**Insulin Resistance After Renal Transplantation**

**Impact of immunosuppressive and antihypertensive therapy**

**OBJECTIVE** — The purpose of the present study was to validate various surrogate estimates of insulin sensitivity (IS) in a renal transplant population and to assess the influence of immunosuppressive and antihypertensive therapy on insulin resistance (IR) after renal transplantation.

**RESEARCH DESIGN AND METHODS** — A total of 167 consecutive renal transplant recipients without previously known diabetes underwent a 75-g oral glucose tolerance test (OGTT) 3 months after renal transplantation. A total of 43 patients also underwent a euglycemic-hyperinsulinemic glucose clamp study. Six OGTT-derived IS indexes were validated against the euglycemic-hyperinsulinemic glucose clamp-derived IS index (ISICLAMP).

**RESULTS** — The OGTT-derived ISI TX correlated closely with the ISICLAMP (r = 0.58, P < 0.001). The other surrogate estimates of IS were also significantly but less well correlated with the ISICLAMP (Spearman’s correlation; r = −0.45 to 0.41, P = 0.003–0.050). In the univariate model, BMI, daily prednisolone dose, creatinine clearance, hypertension, number of antihypersensitive agents, and use of diuretics or β-blockers were negatively associated with ISI TX (P < 0.05). After multiple regression analysis, BMI (P < 0.001), daily prednisolone dose (P < 0.001), cytomegalovirus infection (P = 0.030), and triglycerides (P = 0.034) were shown to be independent predictors of posttransplant IR.

**CONCLUSIONS** — The OGTT-derived ISI TX may be a useful estimate of IS in Caucasian renal transplant recipients. Increasing daily prednisolone dose is an independent predictor of IR after renal transplantation. Hypertension and the use of β-blockers and diuretics may also deteriorate IR in this group of patients.

Renal transplant recipients are insulin-resistant compared with age- and sex-matched controls (1). Impaired nonoxidative glucose disposal explains the development of insulin resistance (IR) in patients with posttransplant diabetes mellitus (PTDM), similar to that observed in patients with type 2 diabetes in the general population (1). Recipients with posttransplant impaired glucose tolerance (IGT) are equally as insulin-resistant as patients with PTDM but have a more preserved insulin response (2).

It is well known that immunosuppressive drugs, primarily prednisolone, provoke glucose intolerance by increasing IR, whereas the mechanisms for the possible diabetogenic effect of cyclosporin A (CsA) and tacrolimus are less well defined (3–5). The combination of prednisolone and CsA may also be deleterious due to mutual inhibition of metabolism (6).

The prevalence of hypertension after renal transplantation is high. After the introduction of CsA, hypertension has been reported in 60–70% of renal transplant recipients and ≤90% of extrarenal transplant recipients (7). In the general population, both hypertension per se and some antihypertensive medications (8) have been reported to be associated with impaired insulin sensitivity (IS). Moreover, in a recently published study, the probability of developing type 2 diabetes was twofold increased in subjects with hypertension, and the group of patients receiving β-blockers had a higher risk of developing diabetes than individuals not treated with antihypertensive drugs (9). β-Blockers and diuretics may worsen IR, whereas calcium channel antagonists have been reported to be neutral and angiotensin-converting enzyme (ACE) inhibitors and α-blockers may have beneficial effects on IS (8).

We have previously reported that prednisolone dose, age, and use of β-blockers are independent predictors of glucose intolerance 10 weeks after transplantation, whereas a positive HLAB27 phenotype and ganciclovir-treated cytomegalovirus (CMV) infections are independent predictors of PTDM (3).

The euglycemic-hyperinsulinemic glucose clamp technique (10) is the gold standard for measuring IS, but this method may be both expensive and cumbersome in larger studies. Various surrogate estimates of IS based on serum insulin and glucose values measured during oral glucose tolerance tests (OGTTs) (11,12) have been reported to correlate well with the corresponding glucose clamp results in nontransplant populations and may be useful in epidemiological studies. However, these studies were...
performed in different populations with varying degrees of glucose tolerance and ethnicity (11–13), and there is no consensus in the literature whether any particular IS index is most appropriate. Moreover, renal transplant patients are characterized by disturbances in both insulin secretion and IR. Revalidation of the previously published IS indexes seems necessary.

The objectives of the present study were, first, to validate six OGTT-derived IS indexes reported in the literature against a euglycemic-hyperinsulinemic glucose clamp–derived IS index (ISICLAMP) in renal transplant recipients and, second, to assess the influence of immunosuppressive and antihypertensive therapy on IR after renal transplantation.

**RESEARCH DESIGN AND METHODS**

**Study population**

A total of 167 consecutive patients, 117 men and 50 women, most of whom were Caucasian (n = 160), underwent a 75-g OGTT at the Rikshospitalet in Oslo, Norway, 10 weeks (70 ± 6 days) after renal transplantation between February 1995 and June 1996. A total of 66 patients (40%) had received a kidney from a living donor. Patients with previously known diabetes were excluded from the study (n = 25).

All patients were given prednisolone; 162 patients were taking CsA (Sandimmun Neoral; Sandoz Pharma [Novartis], Basel), 144 were taking azathioprine, 4 were taking tacrolimus, and 1 was taking mycophenolate mofetil. Oral prednisolone was started on day 1 at 80 mg/day initially and tapered by 10 mg/day until 20 mg/day was reached. Further reduction of 5 mg/day was performed every 2 weeks until 10 mg/day was reached. Rejection episodes were treated with an intravenous bolus of methylprednisolone (500–125 mg/day) for 4–5 days, and oral prednisolone was increased to 30 mg and then tapered by 5 mg every 2 weeks. Biopsy-verified steroid-resistant rejections were treated with antilymphocyte antibodies (ATG/OKT3).

A total of 83 patients (50%) had normal glucose tolerance (NGT); 5 patients (3%) had impaired fasting glucose (IFG), 50 (30%) had IGT, and 29 (17%) had PTDM (14). A subgroup of 43 patients underwent a euglycemic-hyperinsulinemic glucose clamp study. The recruitment procedure, study population, and immunosuppressive protocol have been reported in detail elsewhere (2,3).

The mean age of the patients was 47 years (SD 16, range 18–80), and BMI was 23.5 kg/m² (3.8, 12.8–39.2). The mean daily prednisolone dose was 15 mg (7, 10–30), daily CsA dose was 342 mg (115, 175–1,000), whole blood CsA trough level was 242 μg/l (60, 110–535), and serum creatinine level was 145 μmol/l (44, 64–350). A total of 57% of the recipients had been treated for one or more rejections, and 25% had been treated for CMV infection. A total of 19% (n = 32) of the patients had a first-degree relative with diabetes. A total of 140 patients (84%) had hypertension (defined as repeated blood pressure measurements >140/90 mmHg or use of antihypertensive medication), 130 of whom were taking antihypertensive drugs. A total of 70 patients (42%) were treated with one blood pressure-lowering agent, whereas 60 patients (36%) used two or more antihypertensive drugs. A total of 68 patients (41%) were taking a β-blocker, 58 (35%) were taking a diuretic, 68 (41%) were taking a calcium channel antagonist, 47 (28%) were taking an ACE inhibitor, and 23 (14%) were taking an α-blocker.

All patients gave informed consent to participate in the study, which was performed in accordance with the Declaration of Helsinki (15).

The patients who underwent the euglycemic-hyperinsulinemic glucose clamp examination were comparable with those who only underwent the OGTT in terms of age (P = 0.182), sex (P = 0.267), family history of diabetes (P = 0.605), BMI (P = 0.923), daily prednisolone dose (P = 0.512), CsA trough concentration (P = 0.202), creatinine clearance (P = 0.673), and hypertension (P = 0.182) (using Pearson χ² test or independent samples Student’s t test as appropriate). Of these patients, 6 (14%) had PTDM, 11 (26%) had IGT, 1 (2%) had IFG, and 25 (58%) had NGT (14).

**Procedures**

After an overnight fast, serum glucose and insulin were measured at 0, 1, and 2 h during a 75-g OGTT. The blood samples were collected in SST tubes (Becton Dickinson, Franklin Lakes, NJ) for coagulation and spun for separation of serum within 30 min. Analysis of serum glucose was performed immediately after centrifugation using a glucose dehydrogenase method (Cobas Mira; Roche, Basel). Serum insulin level was determined by a commercial radioimmunoassay (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA). Whole-blood CsA concentrations were measured using a CsA-specific fluorescence polarization immunoassay (TDx analyzer; Abbott Laboratories, Chicago, IL).

The euglycemic-hyperinsulinemic glucose clamp study was performed using a modification of the method described by DeFronzo et al. (2,10). Insulin was infused at a fixed rate of 1 mU·kg⁻¹·min⁻¹ and blood glucose was clamped at 5 mmol/l and maintained for 120 min. Lean body mass was estimated using Hume’s formula (16), which recently has been shown to correlate well with triitated water or electrical bioimpedance measures (r = 0.85 and 0.86, respectively) (17). The glucose disposal rate was calculated from the amount of glucose infused during the last 60 min, and the IS index (ISICLAMP) was calculated as the glucose disposal rate (μmol·kg⁻¹·min⁻¹) divided by mean serum insulin (pmol/l) during the last 60 min of the clamp. The ISICLAMP was correlated with various estimates of IS and IR reported in the literature.

On the basis of the validation study, the most appropriate estimate of IS was used to assess several potential predictors for posttransplant IR.

**IS indexes**

Stumvoll et al. (12) proposed the following equation to be useful in calculating a surrogate estimate of IS in a nonobese Caucasian population (n = 104):

\[
\text{IS}_{\text{STUMVOLL}} = 0.226 - 0.0032 \cdot \text{BMI (kg/m}^2\text{)} - 0.0000645 \cdot \text{InS}_{120} \text{ (pmol/l)} - 0.00375 \cdot \text{Gluc}_{90} \text{ (mmol/l)}
\]

where InS₁₂₀ and Gluc₀ are OGTT serum insulin and glucose values after 120 and 90 min, respectively (12). In the present study, serum glucose was measured after 60 and 120 min, and the latter glucose value was implemented in the slightly modified formula:

\[
\text{IS}_{\text{STUMVOLL}} = 0.226 - 0.0032 \cdot \text{BMI (kg/m}^2\text{)} - 0.0000645 \cdot \text{InS}_{120} \text{ (pmol/l)} - 0.00375 \cdot \text{Gluc}_{120} \text{ (mmol/l)}
\]

Matsuda and DeFronzo (11) derived a composite index that was validated in 153
subjects with varying degrees of glucose tolerance, including 60 subjects with type 2 diabetes:

\[
ISI_{\text{MATSUDA}} = \frac{10,000}{\sqrt{\text{Gluc}_0 \cdot \text{lns}_0 \cdot \text{Gluc}_{\text{mean}} \cdot \text{Ins}_{\text{mean}}}}
\]

where Gluc_0 and Ins_0 represent fasting serum values of glucose and insulin, respectively, whereas Gluc_{mean} and Ins_{mean} are mean concentrations of glucose and insulin during the OGTT.

Furthermore, the IR indexes homeostatic model assessment (IRI_{HOMA} = \lns_0 \cdot \text{Gluc}_0 \cdot \text{IRI}_{\text{AUCGI}} (11), the product of the glucose area under the serum glucose curve and insulin area under the serum insulin curve IRI_{\text{AUCGI}} (11), the fasting insulin IRI_{\text{INSO}} (12), and the 2-h insulin IRI_{\text{INS120}} (12) were validated. Area under the curve was calculated by use of the trapezoidal method.

### Statistical analysis

Correlation coefficients were calculated between the various ISIs and IRIs from the OGTTs and the ISI_{CLAMP}. The Altman-Bland plot was used to evaluate the agreement between the ISIs from the OGTT and the glucose clamp study (18).

Linear regression, unpaired Student’s t tests, and multiple linear regression were used as appropriate in the assessment of different potential risk factors for IR as the dependent variable. Serum triglyceride concentrations were log-transformed to obtain a normal distribution before statistical analysis. Associations between variables in the univariate analyses with \( P < 0.1 \) were included in the multiple regression models. All statistical tests of significance were two-tailed, and \( P < 0.05 \) was considered significant. The analysis was implemented using SPSS version 11.5 for Windows (Chicago, IL).

<table>
<thead>
<tr>
<th></th>
<th>Pearson’s correlation ( (r) )</th>
<th>Spearman’s correlation ( (\rho) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRI_{\text{INSO}}</td>
<td>-0.39#</td>
<td>-0.32#</td>
</tr>
<tr>
<td>IRI_{\text{INS120}}</td>
<td>-0.50#</td>
<td>-0.45#</td>
</tr>
<tr>
<td>IRI_{\text{AUCGI}}</td>
<td>-0.48#</td>
<td>-0.44#</td>
</tr>
<tr>
<td>IRI_{\text{HOMA}}</td>
<td>-0.40#</td>
<td>-0.30#</td>
</tr>
<tr>
<td>ISI_{\text{MATSUDA}}</td>
<td>0.38#</td>
<td>0.41#</td>
</tr>
<tr>
<td>ISI_{\text{STUMOD}}</td>
<td>0.59*</td>
<td>0.58*</td>
</tr>
</tbody>
</table>

* \( P < 0.001 \); † \( P < 0.01 \); ‡ \( P < 0.05 \).

---

Figure 1—A: Correlation between the ISI_{TX} and the ISI_{CLAMP}. B: Altman-Bland plot.
Therefore, the ISI TX was considered appropriate to use as the surrogate estimate of ISA in epidemiological studies in Caucasian renal transplant recipients treated with prednisolone and CsA.

However, the present study has some limitations. First, the number of patients who underwent the glucose clamp was limited (n = 43). Second, the mean daily prednisolone dose and frequency of rejections have probably decreased after the introduction of newer immunosuppressive protocols. Finally, the frequency of CMV infections in our study may be relatively high compared with centers using prophylactic agents.

Increasing BMI correlated strongly with decreasing IS and explained nearly one-third of the variability in ISI TX, which is in accordance with findings in the general population (19). One possible explanation for obesity-induced IR may be the higher levels of free fatty acids, which may deteriorate peripheral IS and promote hepatic glucose production (20).

Because BMI is included in the equation for the dependent parameter ISI TX, an additional multiple regression analysis, excluding BMI, was performed. Increasing prednisolone dose was still independently associated with IR (P = 0.006), whereas triglycerides and CMV infection were shown to be insignificant.

### Table 2—ISI TX as the dependent variable correlated with body weight, renal function, number of antihypertensive agents used, prednisolone dose, and triglycerides by the use of linear regression (n = 167)

<table>
<thead>
<tr>
<th></th>
<th>β (×10⁻³)</th>
<th>95% CI (×10⁻³) (β)</th>
<th>r²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>−3.7</td>
<td>−4.5 to −2.9</td>
<td>0.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Daily prednisolone dose</td>
<td>−1.1</td>
<td>−1.7 to −0.6</td>
<td>0.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>−0.3</td>
<td>−0.5 to −0.1</td>
<td>0.04</td>
<td>0.013</td>
</tr>
<tr>
<td>Number of antihypertensive agents used</td>
<td>−4.9</td>
<td>−8.9 to −0.9</td>
<td>0.04</td>
<td>0.017</td>
</tr>
<tr>
<td>Ln triglycerides</td>
<td>−8.1</td>
<td>−16.2 to −2.2</td>
<td>0.02</td>
<td>0.050</td>
</tr>
</tbody>
</table>

### CONCLUSIONS

To our knowledge, the present study is the first to validate OGTT-derived ISIs in a renal transplant population. The modification of ISI STUMVOLL, ISI TX, which includes BMI, 2-h glucose, and 2-h insulin in the equation, correlated best (r = 0.58) with the ISI CLAMP. Therefore, we suggest that the ISI TX may be used as a surrogate estimate of IS in epidemiological studies in Caucasian renal transplant recipients treated with prednisolone and CsA.

### Table 3—ISI TX in different groups of patients characterized by presence of CMV infection, hypertension, and use of various antihypertensive drugs (n = 167), by independent-samples student’s t test

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>ISI TX (mean ×10⁻²)</th>
<th>Difference (×10⁻²) (mean)</th>
<th>95% CI (×10⁻²) (mean)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diuretic</td>
<td>59 107</td>
<td>4.6 6.0</td>
<td>−1.4</td>
<td>−2.2 to −0.6</td>
</tr>
<tr>
<td>β-blocker</td>
<td>69 97</td>
<td>4.8 6.0</td>
<td>−1.2</td>
<td>−2.0 to −0.4</td>
</tr>
<tr>
<td>Hypertension</td>
<td>140 26</td>
<td>5.3 6.7</td>
<td>−1.4</td>
<td>−2.5 to −0.4</td>
</tr>
<tr>
<td>CMV infection</td>
<td>42 125</td>
<td>4.9 5.7</td>
<td>−0.8</td>
<td>−1.7 to 0.1</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>47 119</td>
<td>5.0 5.8</td>
<td>−0.8</td>
<td>−1.6 to 0.1</td>
</tr>
</tbody>
</table>
tion for the dependent parameter ISITX, one may argue that a correlation between the two variables is inevitable. However, a highly significant correlation between BMI and IR was found ($P < 0.001$), even when using ISIs not including BMI in the equation (11,13).

In the present study, increasing daily prednisolone dose was independently associated with IR. Glucocorticoids promote glucoseogenesis in the liver, inhibit glucose uptake, diminish glycogen synthesis in skeletal muscle cells, and also may attenuate insulin secretion from pancreatic $\beta$-cells (21,22).

Furthermore, steroid treatment may explain the discrepancy between ISISTUMOD and ISICLAMP; on average, the former was 2% lower than the mean ISI STUMOD. However, explanation for some of the discrepancy between ISISTUMOD and ISI CLAMP. However, when interpolating a 90-min serum glucose between the 1- and 2-h values, the mean ISISTUMVOLL was shown to be only 24% higher than the latter. This view is supported by the study published by Henriksen et al. (21), in which IS decreased ~50% after 5 days of treatment with dexamethasone 4 mg/day.

One may argue that the implementation of the 2-h serum glucose value in the ISISTUMOD, compared with the 90-min glucose value in the ISISTUMVOLL, could explain some of the discrepancy between the ISISTUMOD and ISICLAMP. However, when interpolating a 90-min serum glucose between the 1- and 2-h values, the mean ISISTUMVOLL was shown to be only 2% lower than the mean ISISTUMOD ($0.092 \pm 0.094$).

Neither the daily CsA dose nor the whole-blood CsA trough level influenced IS significantly. CsA probably increases the risk for PTDM due to impaired insulin release (4) or worsening of IR (5,23). Our results do not preclude any negative effect of CsA on IS but suggest that any such effect, if present, is not dose-dependent.

Higher levels of triglycerides were significantly correlated with decreasing IS in the multivariate model, which is in accordance with other reports (24,25). Recently, it has been reported that plasma triglyceride concentration is inversely related to the level of plasma lipoprotein lipase (activity and mass) (24). Decreasing plasma triglycerides and nonesterified fatty acids, rather than weight loss per se, has improved IS after biliary-pancreatic diversion (25).

A somewhat unexpected trend for lower IS in patients with ganciclovir-treated CMV infections turned out to be significant in the multiple regression analysis. One cannot find support for any causal relationship between CMV infection and IR in the literature, whereas the hypothesis of virus-induced $\beta$-cell dysfunction is well known (3). One possible explanation may be the higher incidence of rejections and steroid doses in patients with CMV infections.

Hypertension, the number of antihypertensive agents used, and use of diuretics and $\beta$-blockers were all associated with IR, which is in accordance with previous reports from nontransplant populations (8). Moreover, in a recently published large prospective cohort study of 12,550 nondiabetic subjects followed for 6 years, the probability of developing type 2 diabetes was increased twofold in subjects with hypertension, and patients taking $\beta$-blockers had a higher risk of developing diabetes than individuals not treated with antihypertensive drugs (9). However, in our study, the influence of hypertension and antihypertensive agents on IS was shown to be insignificant in the multivariate analysis. This may be explained by a stronger influence of BMI and prednisolone on IS, the low percentage of normotensive patients, and the fact that nearly one-half of the patients (60/130) on antihypertensive therapy used a combination of two or more drugs.

In conclusion, the present study indicates that a modification of the OGTT-derived ISISTUMVOLL may be useful to assess IS in renal transplant recipients and that increasing daily prednisolone dose is an independent predictor of IR after renal transplantation. Hypertension and use of various antihypertensive drugs may also adversely influence IS in renal transplant recipients.

### References

7. First MR, Neylan JF, Rocher LL, Tejani A:


