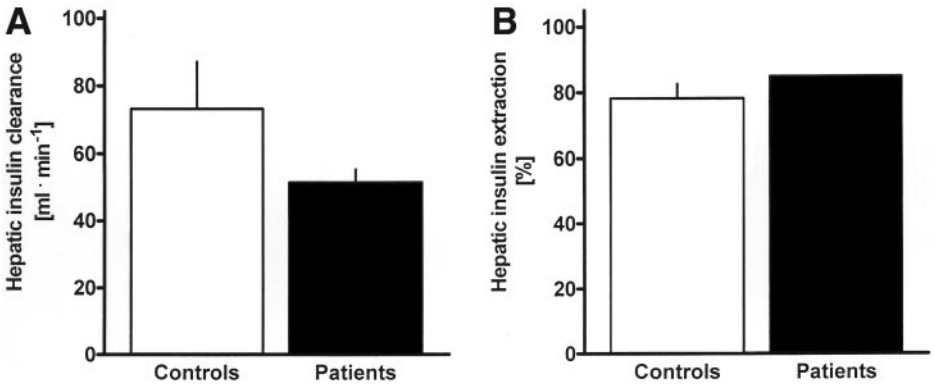

FIG. 10. Absolute hepatic clearance rates (*A*) and fractional insulin extraction (*B*) in two patients after islet autotransplantation and five healthy subjects studied over 40 min in the fasting state. Data are presented as means ± SE.

than that in control subjects as shown by the lower proportion of insulin secretion detected as arising from coordinate secretory bursts under these circumstances (51 ± 5% in patients vs. 85 ± 5% in control subjects, respectively; $P < 0.05$).

There was no relationship between measures of insulin secretion (first-phase insulin or C-peptide responses to glucose or arginine; insulin secretory burst mass or amplitude to glucose; and second-phase insulin or C-peptide responses to glucose) and either the number of islet equivalents transplanted or time since islet transplantation or A1C. However, a strong linear relationship was found between the both the insulin and C-peptide secretory responses to intravenous glucose (administered at basal glucose) and to intravenous arginine (administered at glucose ~150 mg/dl) (Fig. 6). The first-phase insulin responses to glucose and arginine were also closely related to the insulin pulse mass (and amplitude) during the hyperglycemic clamp, consistent with these parameters all being related to the available immediately releasable insulin pool and the fact that this is diminished in islet transplant recipients compared with control subjects (Fig. 7).

Insulin clearance, as estimated from the mean C-peptide–to–insulin ratio in the basal state tended to be reduced in patients compared with control subjects (19 ± 4 vs. 34 ± 6, respectively; $P = 0.09$). Interestingly, there was a strong positive relationship between the percentage of insulin released in pulses and the C-peptide–to–insulin ratio, suggesting decreased insulin clearance with smaller insulin pulses (Fig. 8).

Protocol 2. Basal glucose concentrations in the two patients after islet autotransplantation were 239 mg/dl (patient 1) and 87 mg/dl (patient 2). Consistent with the findings in protocol 1, insulin was secreted in distinct pulses (Fig. 9). Deconvolution of insulin concentrations in the hepatic vein revealed a similar pulse interval in patients (4.17 and 3.92 min, respectively) and control subjects (4.4 ± 0.3 min). Nonpulsatile insulin secretion was similar in patients (18.1 and 7.6 pmol · kg^{-1} · min^{-1}, respectively) and control subjects (15.5 ± 2.4 pmol · kg^{-1} · min^{-1}), whereas pulse mass was somewhat decreased in patients (23.1 and 20.0 pmol/l, respectively) compared with control subjects (33.5 ± 3.5 pmol/l). Likewise, pulse amplitude was lower in both patients (14.7 and 13.3 pmol · kg^{-1} · min^{-1}, respectively) than in control subjects (26.0 ± 3.4 pmol · kg^{-1} · min^{-1}).

The mean splanchic plasma flow was 0.17 l/min in patient 1 and 0.52 l/min in patient 2 and 0.60 ± 0.05 l/min in the control subjects. C-peptide secretion was 1.4 and 0.9 pmol · kg^{-1} · min^{-1}, respectively, in the patients and 1.3 ± 0.2 mol · kg^{-1} · min^{-1} in control subjects, and posthepatic insulin delivery was 0.2 and 0.1 pmol · kg^{-1} · min^{-1}, respectively, in the patients and 0.3 ± 0.1 pmol · kg^{-1} · min^{-1} in control subjects. Thus, insulin clearance was 55 and 48 ml/min in the patients and 73 ± 14 ml/min in control subjects. The corresponding fractional insulin extraction was 84.3, 84.8, and 78.2 ± 4.4%, respectively (Fig. 10).

DISCUSSION

In the present studies, we sought to address whether intrahepatic transplanted human islets secrete insulin in a coordinated pulsatile manner and whether insulin secreted from the islet grafts is delivered to hepatic sinusoids or into the systemic circulation. We report that insulin secretion in islet transplant recipients is pulsatile and that glucose-induced insulin secretion is accomplished through amplification of pulse size. Furthermore, we also observed via a direct transhepatic catheterization approach that hepatic first-pass insulin extraction is similar in patients after intraportal islet autotransplantation and healthy control subjects, implying that insulin secreted from islet grafts is delivered into hepatic sinusoids rather than into the hepatic central vein.

The coordinate release of insulin from islets scattered throughout the liver parenchyma suggests that some synchrony is reestablished among the islet grafts. This inference raises the question of what signals synchronize insulin secretion from topographically separated islets. A number of mechanisms may explain coordination of intrapancreatic islets in health. These include metabolic signals, such as oscillations in glucose or lactate concentrations and coupling through the intrapancreatic neural network (18,19). Clinical studies cannot readily establish the mechanism that accomplishes synchrony of pulsatile insulin secretion by intrahepatic islets. Studies in rats revealed that synchrony was absent shortly after islet transplantation but was reestablished coincident with their innervation 50–200 days later (18). In the present studies, patients were studied at least 9 months after islet transplantation so that innervation of the intrahepatic islet grafts was likely to have been established in these cases.

The presence of pulsatile insulin secretion after intraportal islet transplantation may have clinical implications. Suppression of hepatic glucose production is greater when insulin is presented in a pulsatile compared with constant manner (17,27). Moreover, pulsatile insulin secretion governs the systemic delivery of insulin indirectly because of preferential extraction of insulin bursts (10). Reestablishing a pulsatile pattern of insulin release in patients with

type 1 diabetes through intraportal islet transplantation may therefore be favorable over conventional treatment regimens. Consistent with this notion, hepatic glucose production was 25–30% lower during pulsatile than continuous insulin infusion in patients with type 1 diabetes (28). Under physiological conditions, insulin oscillations in the portal vein are ~10-fold higher than those achieved through pulsatile delivery of insulin into a peripheral vein (15). Thus, the actual impact of pulsatile insulin secretion after intraportal islet transplantation on hepatic insulin action is probably even greater than that inferred experimentally. In this regard, hepatic glucose production is normal in successfully islet transplanted patients with intact islet graft function but increased in patients with islet graft failure (29).

The present experiments show that the insulin secretory responses to both glucose and L-arginine stimulation were closely related to insulin burst mass rather than frequency or basal nonpulsatile secretion. These findings suggest that extremes of pulsatile insulin release are achieved by modulation of insulin pulse size.

Whether the optimal site for islet transplantation is the liver, spleen, kidney capsule, peritoneal cavity, or gut has been debated (6). Important drawbacks in using the portal venous route are the thrombosis, bleeding, and exposure of the islets to high gut-derived environmental toxins and immunosuppressive drugs used to prevent rejection (6). On the other hand, reproducing the physiological route of intraportal insulin delivery might favor this approach, which would putatively expose hepatocytes directly to secreted insulin via fenestrated sinusoids. Binding of insulin to the hepatocyte receptors results in rapid internalization of the insulin-receptor complex, subsequent initiation of the intracellular insulin signaling cascades, and proteolytic degradation of the internalized insulin molecules (30). By these means, the liver acts as the primary insulin-responsive organ and serves as a safeguard against systemic hyperinsulinemia. In contrast, systemic venous drainage after whole-pancreas transplantation results in substantial hyperinsulinemia (23). The present data showing normal first-pass insulin extraction in two patients after islet autotransplantation indicate that islet grafts release the majority of insulin directly into the liver sinusoids, where it undergoes extensive first-pass extraction and presumably directly suppresses hepatic glucose release (29).

Exposure of the liver sinusoids to high local concentrations of insulin secreted from intraportal islet grafts may have other metabolic consequences. Hepatic steatosis occurs in a subgroup of patients after successful intraportal islet transplantation (31,32). Graft failure was associated with reversal of this lesion (32). Such findings could indicate that insulin released by intraportal islet grafts into the liver sinusoids induces lipid deposition, e.g., via promoting the esterification of free fatty acids within the hepatocytes.

In conclusion, these studies show that intrahepatic islets in humans *1*) are coordinated to secrete insulin in a pulsatile manner albeit with detectable impairment of rapid release and *2*) deliver insulin directly to the liver sinusoids. These data imply that chronically implanted intrahepatic islets are capable of restoring a pattern of insulin secretion and clearance that closely reproduces that of the native pancreas.

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