Chronic Peripheral Hyperinsulinemia in Type 1 Diabetic Patients After Successful Combined Pancreas-Kidney Transplantation Does Not Affect Ectopic Lipid Accumulation in Skeletal Muscle and Liver

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OBJECTIVE—So far it is unclear whether chronic peripheral hyperinsulinemia per se might contribute to ectopic lipid accumulation and consequently insulin resistance. We investigated the effects of systemic instead of portal insulin release in type 1 diabetic patients after successful pancreas-kidney transplantation (PKT) with systemic venous drainage on the intracellular lipid content in liver and soleus muscle, endogenous glucose production (EGP), and insulin sensitivity.

RESEARCH DESIGN AND METHODS—In nine PKT patients and nine matching nondiabetic control subjects, intrahepato cellular lipids (IHCLs) and intramyocellular lipids (IMCLs) were measured using 1H nuclear magnetic resonance spectroscopy. Fasting EGP was measured using [6,6-2H2]glucose tracer dilution. A 3-h 75-g OGTT allowed us to assess kinetics of glucose, free fatty acids, insulin, and C-peptide concentrations in plasma and to calculate the clamp-like index (CLIX) for insulin sensitivity and the hepatic insulin resistance (HIR) index.

RESULTS—The PKT patients displayed approximately twofold increased fasting insulin (20 ± 6 vs. 9 ± 3 μU/ml; P < 0.0002) compared with that in nondiabetic control subjects and ~10% increased fasting glucose (P < 0.02) concentrations, but during the OGTT areas under the concentration curves of C-peptide and insulin were similar. IHCL (PKT, 2.9 ± 2.5%; nondiabetic control subjects, 4.4 ± 6.8%), IMCL (PKT, 1.0 ± 0.4%; nondiabetic control subjects, 1.0 ± 0.5%), CLIX (PKT, 8 ± 2; nondiabetic control subjects, 7 ± 3), HIR (PKT, 25.6 ± 13.2; nondiabetic control subjects, 35.6 ± 20 [mg · min⁻¹ · kg⁻¹] × [μU/ml]), and EGP (PKT, 1.6 ± 0.2; nondiabetic control subjects, 1.7 ± 0.2 mg · min⁻¹ · kg⁻¹) were comparable between PKT patients and nondiabetic control subjects. IHCL was negatively correlated with CLIX in all participants (r = -0.55; P < 0.04).

CONCLUSIONS—Despite fasting peripheral hyperinsulinemia because of systemic venous drainage, type 1 diabetic patients after PKT show similar IHCL, IMCL, insulin sensitivity, and fasting EGP in comparison with nondiabetic control subjects. These results suggest that systemic hyperinsulinemia per se does not cause ectopic lipid accumulation in liver and skeletal muscle. Diabetes 59:215–218, 2010

Insulin resistance has been linked to lipid accumulation in insulin-responsive tissues such as liver and skeletal muscle (1–3), but it is not yet clear if ectopic fat accumulation induces insulin resistance and consequently hyperinsulinemia or whether increased intracellular lipid content is rather the result of long-term hyperinsulinemia.

Poorly controlled type 1 diabetic patients (4) as well as insulin-resistant type 2 diabetic subjects and the offspring of type 2 diabetic subjects displayed increased intracellular lipid content in skeletal muscle when compared with healthy individuals (1), whereas well-controlled type 1 diabetic patients exhibited an unchanged intramyocellular lipid content (5).

Pancreatic transplantation in diabetic subjects with end-stage renal disease restores insulin secretion and glucose tolerance (6). Combined pancreas-kidney transplantation (PKT) with systemic venous drainage provides a human model that allows to study the long-term effects of systemic instead of portal insulin delivery on glucose metabolism and intracellular lipid content. It is worth noting that insulin replacement in diabetic patients is commonly administered subcutaneously into the systemic circulation and not through the portal vein (4, 7). It is unclear whether this peripheral route of insulin delivery has clinically relevant consequences.

We studied whether the systemic route of insulin appearance could affect intracellular lipid content in liver and skeletal muscle, as well as endogenous glucose production (EGP) in type 1 diabetes after successful pancreas transplantation.

RESEARCH DESIGN AND METHODS—Following successful combined PKT, nine type 1 diabetic patients were matched for age and BMI with nine healthy control subjects. The PKT patients had received whole pancreas grafts with systemic venous anastomosis to the iliac vein 5.2 ± 1.6 years prior to the study. At the time of examination, the immunosuppressive regimen in the PKT patients included tacrolimus (n = 8) or sirolimus (n = 1) combined with either mycophenolate mofetil (n = 6) or azathioprine (n = 2). None was using insulin or any other antihyperglycemic agent. Five PKT patients received lipid-lowering medication (statins). All subjects had nondiabetic fasting plasma glucose, glycated A1C <6.5%, and...
stable serum creatinine. They gave written informed consent after approval by the local ethics committee.

An oral glucose tolerance test (OGTT) was performed after an overnight fast. After the collection of basal samples, participants were given the standard dose of 75 g glucose in H$_2$O solution (Glukodrink; Roche Diagnostics, Vienna, Austria) within 5 min and remained on bed rest. Venous blood samples were drawn at 0, 10, 20, 30, 40, 60, 90, 120, 150, and 180 min. Blood was rapidly centrifuged, and serum and plasma aliquots were stored at −70°C until further analysis. Insulin sensitivity was estimated by using the clamp-like index (CLIX) (8).

$^3$H-metabolite resonance spectroscopy was used after another 12-h overnight fast to assess the intramyocellular (IMCL) and intrabeatapcellular (IHCL) lipid content at 3-Tesla (Medspec DBX; Bruker Biospin, Ettlingen, Germany) in the soleus muscle and the liver. A STEAM sequence (repetition time [TR] = 4 s, echo time [TE] = 20 ms, mixing time [TM] = 30 ms, 32 averages, voxel volume [VV] = 1.728 ml, and measurement time [TA] = 125 s) (9) was used to acquire muscle spectra. To correct the liver metabolites for their different $T_2$ relaxation times, a series of five breath-hold STEAM experiments (TR = 2 s, TM = 30 ms, four averages, VV = 1.728 ml, and TA = 8 s) with varying TE (15, 20, 30, 50, and 70 ms) were performed. Muscle spectra were evaluated using the linear combination model (9) and were corrected for their $T_2$ relaxation times. IMCL content was then expressed as the percentage ratio of the (CH$_2$)$_n$ group integral to the sum of the integrals of the (CH$_3$)$_n$ group and water resonance from non–water-suppressed spectra of the same position and VV (10). From the processed spectra, the areas under the lipid (0.4–1.8 ppm) and water (3.5–5.6 ppm) resonances were integrated at each TE on the console. IMCL values were calculated from the TE$_2$ corrected signals as the percentage ratio between lipid and the sum of water and lipid integrals. One IMCL and two IHCL measurements in the PKT patients and one IHCL and two IMCL measurements in the non-diabetic control subjects could not be completely finalized because of the participants’ claustrophobia.

Fasting EGP was assessed from a primed continuous infusion [5 min: 3.6 mg (fasting glucose (mg/dl)/90); 115 min: 0.036 mg/min × kg body wt] of $\delta$-[6,6-$^{2}$H$_2$]glucose (98% enriched) (Cambridge Isotope Laboratories, Andover, MA). EGP was calculated from blood samples taken after 2 h of steady-state infusion by dividing $\delta$-[6,6-$^{2}$H$_2$]glucose infusion rate times tracer enrichment by the tracer enrichment in plasma and subtracting the tracer infusion rate (3). A1C and free fatty acids were determined by commercially available radioimmunoassays (insulin: Pharmacia, Uppsala, Sweden; C-peptide: Cis, Gif-Sur-Yvette, France), and free fatty acids (FFAs) with a 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a CP-Sil5 25 m × 0.12 µm capillary column (Chrompack, Middelburg, the Netherlands) and interfaced to a Hewlett-Packard 5971A mass selective detector, as described elsewhere (3).

Fasting portal insulin concentration was estimated as fasting plasma insulin times the portal venous-to-systemic insulin gradient, which was 2.4 in nondiabetic control subjects and 1.0 in the PKT patients (11,12). Hepatic insulin resistance (HIR) was calculated as the product of EGP times fasting insulin (13). To account for the different ways of insulin delivery, we modified HIR using portal venous insulin concentrations estimated from peripheral insulin concentrations (11,12). Total areas under concentration curves of glucose (AUC$_{\text{glucose}}$), insulin (AUC$_{\text{insulin}}$), and C-peptide (AUC$_{\text{C-peptide}}$) were calculated with the trapezoidal rule. Dynamic areas under the curve (ΔAUC) were calculated by subtracting the fasting concentration × 180 min from the total AUC. Data are given as means ± SD. Differences between groups were assessed by the two-tailed Student $t$ test and the $\chi^2$ test for continuous and categorical variables, respectively; $P < 0.05$ is considered significant. Linear correlations were based on Pearson product moment correlations. All the continuous variables were normally distributed according to the Kolmogorov-Smirnov test.

RESULTS
Participants’ characteristics are presented in Table 1. The two groups did not differ in age, BMI, A1C, serum liver enzymes, fasting serum triglycerides, FFA, C-peptide, and HDL cholesterol. The PKT patients had increased serum creatinine, higher systolic blood pressure, and elevated fasting insulin but lower total and LDL cholesterol.

During OGTT (Table 1), the fasting glucose and AUC$_{\text{glucose}}$ in the PKT patients were approximately 10% and −24% higher, respectively, than in the non-diabetic control subjects, whereas ΔAUC$_{\text{glucose}}$ was not different. In the PKT patients, plasma insulin was ~two-fold elevated at fasting, while AUC and ΔAUC of insulin and C-peptide were not different from those of the non-diabetic control subjects. Fasting C-peptide concentrations were not different between the two groups.

No significant differences were found in the comparison of IHCL and IMCL contents between the PKT patients and the non-diabetic control subjects (PKT, IHCL 2.9 ± 2.5% and IMCL 1.0 ± 0.4%; non-diabetic control subjects, IHCL 4.4 ± 6.6% and IMCL 1.0 ± 0.5%). Likewise, fasting EGP, estimated fasting portal insulin concentration, insulin sensitivity (CLIX), and HIR were not different between the PKT patients and non-diabetic control subjects (Table 1).

IMCL was negatively correlated with EGP ($r = −0.67$; $P < 0.02$) and fasting plasma glucose ($r = −0.56$; $P < 0.04$). IHCL was negatively correlated with CLIX ($r = −0.55$; $P < 0.04$) and positively correlated with fasting serum triglycerides ($r = 0.71$; $P < 0.004$). In non-diabetic control subjects, a positive correlation between IHCL and IMCL was found ($r = 0.79$; $P < 0.04$). IHCL was positively correlated with fasting triglycerides ($r = 0.71$; $P < 0.05$), AUC$_{\text{glucose}}$ ($r = 0.84$; $P < 0.01$), and ΔAUC$_{\text{glucose}}$ ($r = 0.81$; $P < 0.02$). In PKT patients, IHCL was positively correlated with AUC$_{\text{insulin}}$ ($r = 0.86$; $P < 0.02$) and ΔAUC$_{\text{insulin}}$ ($r = 0.88$; $P < 0.01$).

DISCUSSION
Our results show that systemic drainage of the transplanted pancreas in type 1 diabetic patients after PKT leads to elevated peripheral insulin concentrations at fasting without affecting intracellular lipid content of liver and soleus muscle. Furthermore, PKT subjects exhibit fasting EGP and whole-body and hepatic insulin sensitivity similar to those in healthy, non-diabetic, and age- and BMI-matched control subjects.

Previous studies in type 1 diabetic patients on subcutaneous (systemic) insulin replacement found alterations of IMCL and IHCL (4,14), which could have resulted from glucose toxicity given the chronic hyperglycemia (7) and/or from peripheral hyperinsulinemia. Systemic venous drainage of PKT allows the investigation of the long-term effects of peripheral insulin delivery on intracellular lipid content; to the best of our knowledge, IHCL and IMCL have never been evaluated in type 1 diabetic patients after PKT. A study in poorly controlled type 1 diabetic patients (A1C = 8.6%) found increased IMCL of the soleus muscle, which was directly correlated with A1C values (4), whereas a study in well-controlled type 1 diabetic patients (A1C = 6.3%) demonstrated unchanged IMCL at fasting and after sustained hyperinsulinemia (5). In line with that study, our PKT patients presenting with normal A1C values and unaltered insulin sensitivity also showed IMCL similar to that of healthy control subjects, suggesting beneficial effects of long-term glycemic control.

Evidence exists that peripheral venous insulin replacement for ~3 days stimulated skeletal muscle lipid accumulation in type 2 diabetic patients (3); thus, insulin per se might also affect ectopic lipid accumulation. However, in non-diabetic humans, hyperinsulinemia only in combination with elevated circulating lipids did increase IMCL storage (15). In our PKT patients, unaltered fasting concentrations of triglycerides and FFAs would indicate similar lipid availability in comparison with non-diabetic

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control subjects. Because PKT patients had normal IMCL despite peripheral hyperinsulinemia, our findings support the hypothesis that hyperinsulinemia alone does not induce IMCL accumulation.

Insulin resistance is associated with hepatic fat accumulation secondary to increased FFA availability and with hyperinsulinemia (3). The reported indirect correlation between IHCL and whole-body insulin sensitivity (3) is confirmed by our study; however, in contrast to other studies in type 1 diabetic and type 2 diabetic patients (3,4), our PKT patients did not differ from healthy, age- and BMI-matched control subjects in hepatic and peripheral insulin sensitivity nor in FFA and portal insulin, therefore exhibiting unaltered IHCL. It is worth noting that the method used for calculation of insulin sensitivity (CLIX) (8) uses C-peptide, which is not influenced by the route of insulin administration, not being cleared in the liver. Previous studies have described insulin resistance in patients after PKT (16,17). However, these studies were performed in patients taking glucocorticoids, which are known to deteriorate insulin sensitivity (18,19). Therefore, the normal insulin sensitivity in our PKT patients confirms the benefits of glucocorticoid-free immunosuppressive treatment.

Fasting EGP was similar in PKT patients and nondiabetic control subjects in spite of systemic insulin delivery, which is in line with previous studies in pancreas-transplanted type 1 diabetic patients with systemic venous pancreas drainage compared with pancreas-transplanted patients with portal venous drainage, kidney-transplanted patients, or healthy control subjects (20,21). On the other hand, increased fasting EGP and reduced insulin-mediated EGP suppression was observed in pancreas-transplanted subjects in comparison with kidney-transplanted patients and healthy control subjects (22); however, those patients were on immunosuppressive therapy including glucocorticoids (22), while our PKT patients were on glucocorticoid-free medication. It is being debated whether insulin exerts different effects on EGP when delivered through the hepatic artery (23) or through the portal vein (24,25) and which of these routes is more important for the regulation of EGP. Studies in type 2 diabetic patients, obese, and healthy humans have shown that short- (3 h) (23) and long-term (3 days) (3) increases in peripheral insulin levels cause a pronounced suppression of EGP indicating that EGP is readily affected by peripheral insulin delivery. In our study, EGP, estimated fasting portal insulin concentration, and hepatic insulin resistance were unchanged in PKT, suggesting normal fasting hepatic glucose metabolism despite systemic instead of portal route of insulin delivery in PKT.

In conclusion, despite displaying fasting peripheral hyperinsulinemia but normal lipids, type 1 diabetic patients after PKT show similar IHCL, IMCL, insulin sensitivity, and fasting EGP in comparison with nondiabetic control subjects. These results suggest that systemic hyperinsulinemia per se does not cause ectopic lipid accumulation in liver and skeletal muscle.
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