Lipotoxicity and Decreased Islet Graft Survival

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Submitted 28 July 2009 and accepted 3 December 2009.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes Care. The American Diabetes Association, publisher of Diabetes Care, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes Care in print and online at http://care.diabetesjournals.org.
**Aim:** To evaluate if baseline serum lipids are associated with islet graft survival in type 1 diabetes mellitus 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 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 didn’t influence islet function. Triglycerides (OR=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 lowering serum lipids may prolong islet graft survival.
Increased free fatty acids (FFA) causes beta-cell dysfunction and death (1-3). Beta-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). As well, FFA-induced endoplasmic reticulum stress has been implicated in beta-cell apoptosis (67), which could be minimized by GLP-1 agonists (8).

Islet grafts infused into the liver directly receive a lipid-rich post-prandial 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 if the lipid profile of type 1 diabetes mellitus (DM) ITx recipients is associated with islet graft survival.

RESEARCH DESIGN AND METHODS

A retrospective cohort study was conducted in 44 type 1 DM subjects [37 ITx alone; 7 islet after kidney (IAK)], post allogeneic ITx between 2000-2007 (follow-up: 40.9±23.5 months). All patients have achieved the goal of glucose stability and avoidance of hypoglycemia and 28 (64%) insulin independence. ITx related procedures were previously described (13). Immunosuppressive regimen consisted of tacrolimus and sirolimus. Three IAK recipients were on corticosteroids 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 University of Miami health research ethics board and informed consent was obtained.

Clinical variables (demography, anthropometry and family history of type 2 DM), insulin dosage/kg islet auto anti-bodies, number of infusions and islet equivalents infused, exenatide use and immunosuppressive medication were recorded. Outcomes were graft dysfunction [positive C-peptide, fasting glucose (FG) >140 mg/dl and/or postprandial glucose >180 mg/dl more than 3 times in one-week period and/or HbA1c >6.5% in 2 consecutive measurements] and graft failure [fasting C-peptide ≤0.10 ng/ml (2 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). Median 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). FP (hexokinase), HbA1c (HPLC, BioRad, Richmond/CA) and auto-antibodies (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 (2-tailed) were significant (SSPS® 16.0).

RESULTS

Age at first ITx was 43.0±8.6 years and DM duration was 30.5±11.7 years.
Eighteen (41%) recipients 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; Figure 1A) 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; Figure 1B). 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 the determinants of shorter graft survival, we compared clinical and laboratory characteristics of subjects below and above their median values (data not shown). Patients with triglyceride and/or VLDL-cholesterol above median were more frequently males and IAK protocol participants, had positive family history of type 2 DM and overweight, were on higher doses of insulin/kg 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 (OR=2.97, 95%CI=1.03-8.52, P=0.044) sustained its association with graft failure, while VLDL-cholesterol (OR=3.06, 95%CI=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, DM duration, BMI, auto-antibodies 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 beta-cells dysfunction and death in type 2 DM (1). Concerns about similar effects in ITx have been raised by post-transplant image studies showing steatosis (9,10). However, the significance of steatosis in humans is not clear, being described either as marker of good function (9) or dysfunction (10).

Recently, lipid toxicity has been studied in an animal model of ITx (14), and liver triglycerides accumulation was associated with poorer islet graft function and histological appearance (reduced beta-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 beta-cell apoptosis (15). This lipoprotein is a component of triglyceride rich VLDL-cholesterol molecule, raising the possibility of an extra mechanism of beta-cell damage.

To the best of our knowledge, this is the first report of serum lipids association with islet graft survival in humans. This findings adds to the understanding of multiple and complex mechanisms of beta-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 FFA, and quantification of liver lipid deposition by spectroscopy. Our
The aim was to proof 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.

ACKNOWLEDGEMENTS

This study was supported by: NIH/NCRR (U42 RR016603, M01RR16587); JDRFI (#4-2000-946, 4-2004-361); NIH/NIDDK (5 R01 DK55347, 5 R01 DK056953); State of Florida, and the Diabetes Research Institute Foundation (Hollywood, FL). CBL was the recipient of a scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Legend:

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

A

B