A 6-year prospective study on new onset diabetes mellitus, insulin release and insulin sensitivity in renal transplant recipients

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Abstract

Background. It is well known that both insulin resistance and insulin deficiency are involved in the pathogenesis of post-transplant diabetes mellitus (PTDM), but the relative importance of the two different mechanisms is still under debate. The present prospective longitudinal study was performed over 6 years to investigate the impact of impaired insulin secretion (ISec) and insulin sensitivity (IS) in the development of PTDM in renal transplant recipients.

Methods. A total of 95 non-diabetic patients underwent a 75 g oral glucose tolerance test (OGTT) 10 weeks post-transplant. Six years later, 63 of these recipients were re-examined, the majority \((n=58)\) with an OGTT. Fasting, 1- and 2-h insulin and glucose levels were measured and used to estimate the insulin secretory response and IS both at baseline and at follow-up.

Results. The proportion of recipients with normal glucose tolerance (NGT) rose from 46\% (baseline) to 65\% (follow-up) \((P=0.008)\), and median fasting and 2-h serum glucose were reduced by 0.7 mmol/l \((P<0.001)\) and 1.3 mmol/l \((P=0.039)\), respectively. The recipients with PTDM at follow-up had a significant decline in the estimated median first and second phase ISec \((-58\text{ and }-47\%, \text{ respectively}, P=0.005\text{ for both})\). The patients who normalized their glucose tolerance from PTDM or IGT at baseline to NGT at follow-up increased their IS significantly \((68\%, P=0.002)\) without significant alterations in ISec.

Conclusions. Impaired ISec seems to be the dominant mechanism in the development of PTDM after renal transplantation. In contrast, normalization of glucose intolerance is associated with improved IS.

Keywords: insulin release; insulin sensitivity; post-transplant diabetes mellitus; renal transplantation

Introduction

Post-transplant diabetes mellitus (PTDM) and impaired glucose tolerance (IGT) are common complications after renal transplantation [1–3]. Both fasting and post-prandial hyperglycaemia may lead to microvascular complications as well as acceleration of macrovascular disease [4,5].

Impaired insulin secretion (ISec) and peripheral insulin resistance have both been identified as potential mechanisms in the pathogenesis of PTDM. It has been argued that the former may be more important than the latter [6]. We have shown recently that defects in insulin release as assessed 10 weeks after renal transplantation indicate a poor prognosis regarding normalization of glucose tolerance during the following year [7]. Moreover, in non-transplanted type 2 diabetics, like the Pima Indians, worsening of glucose tolerance from normal glucose tolerance (NGT) to IGT, and from IGT to diabetes mellitus, is accompanied by a progressive decline in the acute insulin secretory response and an increase in body weight [8]. In contrast, the subjects who remain normoglycaemic over time are characterized by maintained acute insulin response and glucose disposal.

Long-term studies of renal transplant patients are scarce, still questioning whether PTDM may persist over several years in these patients, and whether late-onset diabetes may develop in the individuals who are normoglycaemic shortly after transplantation. The objectives of the present single-centre prospective observational study were first, to assess the glucose tolerance and the occurrence of PTDM in a group of renal transplant recipients over a period of 6 years after transplantation. Secondly, we tested the hypothesis of whether impaired insulin release is more important than insulin resistance in the development of PTDM.
Diabetes mellitus after renal transplantation

Subjects and methods

Subjects

In 1995, 95 Caucasian renal transplant recipients underwent a 75 g oral glucose tolerance test (OGTT) 10 weeks after transplantation. None of these had diabetes prior to transplantation [2]. Six years later, the recipients in this cohort were evaluated for inclusion in the follow-up study. Eighteen patients had died, two had been retransplanted, two were in dialysis and 10 declined to participate, leaving a total of 63 patients to be included in the follow-up study (Figure 1). Each subject gave informed written consent before participating in the study, which was approved by the Regional Committee for Medical Research Ethics, Healthregion South, Norway.

Out of 63 patients, 58 were re-examined with an OGTT, the majority (n = 54) at our centre. The glucose challenge was not performed in five recipients, due to manifest PTDM treated with insulin (n = 4) and lack of patient compliance (n = 1). The OGTT was carried out after an overnight fast and included measurements of fasting, 1- and 2-h insulin and glucose concentrations. There were no statistically significant differences between the recipients included in the study and those who were lost to follow-up in terms of gender, age, blood pressure, use of antihypertensive and immunosuppressive medication, glucose tolerance, serum creatinine and number of rejection episodes.

Stratification of the patients

The recipients were divided into different categories of glucose tolerance according to the criteria given by the Expert Committee [4]: PTDM with fasting serum glucose ≥ 7.0 mmol/l or 2-h serum glucose ≥ 11.1 mmol/l; IGT with fasting glucose < 7.0 mmol/l and 2-h glucose 7.8–11.0 mmol/l; impaired fasting glucose (IFG) with fasting glucose 6.1–6.9 mmol/l and 2-h glucose < 7.8 mmol/l; and NGT with fasting serum glucose < 6.1 mmol/l and 2-h serum glucose < 7.8 mmol/l.

Immunosuppressive therapy

Ten weeks after renal transplantation, all patients (n = 63) were treated with prednisolone and cyclosporin A (CsA) (Sandimmun Neoral®), and 81% with azathioprine. During the follow-up period, six recipients were switched to tacrolimus and two to mycophenolate mofetil, and three patients had their prednisolone treatment withdrawn. Excluding the subjects who changed immunosuppressive therapy from the statistical analyses did not change the overall findings.

Analytical procedures

The analysis of baseline serum glucose was performed using a glucose dehydrogenase method (Cobas Mira, Roche, Switzerland). At follow-up, whole blood glucose was measured with a HemocueAB® B-glucose Analyser (Angelholm, Sweden) in 54 recipients, whereas glucose was analysed in venous serum directly in nine recipients. Whole blood glucose values were recalculated to venous serum values [9].

Serum insulin was determined by a commercial radioimmunoassay (Coat-A-Count™, Diagnostic Products Corporation, Los Angeles, CA) at baseline and by a fluoroimmunoassay (Auto DELFIA™ Insulin, Wallac Oy, Turku, Finland) at follow-up. Creatinine clearance was calculated according to the Cockcroft and Gault formula [10].

Insulin release and sensitivity indices

Insulin release was estimated by the use of three OGTT-derived indices. These equations have been validated in patients with varying degrees of glucose tolerance and correlate well with the results from hyperglycaemic clamp studies [11,12].

Using the trapezoid rule, the area under the curve (AUC) insulin and the AUC glucose during the OGTT were calculated and implemented in the insulin release index: SecrAUC = AUCIns/AUCGluc [11,12]. The first and second phase insulin release were estimated according to the following equations: Secr1.phase = 1194 + 4.724 × Inso – 117.0 × Gluc1 + 1.414 × Ins1; Secr2.phase = 295 + 0.349 × Ins1 – 25.72 × Gluc1 + 1.107 × Inso, where Inso and Ins1 are serum insulin levels at 0 and 1 h after an OGTT, respectively, and Gluc1 represents serum glucose 1 h post-load [12].

The OGTT-derived sensitivity index (IS) index [IS1TX = 0.208 – 0.0032 × body mass index (BMI) – 0.0000645 × Ins2 – 0.00375 × Gluc2], which is a modification of the IS index proposed by Stumvoll et al. [12], was implemented as the surrogate estimate of IS [11,13]. The IS1TX has recently been validated in renal transplant recipients and correlated closely with the results from hyperinsulinaemic euglycaemic clamp (r = 0.58, P < 0.001) [13].

Statistical analyses

Results are given as median and range. For continuous data, the Mann–Whitney or the Wilcoxon matched pairs signed rank sum test was used, whereas Pearson χ² or McNemar’s test was implemented for categorical data as appropriate. Linear regression was implemented in the analysis of potential independent continuous variables, with 2-h serum glucose or IS as the dependent variables. Multiple linear regression was used to assess any independent predictor of change in glucose
tolerance or IS. All variables associated with 2-h serum glucose or IS in the univariate analyses with \( P \)-values < 0.1 were included in the multiple regression model. Two tailed \( P \)-values are reported, and values < 0.05 considered significant. The analyses were performed using the Statistical Package for the Social Sciences (SPSS version 10.0 for Windows, Chicago, IL).

Results

Changes in glucose tolerance from baseline to follow-up

The prevalence of PTDM, IGT and NGT at baseline and follow-up is shown in Figure 2. The proportion of recipients with NGT rose from 46 to 65\% during the 6 years (\( P = 0.008 \)). At baseline, 12 recipients (19\%) had PTDM, IGT was found in 22 recipients (35\%) whereas 29 patients (46\%) had NGT. At follow-up, 14 recipients (22\%) had PTDM, eight recipients (13\%) had IGT and 41 patients (65\%) had NGT. None of the patients had IFG, either at baseline or at follow-up. Five out of 12 patients with diabetes at baseline improved to NGT (\( n = 4 \)) or IGT (\( n = 1 \)) during the follow-up period. Among the 22 recipients with IGT at baseline, 11 patients improved to NGT, whereas six progressed to PTDM. One recipient with NGT at baseline deteriorated to PTDM during the follow-up period. The majority (26 out of 29) of the patients with NGT 10 weeks after renal transplantation were still euglycaemic 6 years later.

In the total patient population, median fasting (\( P < 0.001 \)) and 2-h (\( P = 0.039 \)) serum glucose were significantly lower 6 years after renal transplantation as compared with baseline (Table 1). This coincided with a 50\% decrease in median daily prednisolone dose and CSA whole blood trough concentration, a significant increase in median BMI and a decrement in median serum triglyceride concentration. The renal function improved slightly from baseline to follow-up. When the change in 2-h serum glucose was considered as being the dependent variable, univariate linear regression analysis showed a significant association between reduction in daily prednisolone dose and improvement in glucose tolerance (\( \beta = 0.12, r^2 = 0.069, P = 0.046 \)). On the other hand, neither the increase in BMI nor the decline in CSA whole blood trough concentration were associated with an alteration in glucose tolerance.

Hypoglycaemic therapy

Seven recipients had PTDM both at baseline and at follow-up. Of these, two patients were treated with insulin both at baseline and at follow-up, one recipient was switched from glipizide to insulin during the period and one had metformin withdrawn. The three remaining subjects were not treated with any hypoglycaemic drugs, either at baseline or at follow-up.

None of the five recipients who improved their glucose tolerance from PTDM to either IGT (\( n = 1 \)) or NGT (\( n = 4 \)) were on glucose-lowering drugs at baseline.

One recipient, who deteriorated from IGT to PTDM, was on a combination treatment with insulin and metformin at follow-up.

None of the recipients who were using hypoglycaemic therapy at follow-up went through the OGTT.

Insulin secretion (ISec) and insulin sensitivity (IS)

Calculated indices of ISec and IS for all recipients are given in Table 2. All the indices of ISec were significantly reduced 6 years after renal transplantation (ISec\( _{\text{AUC}} \), \( P < 0.001 \); ISec\( _{\text{AUC}} \), \( P < 0.001 \); Secr\( _{\text{AUC}} \), \( P < 0.001 \)), whereas median IS increased significantly (ISIT\( _{\text{TX}} \), \( P = 0.033 \)).

The changes in BMI and IS from baseline to follow-up were inversely correlated in the univariate model (linear regression: \( \beta = 0.006, r^2 = 0.262, P < 0.001 \)), whereas the reduction in median daily prednisolone dose and triglyceride level tended to be associated with improved insulin action (\( \beta = 0.001, r^2 = 0.067, P = 0.060 \) and \( \beta = 0.008, r^2 = 0.057, P = 0.098 \), respectively). When the change in prednisolone dose, triglyceride concentration and BMI were included in a multiple linear regression model, dose reduction of prednisolone was significantly associated with improved IS, whereas an increase in BMI was associated with a reduction in IS (\( P < 0.001 \) for both, \( r^2 = 0.34 \)). Serum triglycerides were, on the other hand, not related to changes in IS in this model.

None of these parameters were associated with changes in ISec.
Association of changes in ISec and IS with changes in glucose tolerance 6 years after renal transplantation

To assess the relative importance of ISec and IS on changes in glucose tolerance during the follow-up, the recipients were divided into two groups (Table 3). The group of patients who had PTDM at follow-up (n = 14) was labelled ‘diabetic’. The group of recipients who normalized their glucose tolerance, from PTDM or IGT at baseline to NGT at follow-up (n = 15), was labelled ‘normoglycaemic’. At baseline, these two groups were not significantly different in terms of IS, ISec, BMI, fasting and post-prandial serum glucose, kidney function and treatment with antihypertensive medication (P > 0.117).

The diabetic group had a decline in estimated median insulin release of 58% (Secr1.phase) and 47% (Secr2.phase), whereas the ISI TX did not change significantly (Table 3). In contrast, the median ISI TX increased significantly (68%; P = 0.002) whereas the median first and second phase insulin release were unchanged in the normoglycaemic group.

The patients who had NGT both at baseline and at follow-up (n = 26) tended to increase their IS [8.8 (6.1–10.6) vs 9.3 (5.0–12.1) × 10^-2; P = 0.101]. All the ISec indices were significantly reduced during the period (P < 0.001).

Discussion

Glucose tolerance

In general, median fasting and 2-h serum glucose values declined significantly from baseline to follow-up. One half of the recipients with IGT and one-third of the recipients with PTDM improved to NGT. The recipients with PTDM who improved their glucose tolerance were not on glucose-lowering therapy at baseline. The majority of the recipients with NGT at baseline remained normoglycaemic after 6 years. Thus, NGT shortly after renal transplantation indicates a favourable prognosis for normoglycaemia in the long term [3]. On the other hand, about half (n = 7) of the patients with PTDM at follow-up were diabetic already 10 weeks after transplantation. The other half (n = 6) were mainly patients with IGT in the early course who progressed to PTDM, which supports the idea that IGT early after renal transplantation represents a risk factor for later development of PTDM [14,15].

Insulin sensitivity

The IS improved significantly in the cohort in general over the 6 years. We previously have reported that tapering off prednisolone during the first year after renal transplantation is significantly associated with
improved glucose tolerance [3]. The present findings of a significant association between prednisolone tapering and improvement in IS may explain and support our previous data [3].

The group of recipients with PTDM or IGT at baseline who normalized their glucose tolerance was characterized by a significant increase in IS and preserved ISec. Interestingly, they also used higher daily doses of prednisolone than the progressors did in the early course. The subsequent greater dose reduction can be one plausible explanation for the improvement in IS [3]. Furthermore, hyperglycaemia itself predisposes for insulin resistance [16] and, as these patients gradually became normoglycaemic, they may have improved their IS by reducing the glucose exposure in insulin-sensitive tissue. However, the weight gain in both groups may have counteracted the improvement in IS.

Insulin secretion

The OGTT-derived first and second phase ISec decreased by half in the total patient population. Older age is known to be an important determinant of impaired β-cell function [2,17]. However, we were not able to show a significant association between estimated ISec and age in the present study (data not shown). Since the glucose tolerance was improved during the period, the reduction in ISec seems mainly to be caused by increased IS.

It is widely believed that diminution of first phase ISec is the earliest detectable defect of β-cell function in the non-transplant human population [18]. Further, it is widely believed that diminution of first phase ISec is the earliest detectable defect of β-cell function in the non-transplant human population [18]. However, we were not able to show a significant association between estimated ISec and age in the present study (data not shown). Since the glucose tolerance was improved during the period, the reduction in ISec seems mainly to be caused by increased IS.

Although it has been suggested that the mechanism behind the diabetogenic effect of CsA is impaired insulin release [2,13], we were not able to find any association between the change in whole blood CsA trough levels and the change in estimated insulin secretion from baseline to follow-up. If any such an effect exists, it cannot be strictly dose dependent [3].

**Table 3.** Changes in estimated insulin secretion and insulin sensitivity, BMI and daily prednisolone dose according to changes in glucose tolerance

<table>
<thead>
<tr>
<th>Insulin secretion indices</th>
<th>Diabetic (n = 14)</th>
<th>Normoglycaemic (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
</tr>
<tr>
<td>Secr1.phase</td>
<td>858 (67–1776)</td>
<td>359 (−448 to 914)</td>
</tr>
<tr>
<td>Secr2.phase</td>
<td>239 (63–451)</td>
<td>127 (−61 to 257)</td>
</tr>
<tr>
<td>SecrAUC</td>
<td>30 (14–59)</td>
<td>25 (6–57)</td>
</tr>
<tr>
<td>ISITX × 10^−7</td>
<td>7.2 (3.9–9.3)</td>
<td>5.8 (−1.9–9.7)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.9 (19.1–28.1)</td>
<td>24.5 (19.5–41.1)</td>
</tr>
<tr>
<td>Prednisolone (mg/day)</td>
<td>10 (10–30)</td>
<td>5 (0–10)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.0 (0.9–4.2)</td>
<td>1.4 (0.5–3.3)</td>
</tr>
</tbody>
</table>

Values are presented as median (range).

Diabetic: Patients with PTDM at follow-up, who either stayed diabetic or progressed to PTDM from IGT or NGT.

Normoglycaemic: Patients with PTDM or IGT at baseline who normalized their glucose tolerance (NGT) till follow-up.

Statistics: Wilcoxon matched pairs signed rank sum test.
A limitation of the present study is that the surrogate estimates for $I_S$, $I_S$ and $I_S$ may be inferior compared with insulin measures derived from hyperglycaemic and euglycaemic clamp studies. However, the $I_S$ indices have been validated extensively in patients with different degrees of glucose tolerance and are strongly correlated with results from clamp studies [11,12]. In addition, the $I_S$ has recently been validated in renal transplant subjects and found to correlate well with results from hyperinsulinaemic euglycaemic clamp [13].

Moreover, one should be aware that 32 patients were lost to follow-up, of whom 18 died. We do not know the glucose tolerance status of these recipients over the years. This may have biased our conclusions on the transitions between normoglycaemia and hyperglycaemia in the cohort. However, the fact that the persons lost to follow-up were not significantly different from the subjects included in the study at baseline makes it less likely that this is a major flaw of the study.

In conclusion, a decline in insulin secretion seems to be the most important factor for the development of long-term PTDM. On the other hand, improvement in insulin sensitivity together with sufficient insulin release is associated with improved glucose tolerance during the first 6 years after renal transplantation.

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Conflict of interest statement. None declared.

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