The Effect of Systemic Versus Portal Insulin Delivery in Pancreas Transplantation on Insulin Action and VLDL Metabolism

André Carpentier,1 Bruce W. Patterson,1 Kristine D. Uffelman,1 Adria Giacca,1,2 Mladen Vranic,1,2 Mark S. Cattral,3 and Gary F. Lewis1

Combined kidney-pancreas transplantation (KPT) with anastomosis of the pancreatic vein to the systemic circulation (KPT-S) or to the portal circulation (KPT-P) provides a human model in which the chronic effects of portal versus systemic insulin delivery on glucose and VLDL metabolism can be examined. Despite similar plasma glucose and C-peptide levels, KPT-S (n = 9) had an approximate twofold elevation of fasting and intravenous glucose-stimulated plasma insulin levels compared with both KPT-P (n = 7) and healthy control subjects (n = 15). The plasma free fatty acid (FFA) levels were elevated in both transplant groups versus control subjects, but the plasma insulin elevation necessary to lower plasma FFA by 50% was approximately two times higher in KPT-S versus KPT-P and control subjects. Endogenous glucose production was similar in KPT-S and KPT-P, despite ~35% higher hepatic insulin levels in the latter, and was suppressed to a greater extent during a euglycemic-hyperinsulinemic clamp in KPT-S versus KPT-P. Total-body glucose utilization during the euglycemic-hyperinsulinemic clamp was ~40% lower in KPT-S versus KPT-P, indicating peripheral tissue but not hepatic insulin resistance in KPT-S versus KPT-P. Both transplant groups had an approximate twofold elevation of triglyceride (TG)-rich lipoprotein apolipoprotein B (apoB) and lipids versus control subjects. Elevation of VLDL-apoB and VLDL-TG in both transplant groups was entirely explained by an ~50% reduction in clearance of VLDL compared with healthy control subjects. In the presence of increased FFA load but in the absence of hepatic overinsulinization and marked hepatic insulin resistance, there was no elevation of VLDL secretion in KPT-S versus KPT-P and control subjects. These findings suggest that chronic hyperinsulinemia and peripheral tissue insulin resistance with the consequent elevation of plasma FFA flux are insufficient per se to cause VLDL overproduction and that additional factors, such as hepatic hyperinsulinemia and/or gross insulin resistance, may be an essential prerequisite in the pathogenesis of VLDL overproduction in the common form of the insulin resistance syndrome. Diabetes 50:1402–1413, 2001

Currenlty, the most effective established insulin-replacement therapy for patients with type 1 diabetes is achieved with whole-organ pancreas transplantation, which is usually performed together with kidney transplantation (combined kidney-pancreas transplantation [KPT]). Improvements in surgical techniques and immunosuppressive drug regimens over the past 20 years have resulted in an increase in both patient and pancreas graft survival after transplantation as well as freedom from exogenous insulin administration in well over 80% of cases at 1 year posttransplantation in most centers (1,2). The surgical technique most commonly used in North America involves whole-organ transplantation with anastomosis of the duodenal segment to the bladder for drainage of the exocrine secretions and with systemic venous anastomosis to the iliac vein (KPT-S). Because the liver, which normally clears on the first pass 40–60% of the insulin secreted by the pancreas, is bypassed in this procedure, KPT-S results in an overall reduction in insulin clearance (3). Consequently, transplant recipients who undergo this procedure become hyperinsulinemic and have reduced total-body insulin sensitivity (4–9), even when compared with nondiabetic kidney transplant recipients on a similar antirejection drug regimen (7,8). Furthermore, these patients have a reduction in insulin’s antilipolytic activity in peripheral tissues, another feature of systemic insulin resistance (9).

The consequence of KPT-S on lipid metabolism is more controversial. Some investigators have shown a normalization of the lipid profile after transplantation compared with the pretransplantation uremic state (10,11), whereas others have shown increased levels of plasma triglycerides (TGs), apolipoprotein B (apoB), and/or VLDL-TG and VLDL-apoB after transplantation (12,13). Although immunosuppressive agents are thought to play a role in the lipid abnormalities seen after solid-organ transplantation and could explain the findings of the latter studies, it is not clear whether peripheral hyperinsulinemia and the associ-
ated insulin resistance could also play a role in the abnormal lipid metabolism found in pancreas transplant recipients. Insulin plays a pivotal role in controlling the supply of VLDL biosynthetic precursors, such as free fatty acids (FFAs), and affects multiple steps in hepatic VLDL assembly and secretion in a direct and an indirect fashion (14). Individuals with insulin resistance and diabetes are hyperinsulinemic and are resistant to the antilipolytic effect of insulin in peripheral tissues (15,16). Furthermore, hyperinsulinemic and insulin-resistant states are associated with VLDL oversecretion (17–21), which is the main cause of the elevation of TG-rich lipoproteins (TRLs) seen in these patients. The elevation in TRLs, in turn, contributes to their increased risk of cardiovascular disease (22,23).

In 1993, Gaber et al. (24) described a new KPT technique in humans using portal venous anastomosis (KPT-P) and enteric exocrine drainage of the pancreas graft. Others (5,25–27), as well as our group (2), have shown that this new technique results in fewer postoperative complications and less peripheral hyperinsulinemia compared with the systemic-bladder approach. Because hyperinsulinemia is associated with insulin resistance and perhaps with an increased risk of cardiovascular disease (28,29), and because portal anastomosis of the pancreas graft reestablishes a more physiological state of insulin delivery and normalizes peripheral insulin levels, it has been argued that it may lead to normalization of the metabolic profile of the patients and to a reduction of cardiovascular complications (27). However, the effects of KPT-S versus KPT-P on peripheral and hepatic insulin action as well as on VLDL metabolism have not been previously investigated. The aim of the present study, therefore, was to determine the effect of chronic portal versus systemic insulin delivery in KPT recipients on insulin-stimulated glucose disposal, endogenous glucose production, insulin secretion, insulin-mediated suppression of plasma FFA, and VLDL kinetics.

RESEARCH DESIGN AND METHODS

Subjects. The study participants were recruited from among the first 48 patients with type 1 diabetes who underwent combined KPT at the Toronto General Hospital between 1996 and 1998. All healthy non–insulin-requiring KPT recipients were accepted into the study if they were willing to participate; had normal fasting blood glucose levels, normal Hba1c, and normal response to a 75-g oral glucose tolerance test after the transplantation procedure; were taking 10 mg/day or less of prednisone for at least 1 month before the study; had no surgery for at least 3 months; and were not taking lipid-lowering medications for at least 1 month. The complete clinical description and details of the two surgical procedures of the first 40 patients of our cohort have been published previously (2). In brief, the surgical procedures were performed through a midline abdominal incision, with implantation of the kidney first, using the external iliac vessels for vascularization. KPT-S grafts were placed in the right iliac fossa; and the artery and vein were anastomosed to the native common iliac artery and external iliac vein, respectively, and the duodenum was anastomosed to the bladder or to a Roux-en-Y limb of jejunum. In patients undergoing the KPT-P procedure, the portal vein of the pancreas was anastomosed end-to-side to the superior mesenteric vein, whereas the pancreas graft artery was anastomosed to the native common iliac artery. For enteric exocrine drainage, the duodenal segment was anastomosed to a Roux-en-Y limb of jejunum. Patients were not randomly assigned to undergo KPT-S or KPT-P procedures. The first 20 transplants at our institution were performed as KPT-S procedures, with almost all subsequent transplants being performed as KPT-P procedures.

Nine patients with KPT-S and seven patients with KPT-P agreed to participate in the metabolic studies. Two of the nine KPT-S recipients had enteric exocrine drainage of their pancreas graft and the remainder had bladder drainage, whereas all of the KPT-P recipients had enteric exocrine drainage. The subjects were studied between 5 and 19 months after transplantation, and all of them were in stable clinical condition and on a stable drug regimen for at least 1 month before the studies. All of the patients were treated with prednisone and either cyclosporin (seven of nine in KPT-S group and six of seven in KPT-P group) or tacrolimus (two in KPT-S group and one in KPT-P group). The majority of subjects were also treated with either azathioprine (five of nine in KPT-S group and two of seven in KPT-P group) or mycophenolate mofetil (one in KPT-S group and three in KPT-P group) as a third antirejection drug. Every patient also received aspirin (81 mg/day), and most were treated with antihypertensive medication (mainly calcium channel blockers). One patient in the KPT-P group (subject 6) was taking metoprolol (100 mg/day) throughout the studies. No female patient was taking estrogens. Age, gender ratio, BMI, diabetes duration, prednisone dose, cyclosporin dose, or metformin dose, creation time, mean age at transplantation, and BMI were similar between the two transplant groups, as shown in Table 1. Electrolytes and blood pH were normal in all participants.

In addition, eight healthy research volunteers of similar age (36 ± 2 years), gender (five men, three women), and BMI (25.9 ± 1.6 kg/m²) participated as control subjects for the apoB and lipid turnover studies. The results of the two pancreas transplant groups during the graded intravenous glucose infusion study were compared with 15 non-diabetic healthy subjects (12 men, 3 women) of similar age (38 ± 4 years, P = 0.09 vs. KPT recipients) and BMI (27.7 ± 1.3 kg/m²; P = 0.12 vs. KPT recipients) who previously participated in this protocol in our laboratory (30,31). Informed written consent was obtained from all participants in accordance with the guidelines of the Human Subjects Review Committee of the Toronto General Hospital, University of Toronto.

Experimental protocols. Each transplant recipient participated in at least two of three studies performed in random order, on separate days, and within a 2-month period. All studies were performed in the Metabolic Investigation Unit of the Toronto General Hospital, where the patients were admitted on each occasion between 7:30 and 8:30 a.m. after a 12-h overnight fast. They remained fasting during the studies, but had access to water ad libitum. The subjects took all of their medications according to their regular schedule except for prednisone, which was held until completion of each study protocol. For each study, an intravenous catheter was placed in each forearm, one for blood samples and one for intravenous infusions. Blood samples were taken at 10-min intervals throughout the study for measurement of glucose, insulin, C-peptide, and FFA. Blood samples were collected on ice into Vacutainer tubes (Becton Dickinson Vacutainer Systems, Rutherford, NJ) containing Na3EDTA and 30 μg/ml heparin (30,31). The lipase inhibitor Orlistat (tetrahydrolipstatin; Hoffman LaRoche Ltd., Mississauga, ON, Canada) was added to prevent ongoing in vitro lipolysis of the samples.

Euglycemic-hyperinsulinemic clamp and glucose turnover. Eight patients with KPT-S and six patients with KPT-P underwent a euglycemic-hyperinsulinemic clamp with [3-H]glucose infusion so that insulin-mediated glucose disappearance rate (Rd) and endogenous glucose production rate (Rg) could be quantified. A primed (3.3 × 10⁹ dm²/min) continuous infusion (0.33 × 10⁹ dam²/min) of high-performance liquid chromatography–purified [3-H]glucose (New England Nuclear, Boston, MA) was started and maintained throughout the study (32). An aliquot of [3-H]glucose was added to the 20% dextrose infusate (0.388 μCi/kg [3-H]glucose added to 500 ml dextrose solution) to minimize the decline in glucose specific activity (3A) during the clamp (hot glucose infusion rate (GIR) method) (33,34). After a 150-min period of tracer equilibration (time ≈ 150 to 0 min), an infusion of biosynthetic human insulin (Humulin R, Eli Lilly) was started at 10 mU·m²·min⁻¹ for 120 min (time 0–120 min) (low dose), followed by an infusion at 40 mU·m²·min⁻¹ for another 120 min (time 120–240 min) (high dose), and an intravenous glucose infusion was adjusted to maintain plasma glucose at fasting levels. Potassium chloride (KCl) 30 mEq/l was infused at −10 mEq/h in all subjects during the insulin infusion to prevent hypokalemia.

VLDL-apoB and VLDL-TG turnover study. All of the study participants received a standard isocaloric diet containing 30% fat, 20% protein, and 50% carbohydrate for 2 days before each study. During these 2 days, nine patients with KPT-S, seven with KPT-P, and eight healthy control subjects received a primed (6 μmol/kg) continuous [1-¹⁴C]-glucose infusion (0.1 μmol · min⁻¹ · kg⁻¹) (Cambridge Isotope Laboratories, Andover, MA) for 12 h to determine VLDL-apoB secretion and clearance rates while fasting was maintained. On the same occasion, four of the nine patients with KPT-S and the seven patients with KPT-P received a bolus injection of [1,1,2,3,3-¹⁴C]-glycerol (100 μmol/kg) (Cambridge Isotope Laboratories) to determine VLDL-TG
and apoB concentrations.

VLDL, IDL, and HDL fractions, respectively, from total plasma cholesterol, TG, measured by subtraction of cholesterol, TG, and apoB concentrations in an electroimmunoassay (36). LDL cholesterol, LDL-TG, and LDL-apoB were heparin precipitation of the plasma, and plasma apoA1 was measured using results. HDL-TG and HDL cholesterol were measured after manganese/4°C for 18 h. IDL and VLDL fractions were pooled to report the total TRL.

IDL fraction was also separated by ultracentrifugation at d 1.019 at 39,000 rpm and 16°C for 16 h. For the time 0 sample (Table 2), the 1.006 g/ml using a type 50.3 Ti rotor (Ultracentrifuge L870; Beckman, Palo Alto, CA) at 39,000 rpm and 16°C for 16 h. The labeled glucose infusate were assayed together with the plasma samples. The supernatant containing VLDL lipids was separated and dried under nitrogen to be used for isolation of the TG by thin-layer chromatography (TLC). The precipitate of the delipidation procedure (containing VLDL-apoB) was dried and then dissolved overnight in Laemmli’s solution at room temperature, and VLDL-apoB was separated by 4–20% gradient SDS-PAGE (37). Plasma amino acids were separated by cation exchange chromatography, as previously described (38–40). ApoB gel bands were excised and hydrolyzed with 6N HCl in plasma insulin during the graded intravenous glucose infusion study.

Laboratory methods. Glucose was assayed enzymatically at the bedside using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Insulin was measured by radioimmunoassay using a double-antibody separation method (Pharmacia Diagnostic, Uppsala, Sweden). C-peptide was measured by a double-antibody C-peptide radioimmunoassay (Diagnostic Products Corporation, Los Angeles). FFA levels were measured by a colorimetric method (Boehringer Mannheim GmbH Diagnostica). ApoB was measured in each fraction using an electroimmunoassay, as previously described (36). VLDL was isolated from the plasma by ultracentrifugation at d 1.006 g/ml using a type 50.3 Ti rotor (Ultracentrifuge L570; Beckman, Palo Alto, CA) at 39,000 rpm and 16°C for 16 h. For the time 0 sample (Table 2), the IDL fraction was also separated by ultracentrifugation at d 1.019 at 39,000 rpm and 4°C for 18 h. IDL and VLDL fractions were pooled to report the total TRL results. IDL-TG and HDL cholesterol were measured after manganese/heparin precipitation of the plasma, and plasma apoA1 was measured using an electroimmunoassay (36). LDL cholesterol, LDL-TG, and LDL-apoB were measured by subtraction of cholesterol, TG, and apoB concentrations in VLDL, IDL, and HDL fractions, respectively, from total plasma cholesterol, TG, and apoB concentrations.

The VLDL fraction was delipidated with diethyl ether-methanol in acid-washed glass tubes to separate VLDL lipids from VLDL proteins. The supernatant containing VLDL lipids was separated and dried under nitrogen to be used for isolation of the TG by thin-layer chromatography (TLC). The precipitate of the delipidation procedure (containing VLDL-apoB) was dried and then dissolved overnight in Laemmli’s solution at room temperature, and VLDL-apoB was separated by 4–20% gradient SDS-PAGE (37). Plasma amino acids were separated by cation exchange chromatography, as previously described (38–40). ApoB gel bands were excised and hydrolyzed with 6N HCl at 110°C for 24 h. Plasma and apoB hydrolysate amino acids were converted to N-heptanoylhepturobutyl s-propyl esters (38), and enrichment of [1-13C]leucine was measured by electron-impact ionization gas chromatography–mass spectrometry (GCMS) on a Hewlett-Packard (Palo Alto, CA) model 5973 mass selective marker system equipped with a 30-m × 0.25-mm DB-17 capillary column (Supelco, Bellefonte, PA) using ions at m/z 313 and 314. The enrichment was converted to tracer-to-tracer ratios (TTRs) by analysis of suitable [1-13C]leucine isotopic enrichment standards.

VLDL-TG was isolated by TLC on 20-cm × 20-cm silica gel plates (Fisher, #06–600A Gel G TLC plates) using heptane, isopropyl ether, and acetic acid in a ratio of 80:20:2 (41). VLDL-TG glycerol was recovered and converted to heptanoylheptubutyl derivative as previously described (35), and enrichment of [1-13C]glycerol was measured by electron-impact ionization GCMS using a 30-m × 0.25-mm, 0.25-m film DB-17 capillary column (Supelco) on a model 5973 quadrupole GCMS (Hewlett-Packard). Glycerol (m/z 472) and its m/z 473 were measured, and the glycerol TTR was determined by calibration of measured m/z 475/m/z 473 ratios for standards of known isotopic enrichment.

Calculations C-peptide and plasma insulin response during the graded intravenous glucose infusion study. Mean levels of plasma glucose, insulin, and C-peptide were calculated at baseline and during the last 20 min of each 40-min glucose infusion period. Mean insulin and mean C-peptide levels were plotted against the corresponding mean glucose level for each period, thereby establishing a dose-response relationship between glucose and these hormone levels. The dose-response curves were compared as previously described to analyze the results (30,31). The C-peptide–glucose response was used as a surrogate marker to compare qualitatively insulin secretion between the two transplanted groups. This approach is valid because renal function was similar in KPT-S and KPT-P recipients, because C-peptide kinetics are influenced primarily by renal clearance, and because C-peptide kinetics have been shown to be similar in KPT-S patients and in nondiabetic renal transplant recipients on similar immunosuppressive therapy and with similar renal dysfunction (3).

Calculation of the rate of decline of plasma FFA in response to change in plasma insulin during the graded intravenous glucose infusion study.

<table>
<thead>
<tr>
<th>KPT-S recipients</th>
<th>Age (years)</th>
<th>Gender</th>
<th>BMI (kg/m²)</th>
<th>Duration of DM (years)</th>
<th>Prednisone dose (mg/d)</th>
<th>Cyclosporin (tacrolimus) dose (mg/d)</th>
<th>Creatinine clearance rate (normal ≥90)</th>
<th>Time from transplant at which studied (days)</th>
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<tr>
<td>#1</td>
<td>41</td>
<td>M</td>
<td>38.1</td>
<td>37</td>
<td>5</td>
<td>550</td>
<td>52</td>
<td>377</td>
</tr>
<tr>
<td>#2</td>
<td>37</td>
<td>F</td>
<td>25.9</td>
<td>36</td>
<td>5</td>
<td>275</td>
<td>57</td>
<td>368</td>
</tr>
<tr>
<td>#3</td>
<td>38</td>
<td>M</td>
<td>22.6</td>
<td>36</td>
<td>10</td>
<td>(8.0)</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>#4</td>
<td>31</td>
<td>F</td>
<td>23.1</td>
<td>39</td>
<td>7.5</td>
<td>400</td>
<td>96</td>
<td>340</td>
</tr>
<tr>
<td>#5</td>
<td>45</td>
<td>M</td>
<td>23.2</td>
<td>27</td>
<td>7.5</td>
<td>600</td>
<td>57</td>
<td>438</td>
</tr>
<tr>
<td>#6</td>
<td>33</td>
<td>M</td>
<td>26.7</td>
<td>23</td>
<td>7.5</td>
<td>400</td>
<td>81</td>
<td>354</td>
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<tr>
<td>#7</td>
<td>32</td>
<td>F</td>
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<td>41</td>
<td>M</td>
<td>21.3</td>
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<td>237</td>
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<tr>
<td>#9</td>
<td>45</td>
<td>M</td>
<td>21.8</td>
<td>32</td>
<td>7.5</td>
<td>(4.0)</td>
<td>82</td>
<td>313</td>
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<tr>
<td>Mean ± SE</td>
<td>38 ± 2</td>
<td></td>
<td>25.2 ± 1.7</td>
<td>29 ± 2</td>
<td>7.2 ± 0.7</td>
<td>425 ± 43</td>
<td>64 ± 6</td>
<td>378 ± 33</td>
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<table>
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<tr>
<th>KPT-P recipients</th>
<th>Age (years)</th>
<th>Gender</th>
<th>BMI (kg/m²)</th>
<th>Duration of DM (years)</th>
<th>Prednisone dose (mg/d)</th>
<th>Cyclosporin (tacrolimus) dose (mg/d)</th>
<th>Creatinine clearance rate (normal ≥90)</th>
<th>Time from transplant at which studied (days)</th>
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<tr>
<td>#1</td>
<td>39</td>
<td>F</td>
<td>19.7</td>
<td>31</td>
<td>5</td>
<td>500</td>
<td>65</td>
<td>237</td>
</tr>
<tr>
<td>#2</td>
<td>41</td>
<td>F</td>
<td>28.3</td>
<td>26</td>
<td>7.5</td>
<td>700</td>
<td>76</td>
<td>275</td>
</tr>
<tr>
<td>#3</td>
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<td>M</td>
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<td>36</td>
<td>10</td>
<td>600</td>
<td>80</td>
<td>171</td>
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<tr>
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<td>F</td>
<td>21.7</td>
<td>34</td>
<td>5</td>
<td>(6)</td>
<td>82</td>
<td>399</td>
</tr>
<tr>
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<td>M</td>
<td>21.0</td>
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<td>7.5</td>
<td>350</td>
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<td>24</td>
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<td>49</td>
<td>473</td>
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<tr>
<td>Mean ± SE</td>
<td>36 ± 1</td>
<td></td>
<td>23.7 ± 1.4</td>
<td>28 ± 2</td>
<td>7.1 ± 0.7</td>
<td>475 ± 67</td>
<td>64 ± 6</td>
<td>319 ± 38</td>
</tr>
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</table>
To compare the decrease in plasma FFA in response to the increase in plasma insulin between the two transplant groups and control subjects, FFA levels during the graded intravenous glucose infusion were plotted against the corresponding change from baseline of plasma insulin, and the data were fitted to the following monoexponential decay function for each subject:

$$FFA = y_0 + ae^{-\ln S_{NS}}$$

where FFA is the plasma FFA level, $\ln S_{NS}$ is the change of insulin level from baseline, $y_0$ is the asymptote of the function, $a$ is the amplitude of FFA change from $\ln S_{NS} = 0$ to infinity, and $k$ is the slope of the exponential function. The fitting procedure to find the parameters resulting in the least sum of squares was done with Sigma Plot for Windows software, version 4.01, using the Marquardt-Levenberg algorithm. The mean $R^2$ of the fit was 0.83, 0.88, and 0.87 in the KPT-S, KPT-P, and control groups, respectively, suggesting a very good fit of the data. The change in plasma insulin required for a 50% reduction in plasma FFA (INS50%) was then calculated as follows:

$$\ln S_{NS} = \ln(0.5)/k.$$ 

**Estimation of hepatic insulin concentration in KPT-S and KPT-P groups.** We estimated fasting hepatic insulin concentration using the following formulas:

- For control subjects, $[\text{Hepins}]_0 = [\text{Ains}]_0 \times 2.4$
- For the KPT-P group, $[\text{Hepins}]_0 = [\text{Ains}]_0 \times 2.4 \times 75$
- For the KPT-S group, $[\text{Hepins}]_0 = [\text{Ains}]_0 \times 22\% + [\text{Ains}]_0 \times 78\% \times 70\%$

where $[\text{Hepins}]_0$ is the hepatic insulin concentration and $[\text{Ains}]_0$ is the plasma insulin concentration of the arterialized venous blood. The first equation is from DeFeo et al. (42). The second equation is modified after DeFeo et al. (42), and it takes into account the expected ~25% decrease in insulin clearance associated with mild renal failure and immunosuppressive therapy (3). Our equation will likely underestimate hepatic insulin concentrations in the KPT-P group because it assumes that the ~25% decrease in insulin clearance occurs totally in the liver. The second equation assumes that 22% of the hepatic blood flow comes from the hepatic artery and 78% comes from the portal vein (43) and that 30% of the insulin is extracted by the gastrointestinal vascular bed (44).

**Calculation of $R_a$, $R_d$, and assessment of hepatic insulin sensitivity during the euglycemic-hyperinsulinemic clamp.** The SAAM II program (SAAM Institute, University of Washington, Seattle, WA). The model for $\text{[1-13C]}$leucine enrichment (compartment 1) was used as a forcing function that provided a source of tracer for an amino acid precursor pool that served as the source for apoB. After a delay (compartment 2) to account for isotopic dilution of plasma leucine tracer into intracellular pools and synthesis and secretion of VLDL, apoB appeared in plasma VLDL (compartment 3). As in our previous studies (40), a single compartment was adequate to describe VLDL-apoB kinetics. Kinetic heterogeneity requiring more than one VLDL compartment was not evident in the data, based on the 12-h primed constant infusion protocol. The FCR (poors per hour) was determined as the irreversible loss of material from compartment 3.

The FCR of VLDL-TG was estimated from the monoexponential slope of VLDL-TG $\text{[1-13C]}$leucine enrichment immediately after the maximum enrichment. We recently demonstrated that the VLDL-TG kinetics derived from this approach are very well correlated to those derived from $\text{[2-3H]}$glycerol with 48-h sampling when the two tracers are administered simultaneously (35).

VLDL-apoB and VLDL-TG relative secretion rates (RSRs) were calculated by multiplying the mean concentration of VLDL-apoB (in milligrams per deciliter) or VLDL-TG (in micromoles per liter) over the entire 12 h of the study by their respective FCR (per hour) and an assumed VLDL distribution volume of 0.45 dl/kg body wt (50). These values were multiplied by 24 to obtain daily secretion rates. Absolute secretion rates (ASRs) were calculated by multiplying these figures by the body weight of the patient. VLDL-apoB and VLDL-TG clearance rates were calculated in milliliters per minute. **Statistical analysis.** The data were expressed as means ± SE. Unpaired t test was used for comparison between the two transfused groups after appropriate transformation of the data when indicated. For the comparison of the glucose, insulin, and C-peptide response over the 60 min of each insulin infusion period was used to compare the two transplant groups.

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>KPT-S</th>
<th>KPT-P</th>
<th>Control subjects</th>
<th>ANOVA (F)</th>
<th>Difference between groups*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>9</td>
<td>7</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total plasma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.75 ± 0.47</td>
<td>4.62 ± 0.47</td>
<td>3.95 ± 0.32</td>
<td>0.33</td>
<td>—</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.45 ± 0.22</td>
<td>1.70 ± 0.42</td>
<td>0.90 ± 0.19</td>
<td>0.12</td>
<td>—</td>
</tr>
<tr>
<td>ApoB (mg/dl)</td>
<td>87 ± 8</td>
<td>94 ± 9</td>
<td>89 ± 8</td>
<td>0.85</td>
<td>—</td>
</tr>
<tr>
<td>ApoA1 (mg/dl)</td>
<td>122 ± 7</td>
<td>94 ± 19</td>
<td>124 ± 5</td>
<td>0.37</td>
<td>—</td>
</tr>
<tr>
<td><strong>TRL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>0.94 ± 0.13</td>
<td>0.90 ± 0.21</td>
<td>0.34 ± 0.10</td>
<td>0.01</td>
<td>Both KPT-S and KPT-P vs. control subjects</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>0.90 ± 0.17</td>
<td>1.32 ± 0.40</td>
<td>0.52 ± 0.12</td>
<td>0.08</td>
<td>—</td>
</tr>
<tr>
<td>ApoB (mg/dl)</td>
<td>22.2 ± 3.6</td>
<td>19.5 ± 3.4</td>
<td>11.4 ± 1.8</td>
<td>0.03</td>
<td>KPT-S vs. control subjects</td>
</tr>
<tr>
<td><strong>LDL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>2.67 ± 0.38</td>
<td>2.61 ± 0.32</td>
<td>2.39 ± 0.31</td>
<td>0.84</td>
<td>—</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>0.22 ± 0.07</td>
<td>0.12 ± 0.05</td>
<td>0.21 ± 0.06</td>
<td>0.43</td>
<td>—</td>
</tr>
<tr>
<td>ApoB (mg/dl)</td>
<td>65 ± 7</td>
<td>74 ± 6</td>
<td>78 ± 6</td>
<td>0.45</td>
<td>—</td>
</tr>
<tr>
<td><strong>HDL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>1.16 ± 0.07</td>
<td>1.12 ± 0.09</td>
<td>1.22 ± 0.15</td>
<td>0.83</td>
<td>—</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>0.22 ± 0.03</td>
<td>0.33 ± 0.05</td>
<td>0.18 ± 0.02</td>
<td>0.05</td>
<td>KPT-P vs. control subjects</td>
</tr>
</tbody>
</table>

Data are means ± SE, unless otherwise indicated. *P < 0.05 using Scheffe post hoc test to detect difference between groups.

**FIG. 1.** VLDL-apoB multicompartimental kinetic model. 1: plasma amino acid compartment; 2: delay compartment; 3: VLDL compartment.
RESULTS

Graded intravenous glucose infusion study. The baseline plasma glucose levels (Fig. 2A) were similar between the three groups (5.2 ± 0.3 vs. 5.2 ± 0.1 vs. 5.4 ± 0.2 mmol/l in the KPT-S, KPT-P, and control groups, respectively, P = 0.56). Glucose levels increased in a similar fashion in the three groups and reached ∼12.5, ∼11.5, and ∼11.0 mmol/l at the end of the glucose infusion in the KPT-S, KPT-P, and control groups, respectively (ANOVA, P = 0.65).

Fasting insulin levels were not significantly higher in the KPT-S group versus the KPT-P and control groups (75 ± 17 vs. 64 ± 16 vs. 46 ± 4 pmol/l, respectively, P = 0.21), but the insulin levels were significantly higher in the KPT-S group throughout the glucose infusion period compared with the two other groups (ANOVA P < 0.0001) (Fig. 2B). The total area under the insulin curve between 6 and 9 mmol/l of plasma glucose was ∼40% higher in the KPT-S group versus the KPT-P and control groups (522 ± 69 vs. 304 ± 58 vs. 302 ± 28 pmol/l, 6–9 mmol/l glucose interval, respectively, P = 0.002).

The plasma C-peptide levels (Fig. 2C) were similar between the three groups at baseline (0.61 ± 0.11 vs. 0.60 ± 0.15 vs. 0.50 ± 0.09 nmol/l in the KPT-S, KPT-P, and control subjects, respectively, P = 0.75) and during the glucose infusion period (total area under the C-peptide curve versus the 6–9 mmol/l plasma glucose interval: 2.98 ± 0.39 vs. 2.47 ± 0.52 vs. 2.85 ± 0.48 nmol/l, 6–9 mmol/l glucose interval in the KPT-S, KPT-P, and control subjects, respectively, P = 0.75).

The mean fasting plasma FFA levels were similar between the three groups (0.57 ± 0.04 vs. 0.62 ± 0.07 vs. 0.51 ± 0.04 nmol/l in the KPT-S, KPT-P, and control subjects, respectively, P = 0.31). However, both transplant groups had higher plasma FFA than the control subjects over the entire glucose infusion period (ANOVA, P = 0.0008) (Fig. 3A). As shown in Fig. 3B, the relationship between the mean plasma FFA levels versus the mean change in insulin levels from baseline was shifted to the right in the KPT-S group compared with the KPT-P and control groups. The monoeponential decay slope (k) of this relationship was significantly lower in the KPT-S group versus the two other groups (0.010 ± 0.002 vs. 0.023 ± 0.007 vs. 0.022 ± 0.003 in the KPT-S, KPT-P, and control groups, respectively, P = 0.02). INS50% was increased more than twofold in the KPT-S group compared with the KPT-P and control groups (109 ± 34 vs. 48 ± 14 vs. 43 ± 8 pmol/l, respectively, P = 0.02).

Fasting hepatic insulin concentrations. The estimated hepatic insulin concentration at fasting was significantly lower in the KPT-S versus KPT-P group (60 ± 8 vs. 93 ± 15 pmol/l, respectively, P = 0.04). The KPT-S group, but not the KPT-P group, had lower estimated hepatic insulin concentration compared with the healthy control subjects.
(60 ± 8 vs. 110 ± 10 pmol/l in KPT-S vs. control subjects, respectively, $P = 0.001$). Even in the unlikely event that the immunosuppression and mild renal failure present in the KPT-S group would have resulted in complete suppression of the intestinal insulin extraction (0% instead of 30% extraction), hepatic insulin levels would still be lower than in the KPT-P and control groups, respectively (79 ± 11 vs. 93 ± 15 vs. 110 ± 10 pmol/l).

**Euglycemic-hyperinsulinemic clamp study.** In both transplanted groups, the glucose level did not change significantly from baseline during the clamp periods (Fig. 4A). The glucose levels were similar in the KPT-S and KPT-P groups at fasting (5.2 ± 0.1 vs. 5.0 ± 0.1 mmol/l, respectively, $P = 0.20$), but were higher in the KPT-S group than in the KPT-P group both at low dose (5.3 ± 0.1 vs. 4.9 ± 0.1 mmol/l, respectively, $P = 0.005$) and high dose (4.9 ± 0.1 vs. 4.5 ± 0.1 mmol/l, respectively, $P < 0.0001$).

The insulin levels (Fig. 4B) were significantly higher in the KPT-S group compared with the KPT-P group at fasting (87 ± 4 vs. 42 ± 3 pmol/l, respectively, $P < 0.0001$) and during the low-dose period (171 ± 11 vs. 123 ± 5 pmol/l, respectively, $P = 0.001$) but not during the high-dose period of the clamp (382 ± 12 vs. 355 ± 16 pmol/l, respectively, $P = 0.18$).

The plasma glucose SA (Fig. 4C) increased significantly from baseline (by 27 ± 4% ANOVA, $P = 0.008$) during the low-dose period but not during the high-dose period in the KPT-S group. Glucose SA did not change significantly from baseline in the KPT-P group.

The glucose $R_d$ (Fig. 4D) was similar in both groups at

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**FIG. 3.** A: Plasma FFA levels at fasting and during the graded intravenous glucose infusion study. B: Plasma FFA levels in response to glucose-stimulated elevation of plasma insulin levels ($\Delta$ insulin from baseline). ●, KPT-S recipients ($n = 9$); ○, KPT-P recipients ($n = 6$); □, healthy nondiabetic control subjects ($n = 15$).
fasting (13.6 ± 0.4 vs. 12.5 ± 0.6 μmol · kg⁻¹ · min⁻¹ in the KPT-S and KPT-P groups, respectively, \(P = 0.13\)), but \(R_d\) was lower in the KPT-S group than in the KPT-P group during the low-dose period (\(R_d\) 19.8 ± 1.3 vs. 24.9 ± 2.5 μmol · kg⁻¹ · min⁻¹, respectively, \(P = 0.07\)). During the high-dose period of the clamp, the KPT-S recipients had significantly lower \(R_d\) than the KPT-P recipients (35.2 ± 1.5 vs. 46.4 ± 4.0 μmol · kg⁻¹ · min⁻¹, respectively, \(P = 0.008\)). \(G_{\text{ins}}\) values were also lower during the low-dose period (14.6 ± 0.6 vs. 18.9 ± 1.7 μmol · kg⁻¹ · min⁻¹, respectively, \(P = 0.02\)) and the high-dose period (29.0 ± 1.2 vs. 39.9 ± 2.2 μmol · kg⁻¹ · min⁻¹, respectively, \(P <\)
In the KPT-S recipients compared with the KPT-P recipients. The MCR of glucose (not shown) was also 30% lower in the KPT-S group compared with the KPT-P group during the high-dose period (7.1 ± 0.3 vs. 10.1 ± 0.8 ml·kg⁻¹·min⁻¹, respectively, P = 0.008), but this was not the case in the low-dose period.

The endogenous glucose Rₐ (Fig. 4E) was similar in both groups at fasting (13.1 ± 0.3 vs. 12.1 ± 0.6 μmol·kg⁻¹·min⁻¹ in the KPT-S and KPT-P groups, respectively, P = 0.18), during the low-dose period (6.0 ± 0.5 vs. 7.1 ± 0.8 μmol·kg⁻¹·min⁻¹ in the KPT-S and KPT-P groups, respectively, P = 0.27) and during the high-dose period (6.2 ± 0.5 vs. 7.8 ± 1.2 μmol·kg⁻¹·min⁻¹ in the KPT-S and KPT-P groups, respectively, P = 0.21). The level of suppression of Rₐ (Fig. 4F) by insulin from baseline (ΔRₐ from baseline) during the low-dose period was higher in the KPT-S group than in the KPT-P group (7.1 ± 0.6 vs. 5.1 ± 0.6 μmol·kg⁻¹·min⁻¹, respectively, P = 0.03), despite a similar increase in insulin levels from baseline in both groups (84 ± 10 vs. 81 ± 6 pmol/l, respectively, P = 0.80). The ΔRₐ from baseline during the high-dose period was also greater in the KPT-S group than in the KPT-P group (6.9 ± 0.3 vs. 4.4 ± 0.8 μmol·kg⁻¹·min⁻¹, respectively, P = 0.003), despite a similar increase in insulin levels (295 ± 10 vs. 312 ± 17 pmol/l, respectively, P = 0.36).

**VLDL-apoB and VLDL-TG turnover studies.** Fasting total plasma cholesterol and total plasma apoB levels did not differ between the transplant recipients and the control subjects, but the KPT-S and KPT-P groups had higher TRL cholesterol (P = 0.01, significant difference between both transplant groups and control subjects) and TRL-apoB (P = 0.03, significant difference between the KPT-S group and control subjects) (Table 2). They also tended to have higher total plasma TG and TRL-TG compared with the control group (Table 2). There was no significant difference between the three groups in HDL cholesterol and total plasma apoB levels did not differ between the transplant recipients and the control subjects. The HDL fraction in the KPT-P group was more enriched with TG (P < 0.05, significant difference between the KPT-P and control subjects), but there was no significant difference between the three groups in HDL cholesterol and total plasma apoB levels (Table 2).

There was no significant change in VLDL-apoB over the 12-h VLDL turnover study (ANOVA P = 0.98) (Fig. 5A). The VLDL-apoB levels were significantly higher in both transplant groups (mean level 15.4 ± 2.2 and 14.7 ± 3.4 mg/dl in the KPT-S group and the KPT-P group, respectively) than in the control group (mean level 9.1 ± 1.5 mg/dl throughout the course of the study (ANOVA, P < 0.0001; Scheffe test, P < 0.05 for difference between both transplant groups and the control group). The VLDL-TG levels (Fig. 5B) did not change during the 12-h VLDL turnover study (ANOVA, P = 0.97). The VLDL-TG levels were significantly different between all three groups throughout the course of the study (mean level 0.94 ± 0.16 vs. 1.26 ± 0.38 vs. 0.56 ± 0.16 mmol/l in the KPT-S, KPT-P, and control groups, respectively, ANOVA, P < 0.0001; Scheffe test, P < 0.05 for all comparisons). The total plasma TG levels (not shown) were significantly higher in both transplant groups (mean level 1.55 ± 0.20 and 1.85 ± 0.44 mmol/l in the KPT-S group and the KPT-P group, respectively) than in the control group (mean level 0.34 ± 0.20 mmol/l; ANOVA, P < 0.0001; Scheffe test, P < 0.05 vs. both transplant groups), but were not significantly different between the KPT-S and KPT-P groups.

As shown in Table 3, VLDL-apoB FCR was similar between the two transplant groups and tended to be lower than in control subjects (P = 0.08). When both transplant groups were pooled together and compared with the control group, VLDL-apoB FCR was significantly lower in the former (0.308 ± 0.058 vs. 0.504 ± 0.086 pool/h, P = 0.02). Both VLDL-apoB ASR and VLDL-apoB RSR did not significantly differ between the three groups, although the KPT-P group tended to have lower secretion rates. VLDL-apoB clearance was ~40 and ~60% lower in the KPT-S and KPT-P groups, respectively, than in the control group (P = 0.02, significant difference between the KPT-P and control groups).

VLDL-TG FCR (Table 3) was not significantly different among the KPT-S, KPT-P, and control groups. When both transplant groups were pooled together and compared with the control group, VLDL-TG FCR tended to be lower in the former (0.353 ± 0.053 vs. 0.504 ± 0.128 pool/h, P =
Clinical and metabolic predictors of VLDL concentration and kinetics. The effect of the different clinical (age, gender, BMI, creatinine clearance, time from transplant, and dose of immunosuppressive drugs) and metabolic (fasting plasma glucose, insulin, C-peptide, FFA, $R_4$ during the high-dose insulin infusion of the clamp, and suppression of $R_4$ per picomole per liter of increase in insulin levels during the high-dose insulin infusion of the clamp) factors measured in this study on VLDL-apoB and VLDL-TG and on FCR and ASR of both VLDL-apoB and VLDL-TG was examined using bivariate and multivariate linear regression analyses of the pooled data from the KPT-S and KPT-P groups. In bivariate regression analyses, fasting insulin levels correlated positively with log(VLDL-apoB levels) and with log(VLDL-apoB ASR) ($P = 0.01$ and 0.05, respectively). Fasting plasma glucose was positively associated with log(VLDL-apoB ASR) ($P = 0.006$). Fasting plasma FFA was negatively correlated with log(VLDL-apoB FCR) and log(VLDL-TG FCR) ($P = 0.03$ and 0.05, respectively) and positively correlated with log(VLDL-TG) and log(VLDL-TG ASR) ($P = 0.01$ and 0.01, respectively). The daily prednisone dose was positively correlated with log(VLDL-TG) and log(VLDL-TG ASR) ($P = 0.01$ and 0.03, respectively) and tended to correlate inversely with log(VLDL-apoB FCR) ($P = 0.17$) and with log(VLDL-TG FCR) ($P = 0.14$). The daily cyclosporine dose tended to correlate positively with log(VLDL-apoB levels) ($P = 0.15$) and with log(VLDL-TG levels) ($P = 0.05$). None of the other variables correlated significantly with VLDL-apoB or VLDL-TG levels and kinetics (not shown), and similar trends were seen when the type of pancreas transplantation was forced into these analyses (data not shown).

In multivariate analyses, fasting insulin levels and plasma FFA entered the best multivariate model to predict log(VLDL-TG levels) ($R^2 = 0.61$, multivariate model $P = 0.002$; $P = 0.01$ and 0.02 for daily prednisone dose and FFA, respectively). The daily prednisone dose and fasting plasma FFA also entered the best multivariate model to predict log(VLDL-TG ASR) ($R^2 = 0.60$, model $P = 0.01$; $P = 0.04$ and 0.11 for FFA and daily prednisone dose, respectively). No multivariate model was better than FFA levels alone to predict log(VLDL-apoB FCR) or log(VLDL-TG FCR).

DISCUSSION

In the present study, we have shown the following in KPT recipients with normal glucose tolerance and good pancreatic graft function: 1) KPT-P results in normalization of plasma insulin levels, improvement in total-body insulin sensitivity, and normalization of insulin-mediated suppression of plasma FFA levels compared with KPT-S; 2) KPT-P is not associated with differences in fasting endogenous glucose production but results in lower hepatic insulin sensitivity than KPT-S; 3) despite these differences between KPT-S and KPT-P, both procedures result in similar mild elevation of VLDL-apoB and VLDL-TG due to a reduction in VLDL clearance; and 4) KPT-S did not result in VLDL overproduction, despite being associated with marked peripheral tissue hyperinsulinemia and insulin resistance.

The results of the present study clearly show that hyperinsulinemia is associated with KPT-S and confirm our previous findings (2) as well as those of others (5,27). The demonstration of higher insulin response to glucose despite a similar C-peptide response, similar immunosuppressive regimen, and similar renal function in KPT-S recipients versus KPT-P recipients supports the concept that the hyperinsulinemia in KPT-S is due to diminished insulin clearance (3) and not simply from compensatory hypersecretion of insulin due to the immunosuppressive drugs and denervation of the pancreas graft (6). Previous studies have shown lower total-body insulin sensitivity in KPT-S recipients compared with healthy subjects (6–9,51). To our knowledge, the current study is the first to show that portal venous anastomosis of the pancreas leads to better insulin-mediated glucose disposal over KPT-S.

The plasma FFA levels were similarly elevated in KPT-S and KPT-P, which may be related to prednisone treatment (52,53), but KPT-P had normal insulin-mediated plasma FFA suppression, in contrast to the blunted insulin-mediated suppression of plasma FFA in KPT-S compared with healthy subjects. This result constitutes evidence that KPT-
P is not associated with the development of insulin resistance at the peripheral tissues, as is the case with KPT-S. Not all previous studies have demonstrated an impairment of insulin’s antilipolytic action in KPT-S recipients versus healthy subjects (9,51).

Despite the total-body insulin resistance in the KPT-S group, there was no difference in endogenous glucose production during fasting in KPT-S versus KPT-P. We showed a greater suppression of endogenous glucose production during both low-dose and high-dose insulin infusion in the KPT-S group compared with the KPT-P group. This finding may have resulted from the lower estimated fasting hepatic insulin concentration in the KPT-S group versus the KPT-P group (60 ± 8 vs. 93 ± 15, P < 0.05).

Whether hyperlipidemia is present in KPT-S recipients has been controversial (10,11,13,54–57). In the present study, we have shown that the lipoprotein abnormalities in both KPT-S and KPT-P recipients were restricted mainly to the TRL fraction (VLDL + IDL) when compared with healthy subjects. However, LDL-cholesterol, LDL-TG, and LDL-apoB were normal, which may support a defect in the fractional conversion rate of VLDL to LDL. There was also a slight TG enrichment of HDL particles in the KPT-P group, which may have been caused by hypertriglyceridemia in these individuals. HDL-TG enrichment is a frequent occurrence in hypertriglyceridemic states and is felt to be related to the cholesterol ester transfer protein–mediated mass transfer of TGs from the expanded pool of TRL (58). We know of no evidence showing that the immunosuppressive drugs used in these patients impair hepatic lipase activity, although we cannot rule out such an effect in the present study. There have been few previous data comparing lipoprotein profiles in KPT-S and KPT-P recipients. Hughes et al. (59) compared the posttransplantation lipoprotein profiles of KPT-S and KPT-P recipients and showed a significant elevation of VLDL and IDL TG, cholesterol, and apoB levels in the former versus the latter. Those results contrast with our finding of similar VLDL-apoB and lower VLDL-TG between KPT-S and KPT-P, but comparison of these studies is difficult because the patients in the study of Hughes et al. were on higher prednisone doses than those patients in the current study.

In a larger group of patients in our clinic, we found that KPT-P results in a small elevation of VLDL-TG as well as total TG, but not VLDL-apoB or VLDL cholesterol versus KPT-S (60). Due to the small sample size of the present study, it is not possible to exclude a small elevation of secretion and/or a small reduction of clearance of VLDL-TG in the KPT-P versus KPT-S group, which could account for this difference in VLDL-TG. For example, peripheral hyperinsulinemia in the KPT-S group could result in higher LPL activity (not measured) and lead to a relative increase in VLDL clearance in KPT-S versus KPT-P, as has been previously observed in type 1 diabetic patients treated with subcutaneous insulin (61).

The present study demonstrates that the elevation of circulating VLDL-apoB (particle number) in KPT-S and KPT-P recipients is due to impaired VLDL clearance. We have shown a similar trend toward lower VLDL-TG clearance rate in both transplant groups compared with control subjects, although this did not reach statistical significance, perhaps because of the lower number of subjects studied in this protocol. There was very good agreement between our independent determinations of VLDL-apoB FCR and VLDL-TG FCR in the transplant subjects ($R^2 = 0.90, P < 0.0001$), which is expected because the studies were done simultaneously and reflect two very closely linked metabolic processes. We did not assess lipoprotein lipase mass or activity in the current study, and thus the mechanism of the impairment of VLDL clearance in the transplant subjects in our study is unclear.

The daily dose of prednisone was an independent predictor of VLDL-TG levels and tended to correlate inversely with VLDL clearance. Glucocorticoids may decrease in vitro and in vivo lipoprotein lipase expression and activity in adipocytes (62), which may explain this observation. The daily prednisone dose also correlated positively with VLDL-TG ASR, although correcting for FFA levels reduces the strength of this relationship. Several previous studies have linked hyperlipidemia and the use of prednisone after solid-organ transplantation (63–65). We found an association between the VLDL-TG levels and the cyclosporin dose used, and thus cyclosporin may also have played a role in the decrease in VLDL clearance observed in our study (66–69). Creatinine clearance did not correlate with VLDL levels or clearance in our study, but we cannot rule out an effect of the mild renal dysfunction seen in KPT recipients on VLDL clearance (50,70,71). Further studies in nontransplant patients on a similar immunosuppressive regimen would be required to definitively address those issues.

We found no increase in VLDL secretion in either KPT-S or KPT-P recipients compared with healthy nondiabetic subjects. However, VLDL-apoB ASR was correlated with $R_d$ (inverse) and plasma glucose, and VLDL-TG ASR was correlated with plasma FFA and insulin levels. Peripheral tissue insulin resistance may have played a modulatory role on VLDL secretion in KPT recipients, presumably by increasing the FFA flux, but may have been insufficient to frankly increase VLDL secretion. It is possible that, to significantly overproduce VLDL, individuals displaying peripheral tissue insulin resistance need an additional factor, such as hepatic overinsulization and/or gross hepatic insulin resistance. We can exclude hepatic overinsulization and hepatic insulin resistance at least on glucose metabolism (as per the euglycemic-hyperinsulinemic clamp) in the KPT-S compared with the KPT-P group. In keeping with the findings of the present study in humans, chronic systemic insulin infusion in normal rats results in peripheral hyperinsulinemia, peripheral insulin resistance, and resistance to the suppressive effect of insulin on plasma FFA but not hepatic insulin resistance or elevation of VLDL-TGs (72).

Recently, we have shown that increased VLDL secretion in an insulin-resistant hamster model was associated with higher intracellular VLDL-apoB stability and elevated hepatic microsomal TG transfer protein expression, an important enzyme facilitating VLDL assembly (73). Furthermore, our previous human studies also suggested that insulin acutely inhibits VLDL secretion not only through the control of substrates (FFA) from peripheral tissues, but perhaps also through regulation directly at the liver (74). Therefore, although peripheral insulin resistance results in high FFA flux to the liver, liver insulin resistance and/or overinsulization is likely to be required for the channel-
ing of this excess supply toward VLDL synthesis and secretion and thus to establish clinically significant VLDL overproduction.

In conclusion, KPT-S results in more peripheral tissue insulin resistance and less hepatic insulin resistance than KPT-P. Despite these metabolic differences, both procedures were associated with a similar mild elevation of circulating VLDL particles that resulted from an ~50% reduction in VLDL clearance compared with healthy control subjects and that was probably explained by the immunosuppressive drugs used and/or the mild renal failure present in these patients. Furthermore, peripheral tissue insulin resistance with respect to both glucose disposal and FFA suppression in KPT-S recipients did not lead to increased VLDL secretion, as is commonly seen in patients with the insulin resistance syndrome. This result suggests that peripheral hyperinsulinemia per se and consequent peripheral tissue insulin resistance, in the absence of gross hepatic hyperinsulinemia/insulin resistance, is not sufficient to result in the clinically significant VLDL overproduction seen in individuals with the insulin resistance syndrome. Long-term follow-up of patients undergoing either KPT-S or KPT-P is needed to determine whether the metabolic differences we have observed result in differences in clinical outcome, particularly with respect to the subsequent development of cardiovascular disease.

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