Lipotoxicity and Decreased Islet Graft Survival

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OBJECTIVE — To evaluate if baseline serum lipids are associated with islet graft survival in type 1 diabetes islet transplant (ITx) recipients.

RESEARCH DESIGN AND METHODS — Baseline fasting lipid profile was collected from 44 ITx recipients. Comparisons were performed between subjects below and above the median values of each lipid fraction. Differences in outcomes were compared by Kaplan-Meier curves and Cox regression analysis.

RESULTS — Subjects with baseline fasting plasma triglycerides and VLDL cholesterol above the median had shorter islet graft survival (triglycerides: 39.7 ± 6.1 vs. 61.3 ± 6.6 months, $P = 0.029$, and VLDL: 41.5 ± 5.7 vs. 62.8 ± 7.3 months, $P = 0.032$). Total, LDL, and HDL cholesterol did not influence islet function. Triglycerides (odds ratio 2.97 [95% CI 1.03–8.52], $P = 0.044$) maintained its association with graft failure after adjustments for confounders.

CONCLUSIONS — Higher baseline triglycerides are associated with earlier decline in islet graft function. Prospective clinical trials should address whether it is directly caused by lipotoxicity and if strategies focusing on lowering serum lipids may prolong islet graft survival.

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Increased free fatty acids (FFAs) cause β-cell dysfunction and death (1–3). β-Cell lipid accumulation is mediated by defective intracellular lipid oxidation associated with leptin resistance (4). This abnormality can be corrected by insulin sensitizers or leptin therapy (5,6). In addition, FFA-induced endoplasmic reticulum stress has been implicated in β-cell apoptosis (7), which could be minimized by glucagon-like peptide-1 agonists (8). Islet grafts infused directly into the liver receive lipid-rich postprandial blood. Insulin secreted by islet grafts promote triglyceride deposition in surrounding hepatocytes, and multifocal steatosis has been reported in ~20% of islet transplant (ITx) recipients (9,10). Moreover, an insulin resistance phenotype and tendency for higher serum triglycerides, factors associated with steatosis (11), were predictors of shorter graft survival (12). The aim of this study was to determine whether the lipid profile of type 1 diabetic ITx recipients is associated with islet graft survival.

RESEARCH DESIGN AND METHODS

— A retrospective cohort study was conducted in 44 type 1 diabetic subjects (37 ITx alone subjects; 7 islet after kidney [IAK] subjects), post–allogeneic ITx between 2000 and 2007 (follow-up 40.9 ± 23.5 months). All patients have achieved the goal of glucose stability and avoidance of hypoglycemia, and 28 (64%) achieved insulin independence. ITx-related procedures were previously described (13). Immunosuppressive regimen consisted of tacrolimus and sirolimus. Three IAK recipients were on corticosteroid maintenance doses. Fourteen subjects were converted to mycophenolate mofetil or mychophenolic acid, as per protocol ($n = 6$) or due to side effects ($n = 8$). Protocol procedures were approved by the University of Miami Health Research Ethics Board, and informed consent was obtained.

Clinical variables (demography, anthropometry, and family history of type 2 diabetes), insulin dosage per kilogram, islet autoantibodies, number of infusions and islet equivalents infused, exenatide use, and immunosuppressive medication were recorded. Outcomes were graft dysfunction (positive C-peptide, fasting glucose >140 mg/dl and/or postprandial glucose >180 mg/dl more than three times in a 1-week period, and/or A1C >6.5% in two consecutive measurements) and graft failure (fasting C-peptide ≤0.10 ng/ml [two consecutive measurements in absence of hypoglycemia] or stimulated C-peptide ≤0.3 ng/ml).

Fasting lipids (total cholesterol, HDL cholesterol, VLDL cholesterol, and triglycerides) were measured by enzymatic method, and LDL cholesterol was calculated (Friedewald equation). Medians of serum lipids were calculated (total cholesterol: 177 mg/dl, LDL: 96 mg/dl, HDL: 67 mg/dl, VLDL: 13 mg/dl, and triglycerides: 65 mg/dl). Fasting glucose (hexokinase), A1C (high-performace liquid chromatography; BioRad, Richmond, CA), and autoantibodies (radioimmunoassay) were obtained. C-peptide was measured by double antibody radioimmunoassay at fasting and during a mixed-meal test (Boost high protein; Novartis/Sandoz, Nestle Nutrition). Kaplan-Meier curves (log-rank [Mantel-Cox] test) were used to compare time to outcomes (graft dysfunction and failure) between subjects with lipids below and above their median value. Adjustments for confounders were performed with Cox regression analysis. *P* values of <0.05 (two-tailed) were significant (SSPS version 16.0).

RESULTS

— Age at first ITx was 43.0 ± 8.6 years, and diabetes duration was 30.5 ± 11.7 years. Eighteen (41%) recip-

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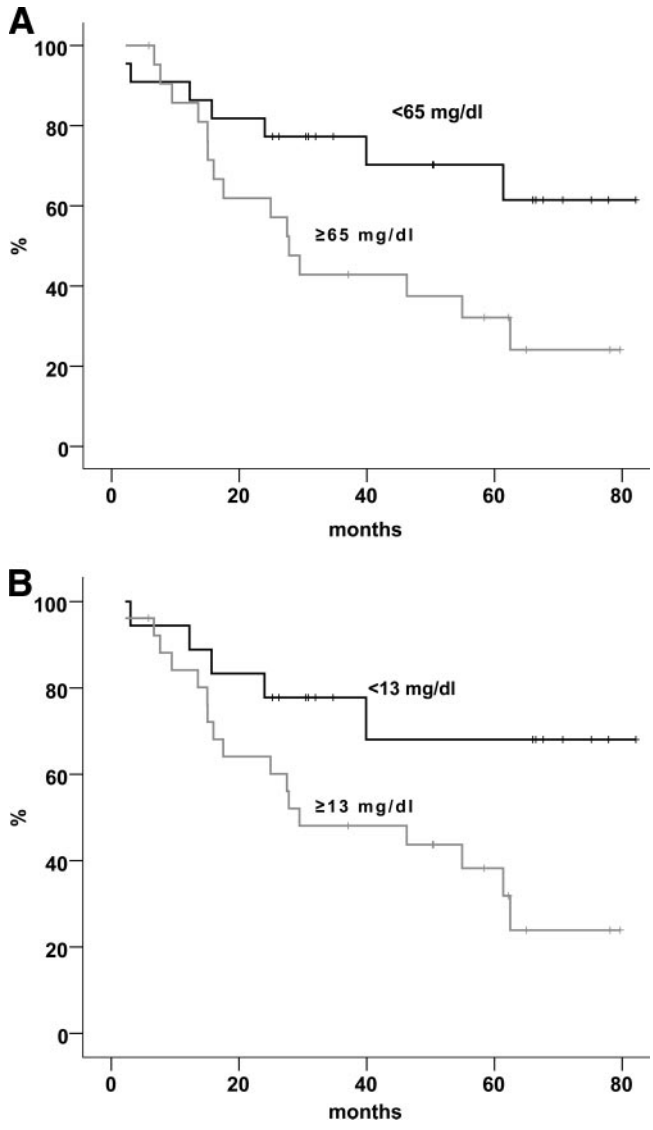

Figure 1—*Islet graft failure according to serum lipids below (black line) and above (gray line) the median values for triglycerides* (A) *and VLDL cholesterol* (B)*. Comparisons were done with Kaplan-Meier curves and log-rank (Mantel-Cox) test.*

ients were male and all were white. Subjects with baseline fasting plasma triglycerides above the median had earlier graft dysfunction (6.1 ± 1.5 vs. 17.3 ± 3.4 months, $P < 0.001$) and failure (39.7 ± 6.1 vs. 61.3 ± 6.6 months, $P = 0.029$) (Fig. 1*A*) in comparison with those with lower values. Similar results were found for VLDL cholesterol (dysfunction: 6.2 ± 1.6 vs. 16.7 ± 3.2 months, $P = 0.001$; failure: 41.5 ± 5.7 vs. 62.8 ± 7.3 months, $P = 0.032$) (Fig. 1*B*). Total, LDL, and HDL cholesterol were not determinants of islet function (data not shown).

To clarify if variables associated with higher triglycerides and/or VLDL cholesterol were determinants of shorter graft survival, we compared clinical and laboratory characteristics of subjects below and above their median values (data not shown). Patients with triglycerides and/or VLDL cholesterol above median were more frequently male and IAK protocol participants, had positive family history of type 2 diabetes and overweight, were on higher doses of insulin per kilogram pre-ITx, and received a longer period of sirolimus/tacrolimus combination. These variables were included in multivariate analysis with time-to-graft-failure as the dependant variable. Triglycerides (odds ratio 2.97 [95% CI 1.03–8.52], $P = 0.044$) sustained its association with graft failure, while VLDL cholesterol (3.06 [0.99–9.45], $P = 0.052$) attained borderline significance. Other variables were analyzed on separate multivariate models based on their biological relevance (HLA mismatches, cold ischemia duration, age, diabetes duration, BMI, autoantibodies, and immunosuppressant's serum trough levels) without modifying the results.

CONCLUSIONS — In ITx recipients, higher baseline triglycerides predict earlier graft dysfunction and failure. VLDL cholesterol produced similar outcomes, probably by the same mechanisms, since VLDL cholesterol is mainly composed by triglycerides.

Lipotoxicity has been pointed as one of the mechanisms responsible for β-cell dysfunction and death in type 2 diabetes (1). Concerns about similar effects in ITx have been raised by posttransplant image studies showing steatosis (9,10). However, the significance of steatosis in humans is not clear, being described either as a marker of good function (9) or dysfunction (10).

Recently, lipid toxicity has been studied in an animal model of ITx (14), and liver triglyceride accumulation was associated with poorer islet graft function and histological appearance (reduced β-cell mass and increased islet fibrosis) (14). Notably, these abnormalities were corrected by therapies targeting lipid supply (restrictive diet) or deposition (leptin gene therapy) (14).

Besides direct toxic effects, another interesting hypothesis connecting higher triglycerides and islet graft survival can be formulated. Lipoprotein apo-C3 provokes human β-cell apoptosis (15). This lipoprotein is a component of triglyceride-rich VLDL cholesterol molecules, raising the possibility of an extra mechanism of β-cell damage.

To the best of our knowledge, this is the first report of serum lipid association with islet graft survival in humans. This finding adds to the understanding of multiple and complex mechanisms of β-cell survival and calls attention to potential new therapies targeting serum lipids, preventing lipotoxicity and possibly leading to longer islet survival.

The study limitations are retrospective analysis and small sample size. We are aware that our results are not definitive and should be confirmed in larger cohorts with more detailed laboratorial analysis, including measurement of FFAs, and quantification of liver lipid deposition by spectroscopy. Our aim was to prove a concept and bring this idea to discussion. Higher baseline triglycerides are

associated with earlier decline in islet graft function. Prospective trials should address whether it is directly caused by lipotoxicity and if strategies reducing serum lipids may prolong islet graft survival.

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