Prediabetes in Pediatric Recipients of Liver Transplant: Mechanism and Risk Factors

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Objective To investigate the role of calcineurin inhibitor exposure and states of insulin resistance—obesity and adolescence—in prediabetes after pediatric liver transplant via oral glucose tolerance testing, which previously has not been done systematically in these at-risk youths.

Study design This was a cross-sectional study of 81 pediatric recipients of liver transplant. Prediabetes was defined as impaired glucose tolerance (IGT; glucose ≥140 mg/dL at 2 hours) or impaired fasting glucose (IFG, ≥100 mg/dL). Corrected insulin response (CIR) was calculated as measure of insulin secretion, corrected for glucose (CIR_{30}, CIR_{60}, CIR_{120}).

Results Subjects were aged 8.1-30.0 years and 1.1-24.7 years post-transplant; 44% had prediabetes—27% IGT, 14% IFG, and 3% both. IGT was characterized by insulin hyposecretion, with lower CIR_{60} and CIR_{120} in IGT than subjects with normal glucose tolerance. Subjects with tacrolimus trough >6 μg/mL at study visit had lower CIR_{120} than those with trough ≤6 μg/mL and those off calcineurin-inhibitors. Mean of tacrolimus troughs preceding the study visit, years since transplant, and rejection episodes were not associated significantly with lower CIR. CIR suppression by tacrolimus was most pronounced >6 years from transplant. Overweight/obese subjects and adolescents who retained normal glucose tolerance had greater CIR than those who were IGT.

Conclusion IGT after pediatric liver transplant is driven by inadequate insulin secretion. It is quite common but not detectable with fasting laboratory values—the screening recommended by current guidelines. Calcineurin inhibitors suppress insulin secretion in these patients in a dose-dependent manner. Given the recent focus on long-term outcomes and immunosuppression withdrawal in these children, longitudinal studies are warranted to investigate whether IGT is reversible with calcineurin inhibitor minimization. *(J Pediatr 2017;182:223-31).*

In adults after liver transplant, diabetes is associated with advanced fibrosis, vascular problems, acute and chronic rejection, and mortality.[1] In pediatric recipients of liver transplant, the prevalence estimates of diabetes have ranged from 1.2% to 14.1%, depending on the definition of diabetes and patient cohort, but existing studies focus on symptomatic diabetes early post-transplant.[2,3] Neither the long-term impact of diabetes nor the prevalence of prediabetes, which includes impaired glucose tolerance (IGT; blood glucose ≥140 mg/dL, 2 hours after glucose load) and impaired fasting glucose (IFG; fasting glucose ≥100 mg/dL), have been studied systematically in these children.[4,5] In children who do not undergo transplant, glucose intolerance in childhood increases the risk of diabetes, cardiovascular disease, and premature death in adulthood.[6,7] To optimize the long-term outcomes of pediatric recipients of liver transplant, conditions like prediabetes—which can progress to clinically dangerous disease but are screened for easily and may be reversible with intervention—are important areas of investigation.

We recently demonstrated that pediatric recipients of liver transplant have a greater odds of IGT than age-, sex-, and race/ethnicity-matched peers (OR 6.50, 95% CI 2.76-15.63, $P < .001$).[8] Their odds of IGT doubled for every 7.5 years on calcineurin inhibitors, which are the mainstay of long-term immunosuppression in all recipients of solid-organ transplants.[8] Calcineurin inhibitors—with tacrolimus implicated more strongly than cyclosporine—are thought to cause IGT by reducing insulin secretion from pancreatic β cells.[9,10] They also may induce β-cell apoptosis.[11,12] Calcineurin inhibitors contribute to post-transplant diabetes in adults, but their relative contribution compared with age-driven risk, obesity, and underlying diseases like steatohepatitis is very difficult to tease out. In addition, these relative contributions likely are not applicable in the pediatric population. Finally, whether β-cell suppression is reversible in subjects with chronic exposure has not been settled.[9,13]

BMI	Body mass index	IR	Insulin resistance
CIR	Corrected insulin response	OGTT	Oral glucose tolerance test
HbA1c	Hemoglobin A1c	NFG	Normal fasting glucose
HOMA	Homeostatic model assessment	NGT	Normal glucose tolerance
IFG	Impaired fasting glucose	WB-ISI	Whole-body insulin sensitivity index
IGT	Impaired glucose tolerance		

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Supported by the National Institutes of Health (NIH)/National Institute of Diabetes and Digestive and Kidney Diseases (K23 DK0990253-A101 [to E.P.]), the American Gastroenterological Association Emmet B. Keeffe Career Development Award in Liver Disease (to E.P.), University of California, San Francisco (UCSF) Liver Center Pilot Funding (P30 DK026743 [to E.P.]), UCSF, and by the NIH/National Center for Advancing Translational Sciences (UL1 TR000004). The content is the responsibility of the authors and does not necessarily reflect the views or policies of funders. The authors declare no conflicts of interest.

http://dx.doi.org10.1016/j.jpeds.2016.11.070

Clarifying contributors to and mechanisms of post-transplant IGT would help us design interventions to prevent the progression of prediabetes. Studying the impact of calcineurin inhibitors is particularly relevant for, and feasible in, pediatric recipients of liver transplants. Most will have decades of exposure to calcineurin inhibitors, usually with greater levels in the immediate post-transplant period (8-12 μg/mL) and lower levels in the long term (3-8 μg/mL, titrated based on liver function tests and biopsy findings); however, recent studies show that pediatric recipients of liver transplants uniquely are tolerant to minimization or complete withdrawal of calcineurin inhibitors.[14-16] Thus far, immunosuppression withdrawal trials have focused on safety for the liver, but improved glucose tolerance and other systemic benefits are important considerations.

In this analysis, we hypothesized that glucose and insulin response to an oral glucose tolerance test (OGTT) would differ by current and cumulative calcineurin inhibitor exposure and conditions associated with insulin resistance, including overweight/obesity and adolescence. Specifically, we theorized that exposure to calcineurin inhibitors would lead to hyperglycemia with reduced insulin secretion. Second, we postulated that insulin resistance, seen particularly in overweight/obese and pubertal subjects, would produce a different pattern of IGT. Subjects who are insulin resistant need to secrete more insulin to achieve the same glucose control. IGT associated with insulin resistance should be characterized by hyperglycemia despite hypersecretion from pancreatic β cells. We also evaluated whether the OGTT predicted hyperglycemia during standard-of-care monitoring after the study visit.

Methods

This study was approved by University of California, San Francisco's Committee on Human Research (12-10290). We performed a cross-sectional study of pediatric recipients of liver transplant aged 8-30 years at study visit. All underwent first liver transplant before age 18 years. At study visit, all were at least 1 year from liver transplant, on stable immunosuppressive regimens for at least 3 months, and on ≤5 mg daily of prednisone. After age-appropriate consent and assent were obtained, subjects were evaluated in University of California, San Francisco's Pediatric Clinical Research Center or during inpatient admission for a surveillance liver biopsy. Subjects who participated in the cross-sectional study with known diabetes (n = 1), liver-kidney transplant (n = 4), or on ≥20 mg prednisone daily (n = 2) were excluded from this analysis to reduce confounding. Of the additional 35 patients aged 8-30 years old followed at our transplant center, 1 was on insulin during the study period, 4 had severe developmental delay preventing participation, 10 were not seen during the study period because they were stable and followed primarily at another center, and 14 were consented but did not complete the study visit (1 nonadherence, 1 diagnosed with post-transplant lymphoproliferative disease, 1 liver-kidney transplant, and 11 stable outpatient without diabetes or known glucose intolerance); 7 were not eligible, only 1 of whom had diabetes.

Fasting serum was obtained after at least an 8-hour fast. Tacrolimus troughs were measured 11-12 hours from the preceding dose. OGTT was performed with weight-based glucose load (1.75 g/kg to maximum 75 g), following the National Health and Nutrition Examination Survey 2011 protocols (http://www.cdc.gov/nchs/nhanes/nhanes2011-2012/manuals11_12.htm). Glucose and insulin levels were drawn immediately before ingestion of the glucose and at 30 (±5) minutes, 1 hour (±5 minutes), and 2 hours (±5 minutes) after start of ingestion. All subjects drank the glucose load in ≤5 minutes. Fasting insulin levels are not affected by calcineurin inhibitors; thus, these measures are valid in our population.[13,17] Hemoglobin A1c (HbA1c) was measured by the use of an ion-exchange high-performance liquid chromatography assay (Bio-Rad Variant II Turbo 2.0; Bio-Rad, Hercules, California).

All subjects had height, weight, and anthropometrics measured following National Health and Nutrition Examination Survey 2011 Anthropometry Procedures (http://www.cdc.gov/nchs/nhanes/nhanes2011-2012/manuals11_12.htm). Overweight and obesity classifications were based on age-appropriate standards. For subjects younger than 18 years at study visit, body mass index (BMI) percentile for age and sex was calculated based on 2000 Centers for Disease Control and Prevention growth charts[18]; subjects were classified as overweight/obese for BMI percentile ≥85th percentile for age and sex.[19] Overweight/obesity in subjects ≥18 years was BMI ≥25 kg/m^2.[20] Pubertal stage was assessed during the study physical examination by 1 of 3 study pediatricians, according to the Tanner scale.

Laboratory data from routine clinical monitoring were extracted from the medical records to investigate whether glucose tolerance predicted hyperglycemia during rejection episodes. For calcineurin inhibitor levels, the lower limits of assay detection were used as the trough value if levels were listed as lower than level measurable, respectively. Data on rejection also were extracted from the medical record.

Glucose and Insulin Measures

Glucose tolerance was determined following the guidelines of the American Diabetes Association. Subjects with normal fasting glucose (NFG) and normal glucose tolerance (NGT) had fasting glucose <100 mg/dL and 2-hour glucose <140 mg/dL. IFG was ≥100 mg/dL. IGT was 2-hour glucose ≥140 mg/dL.[5]

We used corrected insulin response (CIR) as a measure of insulin secretion. CIR was calculated at 30, 60, and 120 minutes postglucose load as (Ins_X * 100)/($Gluc_X$ [$Gluc_X$ – 70]).[21] (**Table I**; available at www.jpeds.com) CIR can be assessed at any time point after the glucose load. It describes the β-cell secretion capacity; lower CIRs suggests insulin hyposecretion for the glucose level, and higher CIR suggests insulin hypersecretion.[21] We thus used CIR to investigate (1) whether insulin hypo- or hypersecretion drove IGT and (2) whether predictors contributed to IGT by reducing insulin secretion or causing insulin resistance. Calcineurin inhibitor exposure was our main predictor of interest; we considered both current exposure (medication, trough at study visit) and chronic exposure (years since transplant, in those on tacrolimus at study visit).