Glucose Metabolism in Renal Transplant Recipients: Effect of Calcineurin Inhibitor Withdrawal and Conversion to Sirolimus

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Cyclosporine A (CsA) and tacrolimus have been associated with an increased risk for diabetes after transplantation, whereas sirolimus is deemed to be devoid of any effect on glucose metabolism. This study was performed to investigate the effect of the withdrawal of calcineurin inhibitors and the switch to sirolimus on peripheral insulin resistance and pancreatic β cell response. Twenty-six patients who received a kidney transplant and discontinued CsA and were converted to sirolimus and 15 recipients of suboptimal kidneys who were treated with tacrolimus plus sirolimus for the first 3 mo after grafting and thereafter with sirolimus alone were enrolled. All patients underwent an oral glucose tolerance test before and 6 mo after the conversion to sirolimus-alone therapy. The withdrawal of CsA or tacrolimus was associated with a significant fall of insulin sensitivity (both \( P = 0.01 \)) and with a defect in the compensatory β cell response, as measured by the disposition index (\( P = 0.004 \) and \( P = 0.02 \), respectively). The increase of insulin resistance and the decrease of disposition index significantly correlated with the change of serum triglyceride concentration after the conversion to sirolimus-based therapy (\( R^2 = 0.30, P = 0.0002; \) and \( R^2 = 0.19, P = 0.004 \), respectively). Clinically, the switch to sirolimus was associated with a 30% increase of incidence of impaired glucose tolerance and with four patients’ developing new-onset diabetes. In conclusion, the discontinuation of calcineurin inhibitors and their replacement by sirolimus fail to ameliorate the glycometabolic profile of kidney transplant recipients. Rather, it is associated with a worsening of insulin resistance and an inappropriately low insulin response.


Materials and Methods

This prospective study enrolled two different groups of kidney transplant recipients.

Group 1

Starting from January 2002 and up to June 2004, all CsA-treated patients who received the histologic diagnosis of chronic allograft
nephropathy (CAN), with serum creatinine (sCr) levels <2.5 mg/dl and daily proteinuria ≤1.0 g, were asked to be converted to sirolimus, without any further modification of the remaining immunosuppressive therapy (low-dose steroids [prednisone 2.5 to 5 mg/d] and MMF [1 to 2 g/d]). Collectively, 32 patients were enrolled. During the follow-up, one patient developed posttransplantation lymphoproliferative disease, one patient had an acute rejection episode and progressed to end-stage kidney disease, three patients discontinued sirolimus because of serious side effects, and one declined consent, leaving a total of 26 patients to be included in the follow-up study.

The conversion protocol consisted of an abrupt CsA discontinuation. Sirolimus therapy was initiated 12 to 16 h after stopping the CNI. All patients were asked to give their written informed consent to affect glucose metabolism, or the absolute need for drugs that are disabling urinalysis, sCr, blood urea nitrogen, and lipid profile) checked at baseline and at the end of the study (6 mo). CsA exposure was monitored by the measurement of whole-blood CsA level obtained 2 h after the morning dose (C2); tacrolimus and sirolimus exposure was evaluated by predose (trough) monitoring.

**Insulin Sensitivity Indexes**

The OGTT-derived insulin sensitivity index for transplantation (ISITX; 0.208 to 0.0032 × BMI [kg/m²] − 0.0000645 × Ins120 [pmol/L] − 0.00375 × Gluc120 [mmol/L]) has been proposed and validated by Hjelmesæth et al. (19) against the gold standard method, the hyperinsulinemic-euglycemic glucose clamp, in a renal transplant population and turned out to correlate best with ISIclamp. ISI was measured also by the ITT. It was derived from linear regression of the rate of the fall of log glucose from 3 to 15 min and calculated from the equation KITT = 0.693/t1/2 × 100 (%/min). Previous studies have found coefficient of variation values for ITT between 6 and 9% and a close relationship between insulin sensitivity as measured by ITT and that measured by the euglycemic-hyperinsulinemic clamp method (20). Finally, we estimated the metabolic clearance rate (MCR) of glucose by the formula MCR (ml × kg⁻¹ × min⁻¹) = 19.2 to 0.281 × BMI − 0.00498 × Ins120 − 0.333 × Gluc120 validated against the hyperinsulinemic-euglycemic glucose clamp (21).

**β Cell Function**

Insulin release was estimated by the use of three equations documented to correlate well (r = 0.70 to 0.75) with insulin secretion as assessed by hyperglycemic clamp studies in patients with varying degrees of glucose tolerance (21). The area under curve (AUC) insulin and the AUC glucose during the OGTT were calculated using the trapezoidal rule and implemented in the insulin release index: SecrAUC = AUCins/AUCGluc (21)

The first-phase and second-phase insulin releases were estimated implementing insulin values at 0 and 60 min and glucose at 60 min (21):

\[
\text{Secr}_{1\text{phase}} = 728 + 3.537 \times \text{Ins}_0 - 120.3 \times \text{Gluc}_{60} + 1.341 \times \text{Ins}_{60} + 21.27 \times \text{BMI} \\
\text{Secr}_{2\text{phase}} = 208 + 0.335 \times \text{Ins}_{60} - 26.33 \times \text{Gluc}_{60} + 0887 \times \text{Ins}_{60} + 3.933 \times \text{BMI}
\]

The acute insulin secretory response increases with decreasing insulin action to maintain NGT, and the relationship between insulin release and insulin sensitivity has been described as hyperbolic (22). The product of the estimates of insulin sensitivity and insulin release, known as the disposition index (DI), is a constant in normoglycemic individuals, whereas the development of glucose intolerance is associated with a decline of the DI (22). In other words, the DI describes the ability of the pancreatic β cell to compensate for various degrees of insulin resistance and therefore may represent a more appropriate measure of β cell function than the absolute insulin release (22). In this study, the DI was estimated as the product of the first-phase insulin release and the ISI_1x (23).

**Statistical Analyses**

Results of quantitative variables are expressed as mean ± SD. Differences between quantitative variables were tested by means of Wilcoxon rank test or Mann-Whitney U test, as appropriate. Correlations
Results
Throughout the follow-up, care was taken to avoid any major modification of pharmacologic therapy. Specifically, no patient required the use of β blockers or diuretics, and the daily steroid dose was not modified in any of the patients. As for antihypertensive drugs, a few patients required the decrease of drug (calcium channel blockers and/or angiotensin-converting enzyme inhibitors), whereas none required de novo prescription of antihypertensive therapy. None of the patients studied had an acute rejection episode or clinically relevant infections throughout the follow-up period. The anthropometric and laboratory features of patients at the start and end of the study are shown in Table 1.

OGTT
In group 1 patients, the withdrawal of CsA and the conversion to sirolimus failed to modify blood glucose and insulin response to oral glucose load (Figure 1). Patients of group 2 showed a significant increase of 2-h blood glucose and insulin concentration 6 mo after the discontinuation of tacrolimus and the switch to full-dose sirolimus (Figure 2).

### Table 1. Anthropometric and laboratory features of the patients examined at the start and end of the study

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Start</td>
<td>End</td>
</tr>
<tr>
<td></td>
<td>44.04 ± 11.82</td>
<td>44.04 ± 11.82</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>16/10</td>
<td>16/10</td>
</tr>
<tr>
<td>Time from transplant (mo)</td>
<td>38.10 ± 29.70</td>
<td>38.10 ± 29.70</td>
</tr>
<tr>
<td>(range, 11 to 114)</td>
<td></td>
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</tr>
<tr>
<td>BMI</td>
<td>25.82 ± 4.82</td>
<td>25.48 ± 4.01</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1.49 ± 0.46</td>
<td>1.63 ± 0.65</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>63.57 ± 20.38</td>
<td>59.20 ± 18.50</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>141.50 ± 66.80</td>
<td>202.70 ± 81.90</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>203.80 ± 51.20</td>
<td>231.40 ± 48.20</td>
</tr>
<tr>
<td>CsA/TAC blood levels (ng/dl)</td>
<td>563.80 ± 124.50</td>
<td>—</td>
</tr>
<tr>
<td>CsA or TAC dose (mg/d)</td>
<td>173.70 ± 54.90</td>
<td>2.90 ± 1.30</td>
</tr>
<tr>
<td>SRL blood levels (ng/dl)</td>
<td>—</td>
<td>9.72 ± 2.64</td>
</tr>
<tr>
<td>SRL dose (mg/d)</td>
<td>—</td>
<td>3.20 ± 1.40</td>
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*Mean cyclosporine A (CsA) whole-blood levels (C2) are the mean of six measures recorded during the 6 mo preceding the discontinuation of the calcineurin inhibitor (CNI). Mean tacrolimus (TAC) trough levels are the mean of six measures recorded every other week in the first 3 mo after engraftment. Whole-blood sirolimus (SRL) trough levels were measured every other week in the first 3 mo after renal transplant (group 2), then at monthly intervals up to the end of the study (groups 1 and 2). BMI, body mass index.

\(P = 0.04.\)

\(P = 0.0003.\)
2). The withdrawal of CNI did not significantly modify any of the above measures.

DI. The DI can be envisioned as a measure of the ability of the \( \beta \) cells to compensate for insulin resistance. The assertion of the foregoing is that the earliest phenotypic \( \beta \) cell defect that may be detected in otherwise glucose-tolerant individuals is a reduced DI.

Renal transplant recipients who were taking CNI, with or without low-dose sirolimus, had an overall normal DI (Table 2), thereby suggesting an adequate feedback loop between the insulin-sensitive tissues and the \( \beta \) cell response. In sharp contrast, patients who were on sirolimus-based immunosuppression showed a brisk decrease of DI (Table 2). Thus, when the effect of insulin sensitivity on \( \beta \) cell function is accounted for, a seemingly appropriate insulin response in fact was to be considered inappropriately low in the face of insulin resistance.

Stratification of Patients According to ADA/WHO Criteria for Classification of Disturbances of Glucose Metabolism

The combined use of FSG and OGTT allowed us to enlarge and refine the diagnosis of the defects of glucose metabolism. At baseline, 18 (43.9%) patients had IFG and 13 (31.7%) showed IGT. The conversion to sirolimus seems to have worsened glucose homeostasis: The proportion of IGT patients rose to 41.5% (17 patients) and four patients developed posttransplantation diabetes (two patients for each group), 17 recipients showing IFG (\( P = 0.009 \), by McNemar test for paired comparisons). Of note, FSG identified only two of four patients with 2-h blood glucose \( \geq 11.1 \) mmol/L.

Correlations between Variables

We first sought a relationship between sirolimus exposure and glucose homeostasis. Mean sirolimus trough levels resulted to correlate weakly with the change of insulin sensitivity (\( \Delta \)-KITT \( \rho = -0.330, P = 0.04 \)), as well as with \( \Delta \)-first phase insulin release (\( \rho = -0.361, P = 0.02 \)). Then, the changes in the parameters of glucose metabolism failed to correlate with the change of either sCr or CrCl over the follow-up period (data not shown).

Finally, because abnormalities of triglyceride storage and increased free fatty acid flux have been shown to lead to impaired insulin sensitivity and impaired pancreatic \( \beta \) cell function (24), we wondered whether the change of serum triglyceride after the conversion to sirolimus would correlate with the change in the parameters of glucose metabolism. Increasing triglycerides correlated strongly with decreasing insulin sensitivity and explained nearly one third of the variability in KITT after sirolimus therapy (Figure 4). Similarly, a positive change in serum triglyceride was associated with a decrease of \( \beta \) cell response, as measured by the change of DI (Figure 4).

Discussion

The mammalian target of rapamycin (mTOR) pathway is emerging as a critical player in the cause of metabolic diseases,
The serine-threonine kinase mTOR plays a key role in the insulin signaling cascade. Thus, sirolimus has the potential to affect strikingly glucose metabolism. Some in vitro evidence would support the above assertion. A sirolimus-sensitive pathway, most likely acting via P70 S6K, has been implicated in the regulation of glycogen synthase kinase 3 and glycogen synthase and in the inactivation of phosphorylase by insulin (31,32). Moreover, sirolimus has been shown to abrogate the insulin-mediated increase in GLUT1 protein synthesis, thereby possibly modulating also insulin-independent glucose transport (33) mTOR and P70 S6K signal transduction pathways also have been shown to control β cell size and proliferation and insulin release; in this way, the inhibition of P70S6K activation by sirolimus might contribute to the onset and development of “insulin resistance” in the β cell (34–36). Finally, Andoh et al. (37) reported that subtherapeutic doses of sirolimus were able to induce glucose intolerance in a rat model of CsA nephrotoxicity and that the addition of CsA strikingly worsened glucose intolerance and the degree of insulin deficiency.

Large clinical trials did not reveal any increase in the incidence of posttransplantation diabetes among patients who were treated with sirolimus (13–16), either alone or in combination with CNI, although recent investigations have challenged this conclusion (38,39). In the above studies, however, posttransplantation diabetes has been defined and recognized only by the patient’s requirement for insulin. As a matter of fact, none of these clinical trials has routinely included OGTT to determine the exact incidence of glycemic abnormalities in renal transplant recipients who are treated with the mTOR inhibitor. Like type 2 diabetes, the onset of posttransplantation diabetes can be insidious, and individuals may be asymptomatic for years before symptoms manifest clinically (1,7). Available evidence from the general population suggests that OGTT levels may be more predictive of an increased risk for cardiovascular disease than the FSG test, especially in individuals with IGT (40–42). Finally, insulin resistance is associated with an increased risk for myocardial infarction and other clinical cardiovascular events, even in patients who do not have hyperglycemia (44).

After the conversion to sirolimus-based immunosuppression, we identified >40% of IGT patients who had a 30% increase of incidence compared with pretreatment values. Our data show...
that if fasting blood glucose alone had been used, then 30% of patients with isolated IGT would have received a diagnosis of having normal glucose tolerance. Four patients had 2-h blood glucose levels compatible with the diagnosis of posttransplantation diabetes, only two of them showing fasting glucose levels >7 mmol/L. Finally, 36 of 41 patients displayed an increase of insulin resistance. Rather unexpected, we found a deterioration of glucose metabolism even among patients who discontinued tacrolimus and were converted to full-dose sirolimus. None of the patients experienced acute rejection or other acute events, such as infection, potentially responsible for the worsening of glucose metabolism; neither was there a deterioration of graft function, which slightly improved. Moreover, it has been demonstrated that long-term use of tacrolimus does not cause chronic, cumulative pancreatic toxicity (30); therefore, an adverse and cumulative effect of the CNI persisting over time, even months after its withdrawal, seems unlikely. Thus, the simplest explanation that we can raise is that full-dose sirolimus (mean trough 11.4 ng/ml) is more “diabetogenic” than a combination of low-dose tacrolimus (trough 6.1 ng/ml) plus low-dose sirolimus (trough 5.2 ng/ml). It may be of interest that a pharmacodynamic study in our laboratory has revealed that tacrolimus hampers the inhibitory effect of sirolimus on P70 S6 kinase in circulating mononuclear cells, which might help to explain the lower diabetogenic impact of low-dose tacrolimus/sirolimus, as compared with full-dose sirolimus (S.D.P. et al., unpublished data).

Previously, an in vitro study had suggested that sirolimus may partially decrease insulin resistance induced by chronic insulin exposure of 3T3-L1 adipocytes, by preventing the reduction of IRS-1 protein levels and Akt Ser-473 phosphorylation, with a partial normalization of insulin-induced glucose transport (45). Although the molecular mechanisms that cause insulin resistance in humans are largely unknown, we may suppose that in vivo

### Table 2. Evaluation of glucose metabolism, insulin resistance, and β cell function in the two groups of patients studied, while taking CNI (CsA or TAC) and 6 months after the discontinuation of CNI and the conversion to SRL-based immunosuppression

<table>
<thead>
<tr>
<th>Group 1 (n = 26)</th>
<th>Group 2 (n = 15)</th>
<th>Controls (n = 70)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CsA</td>
<td>SRL (low-dose)</td>
</tr>
<tr>
<td>AUC glucose (mmol/L)</td>
<td>15.63 ± 3.67</td>
<td>16.21 ± 3.54</td>
</tr>
<tr>
<td>AUC insulin (pmol/L)</td>
<td>1225.70 ± 660.09</td>
<td>1199.13 ± 594.26</td>
</tr>
<tr>
<td>AUC C-peptide (nmol/L)</td>
<td>5.57 ± 3.12</td>
<td>6.31 ± 2.63b</td>
</tr>
<tr>
<td>Secretory AUC</td>
<td>77.22 ± 35.95</td>
<td>72.69 ± 28.24</td>
</tr>
<tr>
<td>First-phase insulin release (pmol/L)</td>
<td>1949.36 ± 797.48</td>
<td>1838.38 ± 671.23</td>
</tr>
<tr>
<td>Second-phase insulin release (pmol/L)</td>
<td>523.25 ± 189.22</td>
<td>484.59 ± 169.55</td>
</tr>
<tr>
<td>DI</td>
<td>100.70 ± 101.23</td>
<td>76.06 ± 101.62c</td>
</tr>
<tr>
<td>MCR (ml/kg per min)</td>
<td>6.77 ± 3.82</td>
<td>6.08 ± 3.95c</td>
</tr>
</tbody>
</table>

*aResults from healthy control subjects, strictly matched for age, gender, BMI, and family history of diabetes, are shown.

AUC, area under curve; DI, disposition index; MCR, metabolic clearance rate.

bP = 0.04, cP = 0.02, dP = 0.004 versus basal values (before the conversion to SRL-based immunosuppression).
several interfering factors may affect the intracellular machinery that is responsible for the response to the hormone. Sirolimus alters the insulin signaling pathway so as to increase adipose tissue lipase activity and/or decrease lipoprotein lipase activity, resulting in in vivo increased hepatic synthesis of triglyceride, increased hypertriglyceridemia, and expanded free fatty acid pool. Free fatty acids, in turn, deteriorate peripheral insulin sensitivity and pancreatic β cell function, leading to impaired glucose metabolism. Accordingly, we found that the change of insulin resistance and β cell response of renal transplant recipients who were taking sirolimus significantly correlated with the increase in serum triglyceride levels. Obviously, our study does not allow us to rule out that, conversely, increased triglycerides are the consequence, rather than the cause, of insulin resistance and inadequate insulin release.

A possible limitation of our study should be discussed, namely the use of a clinic sample with mild renal impairment, which might limit the generalizability of the conclusions to the majority of renal transplant recipients. A recent analysis of patients with varying degrees of renal function impairment demonstrated increased plasma glucose and insulin response to oral glucose load and reduced sensitivity to insulin only in patients with CrCl < 50 ml/min, although previous research suggested that abnormal glucose metabolism may be part of the phenotype of some renal diseases, independent of renal function. More important, neither study was able to find any correlation between CrCl and parameters of glucose metabolism. In the cohort studied here, the conversion to sirolimus was associated with only a slight derangement of glucose homeostasis after the conversion to sirolimus. In conclusion, the results of this study suggest that the mTOR inhibitor sirolimus increases peripheral insulin resistance and impairs pancreatic β cell response and thus possibly worsens glucose homeostasis in renal transplant recipients. These findings support the need for an extensive monitoring of blood glucose levels, both fasting and postload, in all renal transplant recipients.

References


