Insulin secretion rates estimated by two mathematical methods in pancreas-kidney transplant recipients

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Insulin secretion rates estimated by two mathematical methods in pancreas-kidney transplant recipients. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E716–E725, 1998.—After pancreas-kidney transplantation, it is difficult to obtain an accurate estimate of the insulin secretion of the pancreas graft, since several pitfalls are involved using peripheral C-peptide and/or insulin measurements in this determination. In this study, the individual kinetic parameters of C-peptide and then the rates of insulin secretion were estimated by two mathematical methods, the deconvolution method and the “combined model” during slow (oral glucose) and fast (intravenous glucagon) stimulation of the pancreatic graft, resulting in relatively slow and rapid changes in insulin secretion, respectively. ISR was calculated on the basis of the deconvolution technique and the decay curve of C-peptide concentrations, as originally proposed by Eaton et al. (11) and confirmed by Polonsky et al. (21). With this 2-day method, individual C-peptide kinetics are determined in each subject after a bolus injection of biosynthetic human C-peptide while endogenous insulin secretion is suppressed by an infusion of somatostatin. Thereafter, the kinetic parameters are used on the day of experiment to calculate ISR by deconvolution of peripheral C-peptide concentrations (21). Thus, with this method, peripheral insulin measurements are not employed. Another approach uses kinetic modeling of the concomitantly measured concentrations of insulin and C-peptide, termed the “combined model” (28). With this method, ISR and C-peptide and insulin kinetics and an index of hepatic extraction of insulin are calculated on the basis of measured profiles of the two peptides, and in contrast to the deconvolution method, a separate day for assessing C-peptide kinetics is not necessary (28).

In the present study, β-cell function was evaluated in pancreas-kidney transplant recipients from ISR after an oral glucose load and an intravenous glucagon stimulation of the pancreatic graft, resulting in relatively slow and rapid changes in insulin secretion, respectively. ISR was calculated on the basis of the deconvolution technique and the decay curve of C-peptide distribution and degradation (11, 21) and compared with ISR based on the combined model without the use of a bolus injection of C-peptide (28). The results obtained were compared with those in nondiabetic kidney transplant recipients and nondiabetic healthy controls. Because an infusion of somatostatin has been shown to affect renal plasma flow and the glomerular filtration rate (GFR) and thereby potentially C-peptide clearance (27), a group of C-peptide-negative insulin-dependent diabetes mellitus (IDDM) patients was also studied after a bolus injection of C-peptide.

AFTER PANCREAS TRANSPLANTATION, an accurate estimate of insulin secretion is paramount not only for evaluation of the graft function in relation to monitoring the success of the transplantation but also for the studies of the intermediate metabolism after transplantation. Both peripheral insulin and C-peptide concentrations have been used to assess β-cell function after pancreas-kidney transplantation (2–4, 6, 7, 13, 17, 19, 25). However, it has recently been found that there are limitations and pitfalls encountered using these peptide levels as measures of insulin secretion after pancreas-kidney transplantation, since they may falsely overestimate insulin secretion compared with those of normal subjects (2–4, 6, 7). In pancreas-kidney transplant subjects, it therefore seems advisable to determine individual kinetic parameters of C-peptide and then calculate insulin secretion rates (ISR) to get an accurate assessment of insulin secretion.

Insulin secretion can be estimated by several methods (11, 21, 23, 24, 26, 28). The commonest method has been to evaluate β-cell function by calculating ISR by deconvolution of peripheral C-peptide concentrations, as originally proposed by Eaton et al. (11) and confirmed by Polonsky et al. (21). With this 2-day method, individual C-peptide kinetics are determined in each subject after a bolus injection of biosynthetic human C-peptide while endogenous insulin secretion is suppressed by an infusion of somatostatin. Thereafter, the kinetic parameters are used on the day of experiment to calculate ISR by deconvolution of peripheral C-peptide concentrations (21). Thus, with this method, peripheral insulin measurements are not employed. Another approach uses kinetic modeling of the concomitantly measured concentrations of insulin and C-peptide, termed the “combined model” (28). With this method, ISR and C-peptide and insulin kinetics and an index of hepatic extraction of insulin are calculated on the basis of measured profiles of the two peptides, and in contrast to the deconvolution method, a separate day for assessing C-peptide kinetics is not necessary (28).

In the present study, β-cell function was evaluated in pancreas-kidney transplant recipients from ISR after an oral glucose load and an intravenous glucagon stimulation of the pancreatic graft, resulting in relatively slow and rapid changes in insulin secretion, respectively. ISR was calculated on the basis of the deconvolution technique and the decay curve of C-peptide distribution and degradation (11, 21) and compared with ISR based on the combined model without the use of a bolus injection of C-peptide (28). The results obtained were compared with those in nondiabetic kidney transplant recipients and nondiabetic healthy controls. Because an infusion of somatostatin has been shown to affect renal plasma flow and the glomerular filtration rate (GFR) and thereby potentially C-peptide clearance (27), a group of C-peptide-negative insulin-dependent diabetes mellitus (IDDM) patients was also studied after a bolus injection of C-peptide.
METHODS

Subjects

Informed consent was obtained from four groups of subjects. Group 1. This group was made up of six successful pancreas-kidney transplant recipients with systemic delivery of insulin (Px), three of whom had segmental grafting and three whole organ grafting. Two recipients had bladder drainage and four recipients enteric drainage of the exocrine secretion.

Group 2. This group, made up of six nondiabetic kidney transplant recipients with portal insulin secretion, served as the first control group (Kx). All transplant subjects received cadaveric pancreas-kidney and/or kidney grafts. The studies were undertaken 35 ± 6 and 30 ± 8 mo posttransplantation, respectively. The immunosuppressive regimen included prednisolone (5-10 mg/day), cyclosporin A (150-300 mg/day), and azathioprine (50-75 mg/day).

Group 3. This group, made up of six nondiabetic matched controls, served as the second control group (NS).

Group 4. This group, made up of six C-peptide-negative IDDM (type 1) patients with acceptable metabolic control on a multiple insulin injection regimen and with long-standing diabetes mellitus and kidney function, evaluated by serum creatinine levels and GFR like those of the pancreas-kidney and kidney transplant groups, were selected as the third control group. The IDDM group was included to control for the reduced kidney function of the transplant recipients, as assessed from GFR levels, and to evaluate the potential effect of somatostatin on C-peptide clearance during the study day of the bolus injection of C-peptide in the transplant groups. Because the IDDM group has no endogenous insulin secretion, C-peptide kinetics were studied without use of somatostatin. The immunosuppressive regimen included prednisolone (5-10 mg/day), cyclosporin A (150-300 mg/day), and azathioprine (50-75 mg/day).

Study Protocols

Study 1. Assessment of C-peptide kinetics. All experiments were started between 7:00 and 8:00 AM, after the subjects had fasted overnight for 10 h. The IDDM subjects were instructed to take their bedtime insulin injection (around 10 PM) on the evening before the study and not to take their morning insulin injections. No other medications were taken by any of the participating subjects in the morning before the study. The subjects were placed at bed rest and kept supine during the study. Fasting basal blood samples were drawn from an antecubital vein kept patent with 0.9% saline, and the forearm was maintained in a heated box to ensure arterIALIZation of the venous blood. Concentrations of plasma glucose, plasma C-peptide, plasma cyclosporin A, serum creatinine, serum insulin, and HbA1C were measured. In the contralateral antecubital vein, another 17-gauge cannula was used for the somatostatin bolus and infusion as well as the C-peptide injection. Endogenous insulin secretion was suppressed by a bolus injection of 250 µg somatostatin, with a subsequent continuous infusion of 500 µg somatostatin (Durascan Medical Products, Odense, Denmark) for 270 min. Ninety minutes after the start of the infusion, a 50-nmol bolus injection of biosynthetic human C-peptide (Bachem Feinchemikalien, Bubendorf, Switzerland) was administered over 30 s. Blood samples of plasma glucose, C-peptide, and insulin were collected for 270 min. Urine samples were collected from 90 to 270 min to assess urinary C-peptide excretion.

Study 2. Glucagon test (GT). On another day, the subjects in the Px, Kx, and NS groups were studied after an overnight fast for 10 h by means of an intravenous injection of 1 mg glucagon (Novo Nordisk, Bagsvaerd, Denmark) administered over 30 s. Blood samples of plasma glucose, C-peptide, and insulin were collected in the basal state (−10, −5, and 0 min) and for 20 min (2, 5, 6, 8, 10, 12, and 20 min) after stimulation.

Study 3. Oral glucose tolerance test (OGTT). On another day, the subjects in the Px, Kx, and NS groups were studied after an overnight fast for 10 h. The β-cell function was tested for insulin secretion by means of a 4-h OGTT (75 g dextrose). Blood samples of plasma glucose, C-peptide, and insulin were collected in the basal state (−10, −5, and 0 min) and for 240 min (5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 120, 150, 180, 210, and 240 min) after stimulation.

Analytical Determinations

Glucose in plasma was determined in duplicate by the glucose oxidase method (Beckman Instruments, Fullerton, CA). Blood samples of serum insulin and plasma C-peptide were centrifuged immediately at 3,000 rpm for 10 min at 4°C and stored at −20°C, pending analysis. Plasma C-peptide was determined by immunonassay in duplicate, using antiserum M1221 (Novo Nordisk) (12). Serum insulin was measured by the monoclonal insulin ELISA technique (1). HbA1C was analyzed by an HPLC method (normal range 4.1–6.1%). All samples from each pancreas-kidney transplant recipient were measured with samples from one of the control subjects in the same run.

Data Analysis

The first part of the deconvolution method is estimation of the kinetic parameters of the two-compartment model for C-peptide from the decay curve after the bolus of C-peptide (21). The basis of the rate equations for the kinetic model has previously been presented (21). The parameters K1 and K2 represent the rate constants for C-peptide transport from compartment 1 to 2 and vice versa, respectively. K3 is the rate constant for the irreversible disappearance of C-peptide from compartment 1. The calculation of the two volumes, V1 and V2, and the rapid first phase (T1) and the slower second phase (T2) half-lives of C-peptide has previously been reported (21).

The second part consists of calculation of the secretion rate from C-peptide concentrations measured during GT and OGTT. Although the original method (21) used a cubic spline function to smooth the C-peptide concentration profile followed by mathematical deconvolution, we used a cubic spline function to describe the secretion rate (28), which can then be estimated from the measured C-peptide and the individual kinetic parameters for C-peptide by multiple linear regression analysis. The inherent tendency of deconvolution methods to exhibit large random deviations was avoided by choosing the knot points of the splines to be preceded and
INSULIN SECRETION RATES IN PANCREAS TRANSPLANTS

Table 1. Baseline clinical data on the C-peptide kinetic study day

<table>
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<tr>
<td></td>
<td>Px</td>
</tr>
<tr>
<td>Women/men</td>
<td>2/4</td>
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<tr>
<td>Age, yr</td>
<td>43 ± 3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>73.5 ± 4.3</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.6 ± 1.3</td>
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<tr>
<td>Plasma glucose, mM</td>
<td>4.5 ± 0.3*</td>
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<tr>
<td>Plasma C-peptide, pM</td>
<td>774 ± 60*†‡</td>
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<tr>
<td>Serum insulin, pM</td>
<td>112 ± 18*†</td>
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<tr>
<td>HbA1C, %</td>
<td>5.6 ± 0.2*</td>
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<tr>
<td>Serum creatinine, µM</td>
<td>114 ± 6*†‡</td>
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<tr>
<td>Plasma cyclosporine, ng/ml</td>
<td>392 ± 40</td>
</tr>
<tr>
<td>GFR, ml·min⁻¹·(1.73 m²)⁻¹</td>
<td>65.4 ± 2.8</td>
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Values are means ± SE for pancreas-kidney transplant recipients (Px), kidney transplant recipients (Kx), insulin-dependent diabetes mellitus patients (IDDM), and normal subjects (NS). *P < 0.05, compared with IDDM. †P < 0.05, compared with NS. ‡P < 0.05, compared with Kx.

followed by three to four sampling times. The secretion rates were expressed as picomoles per minute per unit of the total distribution volume (V₁ + V₂) (21).

The combined model is defined by means of the rate equations for one-compartment models for insulin and C-peptide, in which k₁ and k₃ are the elimination rate constants for the two peptides (28). The prehepatic secretion rate is expressed per unit distribution volume of C-peptide (V₃), and f is the fraction of the secreted insulin that is not extracted by the liver during the first passage (F) multiplied by the ratio between the distribution volumes, i.e., f = F(V₃/V₁), where V₁ is the distribution volume of insulin. This model was estimated from the measured values of insulin and C-peptide by nonlinear regression analysis where the ISR was represented by a cubic spline function.

From the ISR models, the β-cell function was evaluated by calculating the basal prehepatic ISR, the total and incremental (above basal) amounts of prehepatic insulin during OGTT and GT, and the maximal prehepatic ISR to both β-cell stimuli as well as time to maximum. The contribution of the basal to total insulin secretion during the tests was calculated by extrapolating the basal ISR during the 20 min of the GT and 240 min of OGTT. Because we took the deconvolution method as the "golden standard" in these experiments (21), we also calculated the mean difference between the ISR determined by the two methods. The integrated responses (total and incremental above basal) of insulin and C-peptide were calculated as the area under the curve by means of the trapezoidal rule.

Statistical Methods

Nonparametric statistical tests were employed, the Mann-Whitney rank-sum test in the analysis of unpaired data, the Wilcoxon rank-sum test in the analysis of paired data, and Spearman’s ρ for correlation analysis. All results are presented as means ± SE. Significance of differences was set at P < 0.05.

RESULTS

The clinical characteristics of the subjects at baseline are given in Table 1. HbA1C was significantly increased in the group of IDDM patients. Serum creatinine levels were higher in the Px, Kx, and IDDM groups than in NS (P < 0.05) but also higher in IDDM and Kx than in Px (P < 0.05). GFR values were similar in Px, Kx, and IDDM and significantly lower than the normal range for NS [80–120 ml·min⁻¹·(1.73 m²)⁻¹; P < 0.05]. Plasma cyclosporin levels were identical in the two transplant groups. Fasting plasma glucose, plasma C-peptide, and serum insulin levels within each group were not significantly different between the two study days.

Kinetics of C-Peptide by Deconvolution Method

Baseline data are presented in Tables 1 and 2. Fasting hyperinsulinemia was observed in Kx and Px but not in NS (P < 0.05). Fasting plasma C-peptide levels were increased in Kx and Px, unlike in NS and IDDM (P < 0.05), and in Kx compared with Px (P < 0.05). Before the somatostatin infusion was started, IDDM subjects had fasting insulinemia comparable to that of NS. One IDDM subject, who had high insulin binding antibodies (40% binding capacity to the tracer), has been excluded from the insulin data shown.

Table 2. C-peptide kinetic parameters after bolus injection of 50 nmol C-peptide

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<tr>
<td></td>
<td>Px</td>
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<tr>
<td>K₁, min⁻¹</td>
<td>0.093 ± 0.017*†</td>
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<tr>
<td>K₂, min⁻¹</td>
<td>0.061 ± 0.012*</td>
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<tr>
<td>K₃, min⁻¹</td>
<td>0.048 ± 0.007*†</td>
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<tr>
<td>T₁, min</td>
<td>3.5 ± 0.5</td>
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<tr>
<td>T₂, min</td>
<td>39.7 ± 3.3†</td>
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<tr>
<td>V₁, ml/kg</td>
<td>51.6 ± 1.8</td>
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</table>

Values are means ± SE. K₁, K₂, K₃, rate constants; T₁, T₂, half-lives; V₁, volume (see text for full explanation). *P < 0.05, compared with IDDM. †P < 0.05, compared with NS.
The profiles of plasma glucose and serum insulin and the decay curve of C-peptide after the bolus injection of C-peptide in the four groups are shown in Fig. 1. Plasma glucose declined initially in all groups after initiation of somatostatin and increased thereafter in NS from 3.6 ± 0.2 to 5.9 ± 0.4 mM, in IDDM from 8.1 ± 1.1 to 10.4 ± 1.4 mM, in Kx from 3.5 ± 0.5 to 4.6 ± 0.7 mM, and in Px from 3.7 ± 0.6 to 6.6 ± 1.1 mM, respectively (P < 0.05). Meanwhile, the fasting serum insulin levels were suppressed to levels around detection limit (<5 pM) in the Px, Kx, and NS groups. Likewise, fasting C-peptide decreased to levels around detection limit (<100 pM) before injection of C-peptide. Plasma C-peptide increased to similar peak levels in all groups after the bolus injection of C-peptide (Fig. 1). Urine C-peptide excretion was similar in all groups, constituting 7.6 ± 1.6% in Kx, 6.2 ± 1.1% in Px, 6.4 ± 1.3% in NS, and 7.0 ± 1.2% in IDDM of the amount of C-peptide injected. K₁ was higher in Px compared with NS (P < 0.05), and K₃ was higher in Px and Kx than in NS (P < 0.05, Table 2). No differences were seen in K₁, K₂, and K₃ between Px and Kx, and K₂ was similar in the Px, Kx, and NS groups. K₁, K₂, and K₃ were higher in IDDM than in the other groups (P < 0.05). Distribution volumes (V, ml/kg) and the short half-life (T₁) were similar in all groups, whereas the long half-life (T₂) was significantly increased in IDDM, Kx and Px compared with NS (P < 0.05).

OGTT

Plasma glucose, C-peptide, and serum insulin profiles. Plasma glucose, C-peptide, and serum insulin levels determined by OGTT are reported in Fig. 2 and Table 3. The plasma glucose response was 30% higher in the two transplant groups than in normal subjects (P < 0.05). However, glucose tolerance was normal in the two transplant groups, according to WHO criteria.
The total insulin and C-peptide responses were significantly higher than in NS (P < 0.05), and the total C-peptide response was higher in Kx than in Px (P < 0.05). The corresponding incremental insulin response was twice as high in Kx as in NS (P < 0.05), and the incremental C-peptide response was threefold higher than in NS (P < 0.05). In contrast, the incremental C-peptide responses in Px recipients were not significantly different from those in NS but were 55% lower than those of Kx (P < 0.05). The incremental insulin response was 44% higher in Px than in NS (P < 0.05) and 20% lower than in Kx (P < 0.05).

ISR by deconvolution model. The mean ISR profile during OGTT in Px, Kx, and NS is depicted in Fig. 3A. Basal ISR values were similar in Px and NS, whereas basal ISR was twofold higher in Kx. Both the total amount of insulin secreted and the increment above basal secretion were ∼50% higher in Kx than in Px and NS (P < 0.05). Maximal ISR was also 50% higher in Kx than in Px (P < 0.05), and maximal ISR was 30% lower in Px than NS. Maximal ISR occurred significantly later in Px and Kx than in NS (P < 0.05). There were no differences in basal-to-total ISR between groups. The correlation between basal ISR and total ISR was r = 0.93 (P < 0.00001), between basal ISR and the increment in ISR was r = 0.80 (P = 0.0002), and between basal ISR and maximal ISR was r = 0.65 (P = 0.0048; n = 18).

ISR and insulin and C-peptide kinetics by combined model. The mean ISR profile during OGTT in Px, Kx, and NS is shown in Fig. 3B and Table 4. The mean elimination rate constant of insulin, kᵢ, did not differ
correlation coefficient was \( r = 0.47 (P < 0.0661) \), and the basal ISR correlated highly with the time to the maximal ISR \( (r = 0.83, P = 0.0001; n = 18) \). Comparison between ISR estimated by deconvolution method and combined model during OGGT. The mean difference between the calculated ISR determined by the two methods is shown in Fig. 3C. The combined model gave the highest rates during the first 15 min \( (P = 0.05 \text{ at } 5 \text{ min}) \), whereas the deconvolution method gave the highest mean rates thereafter, without reaching statistical significance. The profiles in the separate groups in Fig. 3, A and B, showed that the combined model gave significant higher secretion in the Px group in the early part of the test and significantly lower secretion in the NS group from 45 to 150 min than did the deconvolution method, but there were only small differences in the Kx group.

**GT**

Plasma glucose, C-peptide, and serum insulin profiles. Plasma glucose, C-peptide, and serum insulin levels determined by GT are reported in Fig. 4 and Table 3. The glucose response to intravenous glucagon resulted in higher glucose concentrations at the end of the 20-min study period in Kx \((8.9 \pm 0.7 \text{ mM})\) than in NS \((7.0 \pm 0.5 \text{ mM})\) and Px \((7.6 \pm 0.6 \text{ mM})\), \(P < 0.05\). The total insulin response in Kx was 3-fold higher than in NS and 2.5-fold higher than in Px, respectively \((P < 0.05)\), and 2-fold higher in Px than NS \((P < 0.05)\). The total C-peptide response was 2.5- and 1.5-fold higher in Kx than in NS and Px, respectively \((P < 0.05)\). In contrast, only incremental insulin and C-peptide responses were higher in Kx than in NS \((P < 0.05)\).

ISR by deconvolution model. The ISR profile during GT in Px, Kx, and NS is demonstrated in Fig. 5A and Table 3. Basal ISR values were similar in Px and NS but twofold higher in Kx \((P < 0.05, \text{ Table } 4)\). Total and incremental amounts of insulin secreted were significantly higher in Kx than in Px and NS \((P < 0.05)\), which had not statistically different responses. Maximal ISR values were significantly lower in Px than in Kx \((P < 0.05)\).

**Table 4. Insulin and C-peptide kinetic parameters and physiological equivalents estimated from combined model of insulin secretion**

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<tr>
<td></td>
<td>Px</td>
</tr>
<tr>
<td>Oral glucose tolerance test</td>
<td></td>
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<tr>
<td>( k_{c}, \text{ min}^{-1} )</td>
<td>0.100 ± 0.014</td>
</tr>
<tr>
<td>( k_{c}, \text{ min}^{-1} )</td>
<td>0.016 ± 0.002</td>
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<tr>
<td>( f = F(Vc/VI) )</td>
<td>0.82 ± 0.09*†</td>
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<tr>
<td>Glucagon test</td>
<td></td>
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<tr>
<td>( k_{c}, \text{ min}^{-1} )</td>
<td>0.046 ± 0.015†</td>
</tr>
<tr>
<td>( k_{c}, \text{ min}^{-1} )</td>
<td>0.017 ± 0.002†</td>
</tr>
<tr>
<td>( f = F(Vc/VI) )</td>
<td>0.75 ± 0.10*</td>
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</table>

Values are means ± SE. \( k_{c}, k_{c} \), rate constants for insulin and C-peptide secretion, respectively; \( f = F(Vc/VI) \), fraction of secreted insulin (see text for further explanation). *\( P < 0.05 \), compared with NS. †P < 0.05, compared with Kx.
0.05), but the difference between Px and NS failed to reach statistical significance. No differences were observed in the basal-to-total ISR or in time-to-maximal ISR between groups.

ISR and insulin and C-peptide kinetics by combined model. The ISR profile during GT is demonstrated in Fig. 5B and Tables 3 and 4. Kinetic parameters were not significantly different compared with the OGTT day. No differences between groups were observed in the calculated basal ISR, whereas the total and incremental amounts of insulin secreted were significantly lower in Px than in Kx and NS (P < 0.05) and lower in NS than in Kx (P < 0.05). Although maximal ISR was lower in Px than in Kx and NS, these differences were not significant. The time-to-maximal ISR was similar in the three groups.

Comparison between ISR estimated by deconvolution method and combined model during GT. The mean difference between the calculated ISR determined by the two methods during GT is shown in Fig. 5C. The combined model gave the highest rates during the first 5 min (P < 0.001 at 5 min), whereas the deconvolution method gave the highest mean rates thereafter (P < 0.05 at 8, 10 and 12 min). There was also a significantly lower basal secretion with the combined model relative to the deconvolution method. Figure 5, A and B, shows that the profile for the NS group obtained from the combined model peaked earlier than the deconvolution profile (at 4 min).

DISCUSSION

In the present study, ISR was estimated in pancreas transplant recipients by two mathematical methods, the deconvolution technique and the combined model, during slow and fast changes in insulin secretion. Compared with that in nondiabetic kidney transplant recipients, incremental ISR in pancreas-kidney transplant recipients was absolutely reduced using both approaches during oral glucose load as well as after intravenous glucagon. In contrast, calculations of incremental ISR in normal subjects and pancreas-kidney
transplant recipients induced by the two \( \beta \)-cell stimuli were about the same.

After pancreas-kidney transplantation, it is important to obtain an accurate estimate of the insulin secretion of the pancreas graft. The present study clearly illustrated the problems of using peripheral measurements of insulin and C-peptide in the interpretation of \( \beta \)-cell function in pancreas-kidney transplant recipients. The total insulin and C-peptide responses in pancreas-kidney transplant recipients to oral glucose both indicated higher insulin secretion than in normal subjects, whereas incremental C-peptide responses did not. In fact, the insulin data merely reflect the effect of systemic delivery of insulin with no first-pass hepatic uptake of insulin, and the C-peptide data are influenced by the reduced C-peptide metabolic clearance rate. This is also evident when peripheral insulin and C-peptide responses are compared between the pancreas transplant recipients and the non-diabetic kidney transplant recipients. No statistical difference in total insulin response was demonstrated, whereas the C-peptide response was reduced with \( \sim 50\% \) in the pancreas transplant recipients. However, when the kinetics of the peptides are taken into consideration in calculation of the actual ISR, no major differences in ISR were demonstrated between pancreas transplant recipients and normal subjects. Both the total amount of insulin secreted and the incremental insulin secretion were \( \sim 50\% \) higher in the kidney transplant recipients than in the pancreas transplant recipients and normal subjects. The same pattern was demonstrated when analyzing the peptide responses to intravenous glucagon, in which the peripheral peptide measurements in pancreas-kidney transplant recipients indicated improved responses compared with normal subjects, but the actual ISR values were similar. Both peripheral peptides and insulin secretion were reduced in pancreas-kidney transplant recipients compared with kidney transplant recipients.

In the basal state, the increased ISR seen in kidney transplant recipients might be the result of the immunosuppressive treatment inducing insulin resistance, whereas the peripheral hyperinsulinemia in pancreas-kidney transplant recipients, caused by a combination of systemic insulin delivery and immunosuppressive induced insulin resistance, did not to the same extent result in higher basal ISR, as previously demonstrated (2, 3, 6–8). This was demonstrated by both mathematical methods, but especially using the deconvolution model.

The incremental ISR to oral glucose was higher or similar, respectively, in the kidney and pancreas-kidney transplant groups than in normal subjects, but these responses have to be related to the glycemic responses and insulin resistance. By doing so, an impairment in ISR in both transplant groups was obvious, independent of the method used to evaluate insulin secretion, since the hyperglycemic responses were increased by 30% in the transplant groups. The hyperglycemic response and impairment in insulin secretion in pancreas-kidney transplant recipients have been reported in other study designs (2, 3, 6, 19). The predominant causes of this impairment have previously been discussed in detail, the main components being the number of functioning \( \beta \)-cells, reduced by the amount transplanted (segmental vs. whole pancreas graft), and/or by the number and severity of rejection episodes (2, 3, 6, 8, 19). We and others (8, 9, 15) have previously shown a markedly reduced insulin sensitivity in these recipients. A dose relationship between insulin secretory capacity and insulin resistance has been shown in subjects with normal glucose tolerance (8). Therefore the present data also suggest that the transplanted \( \beta \)-cells have not fully adapted to the concomitant insulin resistance. A direct effect of the immunosuppressive treatment on the \( \beta \)-cells has also been suggested as a mechanism inducing impaired \( \beta \)-cell function and insulin sensitivity as seen in our non-diabetic kidney transplant recipients (18, 30). Also, as previously reported, basal insulin secretion constituted significantly more of the total amount of insulin secreted in pancreas transplant recipients than in the control groups calculated with the combined model, whereas a similar, though not a significant difference, was seen with the deconvolution method (2, 3, 6, 8).

The fast insulin secretory responses after intravenous glucose demonstrated a higher similarity between the groups than did the slower changes seen after OGGT and were comparable to previous reports (5, 17). Thus insulin secretory responses to glucagon were similar in normal subjects and pancreas-kidney transplant recipients despite different peripheral levels of insulin and C-peptide.

Several pitfalls are involved using peripheral C-peptide and/or insulin measurements in the evaluation of insulin secretion, and the present results demonstrate that the seemingly higher insulin secretion in pancreas-kidney transplant recipients and non-diabetic kidney transplant recipients is explained by different mechanisms (22). The systemic delivery of insulin in pancreas-kidney transplant recipients avoids first-pass hepatic extraction unlike portal insulin delivery in non-pancreas-kidney transplant subjects and therefore makes peripheral insulin measurements unsuitable for this evaluation (2–4, 6, 7). Instead, C-peptide has been used as a marker of insulin secretion after pancreas-kidney grafting (2–4, 6, 7, 13, 17, 19, 25). C-peptide is cosecreted from the \( \beta \)-cells in equimolar amounts with insulin, and hepatic extraction of C-peptide is negligible and its metabolic clearance rate constant over the physiological range of concentrations (20). However, pancreas-kidney transplant recipients still have slightly reduced kidney function, since they receive only one kidney, and kidney function may further be adversely affected by cyclosporin (2, 3, 25). Accordingly, the kinetics of C-peptide differ between pancreas-kidney transplant recipients and normal subjects, resulting in an increased half-life of C-peptide (2, 3, 6–8), as demonstrated in the present study as well and independent of the model approach. The prolonged half-life results in overestimation of insulin secretion in the transplant recipients. Furthermore, during non-steady-state conditions the peripheral concentration of C-
peptide does not change in proportion to its secretion rate, because C-peptide is distributed outside the plasma compartment and because of the long half-life of C-peptide (16). Last, the faster the changes in insulin secretion, the less accurate is the estimate of ISR directly from C-peptide concentrations (20). Therefore C-peptide levels may be representative only in the basal state of ISR and comparable only among subjects with normal kidney function. For these reasons, it can be stated that insulin secretion cannot be accurately assessed from peripheral plasma insulin or C-peptide concentrations and that the peptide comparisons between pancreas transplant recipients with systemic or portal insulin delivery will not be valid unless mathematical modeling based on the individual kinetics of the peptides is used for reliable assessment of insulin secretion.

As previously mentioned, ISR can be obtained by various methods (11, 21, 23, 24, 26, 28). The insulin secretion model described by Rudenski et al. (24) is inapplicable, since it uses only insulin measurements and does not take account of the lack of first-pass hepatic insulin extraction in pancreas-kidney transplant recipients with peripheral insulin secretion. A simplified version of the deconvolution method has previously been published (26). Being based on standardized kinetic parameters, it eliminates the separate experimental day for assessment of C-peptide kinetics (26). However, this model cannot be used in subjects with impaired kidney function, such as pancreas-kidney transplant recipients (26). At present, it therefore seems that only the two models employed in the present study can account for the various physiological and anatomical circumstances in pancreas-kidney recipients and kidney transplant recipients. The deconvolution method has previously been applied to pancreas-kidney transplant recipients demonstrating a reduced clearance of C-peptide (2), but it is time consuming because of the separate day reserved for estimation of the kinetics of C-peptide and is also expensive because of the costs of human biosynthetic C-peptide and somatostatin (21). The combined model has previously been applied in a study of subjects with altered hepatic extraction of insulin and peripheral hyperinsulinemia and also after pancreas-kidney transplantation (3, 6–8, 29).

When the estimated ISR values from the two mathematical methods are compared, the insulin secretion profiles for the OGTT showed steeper increases when calculated from the combined model, and the secretion rates for the normal subjects and kidney transplant subjects decayed more rapidly using the combined model than using the deconvolution approach. On the much shorter time scale of the GT study, the profiles from the combined model also showed faster increases and subsequent decays than those based on deconvolution. Higher peak values were observed in all groups using the combined model. In relation to the overall mean differences between the two methods, there were some differences in the subgroups. Thus the combined model seemed to overestimate ISR in the early post-stimulatory phase of insulin secretion and to underestimate ISR in the later phase of insulin secretion compared with the deconvolution method, this being most pronounced during fast changes in insulin secretion. These findings are probably explained by the fact that the estimated ISR in the combined model are based on the single compartment approximation for C-peptide rather than the two-compartment model on which the deconvolution method is based. Therefore the dynamic differences between the mean secretion profiles obtained with the two methods are probably due to the different C-peptide kinetic modeling. In agreement with this explanation it is also apparent that the dynamic differences between the two methods was more pronounced in the short GT than in the OGTT.

From this study alone, one cannot determine which of the two methods gives the most correct and precise results. The deconvolution method should in theory give more accurate secretion rates than the combined model, since it is based on the two-compartment model for C-peptide, which seems to be required for an adequate description of the C-peptide kinetics after intravenous bolus administration (26). It has been validated and used extensively in many clinical studies; however, the kinetic parameters obtained from this test may be influenced by the continuous infusion of somatostatin, which, of course, is not infused during the OGTT or GT. This might lead to some bias in the estimation of the secretion rates. The combined model was designed with approximate one-compartment models for C-peptide and insulin, but a validation study in dogs showed that it gave accurate estimates of portally infused insulin and C-peptide rates resembling responses to OGTT (28). These findings have later been reiterated with insulin and C-peptide infusions in humans (14). On the basis of the present data, the two methods yield relatively large dynamic differences in the GT, and it therefore seems very likely that the deconvolution method gave the most reliable estimates of the fast changes in insulin secretory responses. This conclusion, however, will be dependent on the accuracy of the C-peptide assay and on the assumption that somatostatin does not influence C-peptide kinetics. The latter seems to be confirmed by the findings of similar GFR and C-peptide decay curves in the transplant groups and the C-peptide-negative IDDM group, which did not receive somatostatin; these findings, however, contrast with previous reports (27). The combined model may give sufficiently accurate results in many situations with slow changes in insulin secretion, i.e., OGTT, and might be the method of choice when the additional efforts required to determine individual C-peptide kinetics are not warranted.

In conclusion, several limitations and pitfalls exist in the evaluation of insulin secretion after pancreas-kidney transplantation with systemic delivery of insulin. In general, the use of the stimulated peripheral insulin and/or C-peptide measurements as an index of β-cell function cannot be recommended. We suggest that, to facilitate the interpretation of peripheral measurements of these peptides, at least the individual kinetics of C-peptide should be estimated in pancreas-kidney transplant recipients followed by calculation of ISR by deconvolution. Alternatively, the combined model...
can be employed especially in situations with slow changes in insulin secretion. The present study has clearly demonstrated that insulin secretion after pancreas transplantation is not entirely normalized.

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