Insulin sensitivity index in type 1 diabetes and following human islet transplantation: comparison of the minimal model to euglycemic clamp measures

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Insulin sensitivity index is an underappreciated feature of type 1 diabetes (T1D) (7, 13, 32, 33), resulting at least in part from the absence of endogenous insulin secretion, frequent periods of sustained hyperglycemia (12, 21, 31), and impaired sensitivity of lipolysis to inhibition by insulin, resulting in elevated free fatty acids (FFA) (24, 33). Islet transplantation restores endogenous insulin secretion that can correct the hyperglycemia of T1D and normalize FFA metabolism (15, 20, 27), effects that have been associated with improved minimal model indexes of insulin sensitivity derived from an insulin-modified frequently sampled intravenous glucose tolerance (FSIGT) test in T1D recipients of islet transplants. More recently, employing the hyperinsulinemic-euglycemic clamp technique with a stable glucose isotope, our group has confirmed this correction of impaired insulin sensitivity in T1D by islet transplantation and shown that the improvement is mediated by effects at the liver and skeletal muscle (18). Extension of these findings to larger populations and to other therapeutic interventions for T1D may be more readily accomplished using the FSIGT test that can be more easily standardized across clinical sites.

Whereas the minimal model index of insulin sensitivity, $S_I$, can be derived in T1D subjects using an insulin-modified frequently sampled intravenous glucose tolerance (FSIGT) test with good parameter resolution (10, 20, 28), $S_I$ has not before been evaluated against measures of insulin sensitivity derived from the gold-standard hyperinsulinemic-euglycemic clamp in T1D as has been performed in subjects with type 2 diabetes (T2D) (22). Because the development of absolute insulin deficiency in T1D is distinct from the relative insulin deficiency present in T2D, a comparative analysis of clamp and minimal model-derived indexes of insulin sensitivity in T1D subjects is critical to the ongoing application of the minimal model approach to the study of insulin sensitivity in this population. The minimal model-derived $S_I$ does not distinguish between insulin action to suppress glucose production (primarily from the liver) and that to enhance peripheral glucose disposal (primarily in skeletal muscle), which requires isotope tracer methodology but does provide an estimate of total body insulin sensitivity that is believed to largely represent peripheral insulin action needed for disposal of the injected glucose load. We sought to determine how well the FSIGT minimal model-derived $S_I$ compared with total body and peripheral insulin sensitivity estimates derived from the hyperinsulinemic-euglycemic clamp with a stable glucose isotope in subjects with T1D and following islet transplantation.
MATERIALS AND METHODS

T1D subjects included those with long-standing C peptide-negative disease complicated by hypoglycemia unawareness and frequent severe hypoglycemia events who had normal kidney function and were initially considered as potential candidates for islet alone transplantation \((n = 21)\). A subgroup of these subjects received intrahepatic islet transplants \((n = 12)\) as part of the Clinical Islet Transplantation (CIT) Consortium protocols being conducted at the University of Pennsylvania \((1)\). The study protocols were approved by the Institutional Review Board of the University of Pennsylvania, and all subjects gave their written informed consent to participate.

The islet transplant recipients included all \(11)\) subjects participating in the CIT07 protocol from our institution that included thymoglobulin and rituximab for induction and was later retransplanted under an Edmonton protocol with CIT05 protocol consisting of thymoglobulin and rituximab for induction and was later retransplanted under an Edmonton protocol with basiliximab induction. The transplant recipients underwent one or two intraportal infusions of islets to achieve insulin independence. Maintenance immunosuppression consisted of low-dose tacrolimus (12-h blood trough target 3–6 \(\mu\)g/l) and sirolimus (24-h blood trough target 10–15 \(\mu\)g/l for the first 3 mo and 8–12 \(\mu\)g/l thereafter).

Healthy nondiabetic control subjects for the FSIGT \((n = 11)\) were selected for gender, age, and body mass index (BMI) from other studies conducted by our group \((23, 25)\) to match that of the T1D subjects.

Metabolic studies. All 21 T1D subjects underwent FSIGTs and hyperinsulinemic-euglycemic clamps between 2 days and 1 mo apart. When performed the same week, the FSIGT was conducted on the first day, and menstruating women underwent both tests during the follicular phase of their menstrual cycle \((26)\). The 12 subjects who underwent islet transplantation each repeated the FSIGT between 2.5 and 7 mo posttransplant and the euglycemic clamp between 6 and 7 mo posttransplant. The median (interquartile range) time between the posttransplant FSIGT and euglycemic clamp studies was 3 \((2.5–3.5)\) mo. For both the euglycemic clamp and FSIGT, T1D subjects and insulin-dependent islet transplant recipients were admitted to the University of Pennsylvania Clinical and Translational Research Center (CTR) the afternoon before study and fasted overnight after 2000 for 12 h before testing. At 2100 subjects were converted from subcutaneous insulin to a low-dose intravenous insulin infusion protocol to target blood glucose at 81–115 mg/dl. Insulin-independent islet transplant recipients and normal controls had the option of fasting overnight either in the CTR or at home with arrival in the CTR by 0630 the morning of study. By 0700, one catheter was placed in an antecubital vein for infusions, and one catheter was placed in a hand vein for blood sampling, with the hand placed in a thermoregulated box \((-50^\circ C)\) or heating pad to promote arterIALIZation of venous blood.

FSIGT. When used overnight, the insulin infusion was discontinued 20 min before testing. All other medications were withheld until later in the morning. After baseline blood sampling at \(-10, -5\), and \(-1\) min, 0.3 g/kg of 50% glucose was injected over 1 min starting at \(t = -30\) s, and 0.03 U/kg of insulin \((1 U / 1 m l\) solution) was injected over \(30\) s starting at \(t = 20\) min. Additional blood samples were collected at \(t = 1, 2, 3, 4, 5, 7, 10, 12, 14, 16, 18, 20, 22, 25, 30, 40, 50, 70, 100, 140,\) and \(180\) min after the injection of glucose \((20, 22, 26)\). All samples were centrifuged at \(4^\circ C\), separated, and frozen at \(-80^\circ C\) for subsequent analysis. Serum glucose was measured by the glucose hexokinase method using an automated glucose analyzer (Roche Module P; Roche Diagnostics, Indianapolis, IN) and serum insulin by two-site immune-enzymometric assay using a Tosoh 2000 autoanalyzer (Tosoh Biosciences, San Francisco, CA) at the Northwest Lipid Research Laboratory (University of Washington, Seattle, WA).

Euglycemic clamp. At \(t = -120\) min a primed \((5 mg/kg \cdot fasting\) plasma glucose in mg/dl \(\times 0.09\) mg/dl over 5 min) continuous \((0.05 mg/kg \cdot min^{-1}\) for 35 min) infusion of the stable glucose isotope tracer \([6,6-^{2}H_{2}]\) glucose \((99%\) enriched; Cambridge Isotope Laboratories, Andover, MA) was administered to assess endogenous glucose production before and during the induction of hyperinsulinemia \((4, 18)\). When used overnight, the insulin infusion was continued during this period to maintain stable normoglycemia until \(t = 0\). After baseline blood sampling at \(-20, -10,\) and \(-1\) min, at \(t = 0\) min a continuous infusion of insulin was initiated at 1 \(mU / k g \cdot min^{-1}\) for 240 min to produce hyperinsulinemia. Subsequently, a variable-rate infusion of 20% glucose was administered according to the glycemlc clamp technique \((8)\) to maintain the plasma glucose \(-90 mg/dl\). To reduce changes in plasma enrichment of \([6,6-^{2}H_{2}]\) glucose during the clamp, the 20% glucose solution was enriched to 2.0% with \([6,6-^{2}H_{2}]\) glucose \((4)\). Morning immunosuppression medications were taken after baseline blood sampling if applicable. Blood samples were taken every 5 min, centrifuged, and measured at bedside with an automated glucose analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH) to adjust the glucose infusion rate and achieve the desired plasma glucose concentration. Additional blood samples were taken every 20 min for biochemical analysis. All samples were collected on ice in chilled tubes containing EDTA and Protease Inhibitor Cocktail (Sigma-Aldrich, St. Louis, MO), centrifuged at \(4^\circ C\), separated, and frozen at \(-80^\circ C\) for subsequent analysis. Plasma glucose was verified in duplicate by the glucose oxidase method using an automated glucose analyzer (YSI 2300; Yellow Springs Instruments), and plasma insulin was measured in duplicate by double-antibody radioimmunoassay (Millipore, Billerica, MA) as previously described \((18)\). Enrichment of \([6,6-^{2}H_{2}]\) glucose was measured by gas chromatography-mass spectrometry at Metabolic Solutions (Nashua, NH) or the Metabolic Tracer Resource at the University of Pennsylvania.

Calculations and statistics. Basal levels of glucose and insulin were calculated from the mean of the baseline samples preceding \(t = 0\) for the FSIGT. Intravenous glucose tolerance was evaluated from the FSIGT by the glucose disappearance rate \(K_{g} = \ln [\text{glucose}] / \text{min} \times 100\), calculated as the slope of the natural log of glucose values between 10 and 40 min with least-squares linear regression \((29)\) using Origin software (Northampton, MA). The FSIGT parameters for the acute insulin response to the injection of glucose \((AIR_{p})\), insulin sensitivity \((S_{i})\), disposition index \((D_{I} = AIR_{p} \times S_{i})\), and glucose effectiveness \((S_{c})\) were derived from Bergman’s minimal model using MINMOD Millennium software \((6)\) as previously described \((20, 25, 26)\).

The rate of appearance \((R_{A})\) of glucose during the euglycemic clamps was calculated using Steele’s nonsteady state equation modified for the use of stable isotopes: \(R_{A} = (F-V[(C_{2}+C_{1})/2][(E_{2}-E_{1})/(t_{2}-t_{1})])/(E_{2}+E_{1})/2\), where \(C_{2}\) and \(C_{1}\) are the glucose concentrations at the times \(2\) and \(1\), respectively (in mg/ml), \(V\) is the fractional volume of distribution of glucose (40 ml/kg), \(F\) is the tracer infusion rate, and \(E\) represents the isotopic enrichment at the respective time points \((30)\). The rate of disposal \((R_{D})\) of glucose during the euglycemic clamps was calculated using Steele’s nonsteady-state equation \(R_{D} = R_{A} - V[(C_{2}+C_{1})/(t_{2}-t_{1})]\). Total body insulin sensitivity was calculated from the final hour of the euglycemic clamp as \(S_{IClamp} = M / (\Delta G \times G)\) where \(M\) is the glucose infusion rate \((GIR)\), \(\Delta G\) is the change in insulin concentration from basal to the steady-state condition during the final hour of insulin infusion, and \(G\) is the steady-state glucose concentration during the final hour \((2)\). Accounting for space correction \((SC)\), such that \(M = GIR - SC\) where \(SC\) = \((G_{2} - G_{1}) \times 1.9/(t_{2} - t_{1})\) calculates the glucose removed from or added to the glucose space between each time interval where \(G_{2}\) and \(G_{1}\) are the glucose concentrations at the \(times\) \(2\) and \(1\), respectively (in mg/dl) \((8)\), resulted in nearly identical measures for \(S_{IClamp}\) \((r^2 = 0.997; P < 0.00001)\). Peripheral (primarily skeletal muscle) insulin
sensitivity was calculated using the steady-state tracer-derived glucose disposal as \( S_{IP(clamp)} = \Delta R_g(\Delta I \times G) \) (3).

Whereas both minimal model (SI) and clamp \( S_{IC(clamp)} \) and \( S_{IP(clamp)} \) measures reflect insulin sensitivity that is dominated by extrahepatic effects of insulin (3), they are not directly comparable because they are expressed in different units \( [\mu U/ml]^{-1} \) per min and \( ml/min \cdot kg^{-1} \) per \( \mu U/ml \), respectively. This is due to each parameter being normalized to a different measure of body size, with the volume of distribution of glucose used in the minimal model and body weight in the clamp calculations. To convert them to a common index of insulin sensitivity, \( S_{IC} \), reflecting the unitary change in insulin to cause a given increment in glucose clearance \( (dl/min) \) per \( \mu U/ml \), we followed the procedure reported by Bergman’s group (3, 22) such that \( S_{IC(minmod)} = S_I \times V_D \) where the volume of distribution of glucose is calculated as \( V_D \) \( = \) dose of glucose injected in milligrams divided by \( G_0 - G_n \) in mg/dl, where \( G_0 \) is the peak and \( G_n \) the basal glucose derived from the minimal model, \( S_{IC(clamp)} = S_{IC} \times \) body weight in kilograms, and \( S_{IP(clamp)} = S_{IP} \times \) body weight in kilograms. Because the conversion factors used for the minimal model and clamp-derived estimates of insulin sensitivity are independent, this procedure excludes bias in the statistical comparison of the two methods for measuring \( S_{IC} \) (3).

All data are expressed as means ± SE. Comparison of results between pre- and posttransplant T1D subjects was performed with paired Student’s t-tests or Wilcoxon Match Pairs tests for nonparametric data, and comparison of results between each T1D group and controls was performed with unpaired Student’s t-tests or the Mann–Whitney U-test as appropriate using Statistica software (StatSoft, Tulsa, OK). Comparison of the minimal model and clamp-derived indexes of insulin sensitivity were performed by least-squares linear regression using Origin software. Significance was considered at \( P < 0.05 \) (2-tailed).

RESULTS

Subject characteristics. The T1D subjects were of comparable gender distribution, age, body weight, and BMI to the control subjects (Table 1). Those T1D subjects who underwent islet transplantation had a decrease in body weight with resulting decrease in BMI (\( P < 0.01 \); Table 1), although values of both remained not different from normal. The T1D subjects had ∼30 years of disease duration and an insulin requirement

Table 1. Subject characteristics at the time of FSIGT testing

<table>
<thead>
<tr>
<th>TID</th>
<th>Allevated (n = 21)</th>
<th>Pretransplant (n = 12)</th>
<th>Posttransplant (n = 12)</th>
<th>Normal Controls (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>9/12</td>
<td>5/7</td>
<td>5/7</td>
<td>6/5</td>
</tr>
<tr>
<td>Age, yr</td>
<td>46 ± 2</td>
<td>46 ± 3</td>
<td>47 ± 3</td>
<td>40 ± 2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>70 ± 3</td>
<td>71 ± 3</td>
<td>66 ± 3</td>
<td>71 ± 3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25 ± 1</td>
<td>25 ± 1</td>
<td>23 ± 1</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>HbA₁c, %</td>
<td>6.8 ± 0.2</td>
<td>7.1 ± 0.2</td>
<td>5.5 ± 0.1</td>
<td>ND</td>
</tr>
<tr>
<td>T1D duration, yr</td>
<td>31 ± 2</td>
<td>29 ± 4</td>
<td>31 ± 4</td>
<td>ND</td>
</tr>
<tr>
<td>Insulin use, U/kg·day⁻¹</td>
<td>0.50 ± 0.03</td>
<td>0.48 ± 0.05</td>
<td>0.02 ± 0.02*</td>
<td>ND</td>
</tr>
<tr>
<td>IE/kg</td>
<td>9.64 ± 666</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tacrolimus, μg/l</td>
<td>4.8 ± 0.4</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sirolimus, μg/l</td>
<td>9.2 ± 0.7</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SE; n, no. of subjects. FSIGT, frequently sampled intravenous glucose tolerance; T1D, type 1 diabetes; M, males; F, females; BMI, body mass index; IE/kg, islet equivalents transplanted/kg recipient body wt whereby an IE approximates a standard islet diameter of 150 μm. *P < 0.01 for comparison with T1D pretransplant. †One subject was converted from sirolimus to mycophenolate mofetil because of the development of interstitial pneumonia 4 wk posttransplant that subsequently resolved (19).
Following correction to common indexes of insulin sensitivity, the common insulin sensitivity index derived from the minimal model, $S_{IC(minmod)}$, was highly predictive of the common measures $S_{IC(clamp)}$ ($r^2 = 0.74$; $P < 0.00001$; Fig. 3A) and $S_{ICP(clamp)}$ ($r^2 = 0.77$; $P < 0.00001$; Fig. 3B) derived from the euglycemic clamp when examining all 21 T1D subjects, those not transplanted ($n = 9$), and those pretransplant ($n = 12$). This was also the case when considering individual T1D subjects who were not transplanted ($n = 9$) and those pretransplant ($n = 11$) for comparison of $S_{IC(minmod)}$ with both $S_{IC(clamp)}$ ($r^2 = 0.66$; $P < 0.00001$; Fig. 3C) and $S_{ICP(clamp)}$ ($r^2 = 0.70$; $P < 0.00001$; Fig. 3D).

When examining the change in uncorrected insulin sensitivity measures in the transplanted subjects ($n = 11$), $\Delta S_{IC(minmod)}$ correlated with $\Delta S_{IC(clamp)}$ ($r = 0.61$; $P < 0.05$; Fig. 4A) and more weakly with $\Delta S_{ICP(clamp)}$ ($r = 0.50$; $P = 0.07$; Fig. 4B).

For the change in corrected insulin sensitivity measures from pre- to posttransplant ($n = 11$), $\Delta S_{IC(minmod)}$ correlated with both $\Delta S_{IC(clamp)}$ ($r = 0.60$; $P < 0.05$; Fig. 4C) and with $\Delta S_{ICP(clamp)}$ ($r = 0.63$; $P < 0.05$; Fig. 4D). Similar to the results with $S_{IC(minmod)}$ (Table 2), after adjusting for Vd of the injected glucose, insulin sensitivity measured as $S_{IC(minmod)}$ was lower in TID pre- compared with posttransplant and with normal (2.71 ± 0.63 vs. 4.72 ± 0.44 vs. 5.36 ± 0.88 dl/min per $\mu$U/ml; $P < 0.05$ for both).

**DISCUSSION**

The present study is the first to demonstrate a high correlation of the minimal model-derived $S_{IC}$ to euglycemic clamp-
derived measures of insulin sensitivity in T1D and islet transplant recipients. These results confirm prior reports of improved insulin sensitivity following islet transplantation for T1D that used minimal model-derived estimates from the FSIGT (15, 20) and validate the minimal model-derived SI as a measure of both total body and peripheral insulin sensitivity in T1D. In fact, the correlations shown here were slightly stronger with clamp-derived peripheral insulin sensitivity, SIP-(clamp), based on tracer-calculated glucose disposal, Rd, than clamp-derived total body insulin sensitivity, SI(clamp), based on glucose infusion rate. Importantly, the relationships between the minimal model and clamp-derived estimates were stronger when considering the volume-corrected SIC, particularly when evaluating untransplanted and posttransplant subjects together, an effect explained by the different VD for the injected glucose between these groups. Indeed, the SIC measure is more appropriate than the standard SI when comparing T1D subjects with varying VD, since similar relationships were present using SIC whether considering only T1D subjects or including those postislet transplantation.

Prior reports from our group and others have established that the minimal model can adequately resolve the parameter S1 in T1D (10, 20, 28), despite a late rise in glucose during the FSIGT as seen in our study (Fig. 1). Other studies involving T2D have encountered estimates of SI equal to zero, a problem avoided in the present work we believe by the use of an overnight insulin infusion targeting near-normal glycemia that was present at the start of testing. It is important to note that the minimal model SI underestimates insulin sensitivity as measured by the clamp when the values were normalized to identical units with the slope for correlations of SIC(minmod) against SIC(clamp) and SIP(clamp) both being around 0.5 (Fig. 2). A similar underestimation was also reported for the analysis comparing the minimal model and clamp-derived measures of insulin sensitivity in subjects that ranged from normal with those with impaired glucose tolerance, including overt T2D (22). This underestimation is likely explained by the difference in the effect on glucose disposal of an insulin bolus, as given with the FSIGT, compared with a continuous infusion of insulin.
during the euglycemic clamp since insulin-stimulated glucose disposal increases with time (9). This same difference in insulin administration may also explain the greater magnitude of increase in insulin sensitivity seen with the minimal model than with the euglycemic clamp measures and suggests that the FSIGT may be a more sensitive methodology for identifying changes in insulin sensitivity in T1D.

Given these differences in performance characteristics, the minimal model SI should still be regarded as an index of insulin sensitivity and not be directly substituted for direct measures obtained from euglycemic clamp studies.

This is the first study to show that the impaired SG seen in T1D (10, 28) can be normalized following islet transplantation. The low SG derived from the FSIGT in T1D may in part be a consequence of absent insulin secretory function, since greater AIRg in normal subjects may lead to an overestimation of SG by the minimal model approach (11). In our prior study of islet transplant recipients where SG was less than normal, the AIRg was significantly reduced (20). In the present study, AIRg was not different from normal, although individual subjects did have reduced responses, and so demonstrates that, in the presence of near-normal β-cell function, there is no impairment of SG in islet transplant recipients. Glucose effectiveness is comprised of peripheral and hepatic components, with glucose autoregulation of glucose production by the liver contributing importantly to overall endogenous glucose production (5). Because glucose effectiveness describes the capacity for glucose to mediate its own disposal independent from insulin, the normalization of SG reported here provides additional evidence to the already reported improvements in hepatic and peripheral insulin sensitivity (18) against a detrimental effect of the intrahepatic site of transplantation or immunosuppression regimen on the maintenance of glucose homeostasis in islet recipients.

In conclusion, the minimal model-derived index of total body insulin sensitivity, SIC, is highly predictive of clamp-derived measures of both total and peripheral insulin sensitivity in T1D and following islet transplantation. When employing the FSIGT to generate the minimal model Si, simultaneous
generation of $S_G$ is possible, with both indexes impaired in T1D and capable of correction as shown here with islet transplantation. The FSIGT parameter $AIR_g$ also provides a measure of islet graft $\beta$-cell function that is predictive of insulin requirements (14) and so may also be of interest during postislet transplant metabolic assessment. Thus, our data indicate the usefulness of the minimal model for longitudinal or cross-sectional studies in T1D populations when performance of the euglycemic clamp is not feasible for economic or practical reasons. Finally, the FSIGT may be the desired methodological approach in T1D for additional consideration of potential therapeutic effects on glucose effectiveness and $\beta$-cell function.

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DISCLOSURES

No potential conflicts of interest relevant to this article were reported.

AUTHOR CONTRIBUTIONS


REFERENCES