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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 (CIR₃₀, CIR₆₀, CIR₁₂₀).

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 CIR₆₀ and CIR₁₂₀ in IGT than subjects with normal glucose tolerance. Subjects with tacrolimus trough >6 μ g/mL at study visit had lower CIR₁₂₀ than those with trough ≤ 6 μ g/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}

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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		

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 $\mu\text{g}/\text{mL}$) and lower levels in the long term (3-8 $\mu\text{g}/\text{mL}$, 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 charts¹⁸; subjects were classified as overweight/obese for BMI percentile ≥ 85 th percentile for age and sex.¹⁹ Overweight/obesity in subjects ≥ 18 years was BMI ≥ 25 kg/m².²⁰ 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 $(\text{Ins}_x \times 100) / (\text{Gluc}_x [\text{Gluc}_x - 70])$.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).

We also evaluated fasting measures of insulin resistance (homeostatic model assessment [HOMA] of insulin resistance [IR]) and pancreatic β -cell function (HOMA of β -cell function). We calculated the whole-body insulin sensitivity index (WB-ISI) to evaluate insulin resistance over the entire OGTT (Table I).^{4,21-24} In analysis of rejection-associated hyperglycemia, hyperglycemia was considered any glucose ≥ 200 mg/dL.^{4,5}

Statistical Analyses

Categorical variables were compared with McNemar χ^2 tests. For continuous variables, all data are presented with median and IQR, and groups were compared with Kruskal-Wallis tests given the small sample sizes. Wilcoxon rank-sum was used as a nonparametric test for trend in CIR across the OGTT. In the box plots, boxes demarcate the median and 25th/75th percentile; whiskers mark the most extreme values within $1.5 \times$ IQR (75th-25th percentile), and dots mark outliers.

In multivariable analysis, we used generalized estimating equations with robust SEs to investigate the impact of predictors on CIR, using CIR as a repeated-measures outcome and controlling for within-person correlations. The model accounts for a statistical interaction between time (30, 60, 120 minutes) and calcineurin inhibitor category. Sex, ethnicity, and alanine aminotransferase at study visit were considered but were not significant predictors and did not change other predictors appreciably. An interaction between time and years since transplant was not significant and thus excluded. Stata 14 (StataCorp, College Station, Texas) used for all analyses. *P* values $<.05$ were considered statistically significant.

Results

This cross-sectional study included 81 recipients of liver transplant, 8.1-30.0 years of age and 1.1-24.7 years post-transplant at study visit (Table II). No subjects were on oral hypoglycemic medications or had a diagnosis of post-transplant diabetes. Three subjects were on prednisone, ≤ 5 mg daily. One subject missing Gluc₁₂₀ was classified as NGT because Gluc₆₀ was 92 mg/dL. Four more missing Gluc₁₂₀ could not be classified by glucose tolerance status.

Glucose Tolerance

Of the 77 patients with complete OGTT data, 27% had IGT despite NFG, 14% had IFG with NGT, 3% had both IFG and IGT, and 56% had NFG/NGT. Of note, all subjects had a normal HbA1c (range 3.8%-5.7%). We evaluated the impact of factors that can falsely lower HbA1c—including anemia and aspirin use. Our prevalence of anemia was low ($n = 5$ with hemoglobin <11 mg/dL), but HbA1c levels did not correlate with hemoglobin ($P = .95$). Six patients were on aspirin at study visit, but their HbA1c distribution also was not significantly different than those not on aspirin (median 5.2, IQR 4.5-5.3% vs 4.9, IQR 4.6-5.2%, $P = .37$).²⁵

The subjects with IFG had elevated fasting glucose, by definition, but the impairment was mild (range 100-105 mg/dL). They were more insulin resistant by fasting variables (HOMA-IR), without a significant difference

Table II. Demographics, transplant, and post-transplant characteristics of cohort

Characteristics	All (n = 81)*
Age at visit, y	15.6 (11.5-18.5)
Female	46%
Hispanic	42%
Race	
White	36%
Black	7%
Asian	15%
Other†	27%
Multiracial	15%
Indication for transplant‡	
Biliary atresia	35%
Metabolic disease	16%
Cholestatic disease	6%
Acute liver failure, tumor, other	43%
Overweight/obese by BMI	26%
Acanthosis nigricans	23%
Family history of diabetes (first or second degree)	60%
Years since first liver transplant	11.2 (7.9-15.7)
Years on calcineurin inhibitors	10.7 (7.5-14.8)
Calcineurin inhibitor	
Tacrolimus	79%
Cyclosporine	10%
None	11%
Tacrolimus trough at visit, μ g/L, n = 64	4.2 (2.5-6.2)
Mean recent tacrolimus trough, μ g/L, n = 64§	4.7 (3.4-6.1)
Cyclosporine trough at visit, μ g/L, n = 8	55 (45-95)
Mean recent cyclosporine trough, μ g/L, n = 8§	89 (43-157)
Retransplant	6%
Biopsy-proven acute rejection episodes	
0-1	69%
≥ 2	31%
Chronic rejection	7%
AST, IU/L	33 (25-47)
ALT, IU/L	30 (21-45)
GGT	23 (13-45)
Total bilirubin	0.9 (0.6-1.3)
Creatinine, mg/dL	0.57 (0.47-0.79)
Hematocrit, %	39.5 (36.8-42.7)
Platelets	191 (148-217)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase.

*Data represent proportion or median, IQR. McNemar χ^2 test for categorical variables, Kruskal-Wallis for continuous variables.

†Race and ethnicity self-reported. Other ethnicity includes Native American, Alaskan, Pacific Islander, Hawaiian, multiracial, and unknown.

‡Metabolic liver disease includes alpha-1-antitrypsin deficiency, Crigler-Najjar syndrome, cystic fibrosis, glycogen storage disease, inborn errors in bile acid metabolism, neonatal hemochromatosis, primary hyperoxaluria, tyrosinemia, urea cycle defects, and Wilson disease. Cholestatic conditions include Alagille syndrome, Byler disease, progressive intrahepatic cholestatic syndromes, total parenteral nutrition cholestasis, sclerosing cholangitis, and idiopathic cholestasis. Other liver disease includes congenital hepatic fibrosis, Budd-Chiari syndrome, autoimmune hepatitis cirrhosis, drug toxicity, hepatitis C cirrhosis, and unknown cirrhosis.

§Mean of 3 most recent trough levels before study visit. One subject with NGT was on sirolimus. Nine also were on mycophenolate mofetil: 6 were NGT, 1 IFG, and 2 IGT.

in measures of β -cell function (HOMA of percent β -cell function; CIR at any time point) (Table III and Figure 1; available at www.jpeds.com). Patients with IFG had greater peak glucose than patients with NFG/NGT, with no difference in insulin levels, or insulin levels at any point during the OGTT—suggesting insulin hyposecretion for the degree of hyperglycemia (Table III).

The 2 subjects with IFG/IGT were both normal weight, not on prednisone. Both had low insulin levels despite their hyperglycemia (1: Gluc₀ 107 mg/dL, Ins₀ 13.6 mU/L; Gluc₁₂₀ 144 mg/dL, Ins₁₂₀ 3.7 mU/L on tacrolimus with trough

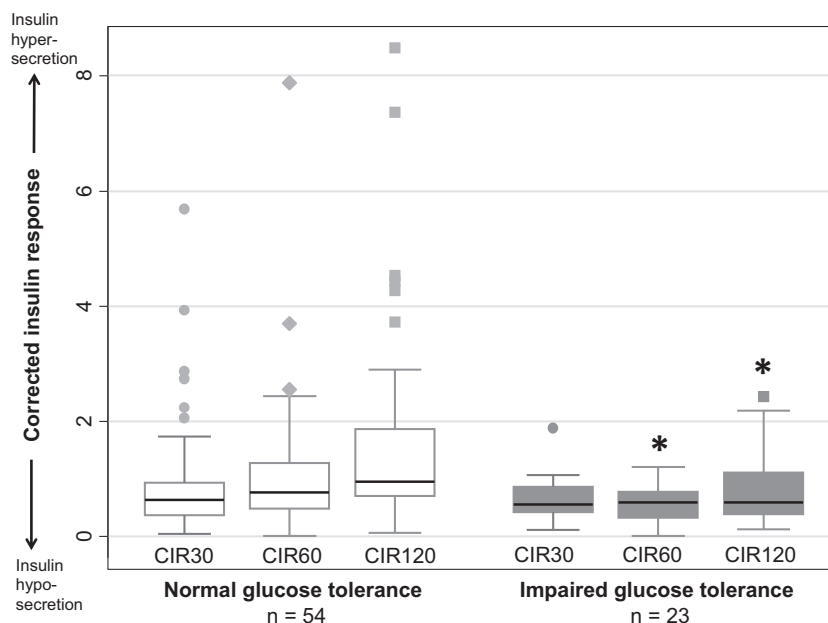


Figure 2. CIR at 30, 60, and 120 minutes after an oral glucose load (OGTT) in pediatric recipients of liver transplant, by glucose tolerance. Those with IGT had lower CIR₆₀ and CIR₁₂₀, suggesting insulin hyposecretion. * $P < .05$ for difference between IGT and NGT subjects at each time point.

4.1 $\mu\text{g}/\text{mL}$, 11.3 years on tacrolimus; 2: Gluc₀ 100 mg/dL, Ins₀ 5 mU/L; Gluc₁₂₀ 142 mg/dL, Ins₁₂₀ 15.7 mU/L on cyclosporine with trough 70 $\mu\text{g}/\text{L}$, 20.5 years on cyclosporine).

Glucose intolerance was not associated significantly with age at visit, sex, self-reported race/ethnicity, indication for transplant, overweight/obesity, or family history of diabetes (data not shown). None of the patients had cystic fibrosis. Two were transplanted for gestational alloimmune liver disease; one was obese with IFG and insulin resistance but NGT, the other had normal fasting glucose and did not complete the OGTT. All subjects had stable serum transaminases and liver function at study visit (Table II), and glucose tolerance was not associated with serum transaminases. Years since transplant, age at visit, type of calcineurin inhibitor, trough at visit, and mean of 3 most recent did not differ by glucose tolerance status (data not shown).

IGT Is Characterized by Insulin Hyposecretion

Subsequent analyses focus on glucose tolerance status, with classification as NGT or IGT based on the 2-hour glucose. Fasting glucose and insulin measures did not differentiate IGT from subjects with NGT (Table II). In subjects with NGT, CIR increased during the OGTT ($P = .001$ test for trend). In subjects with IGT, however, CIR did not increase ($P = .60$ test for trend). In subjects with IGT, CIR₆₀ and CIR₁₂₀ were significantly lower than in subjects with NGT, suggesting relative hyposecretion of insulin for the degree of hyperglycemia (Figure 2). This difference remained significant when the subjects with IFG/NGT were excluded from the NGT group (Figure 1). The subjects with IGT were not more insulin resistant than the subjects with NGT, by either fasting (HOMA-IR) or stimulated (WB-ISI) measures (Table III).

Insulin Hyposecretion Is Associated with Calcineurin Inhibitor Exposure

Seventy-nine percent of the cohort was on tacrolimus, 10% on cyclosporine, and 11% off of calcineurin inhibitors after immunosuppression withdrawal. Because lower CIR₆₀ and CIR₁₂₀ distinguished the subjects with IGT from the subjects with NGT, we next investigated factors associated with CIR.

Subjects with tacrolimus trough $>6 \mu\text{g}/\text{mL}$ at study visit had lower CIR₆₀ and CIR₁₂₀ than those off of calcineurin inhibitors and those on tacrolimus with trough $\leq 6 \mu\text{g}/\text{mL}$ (Figure 3, A). Their CIR remained flat during the OGTT ($P = .48$ test for trend), compared with increasing CIR over time for those with low tacrolimus troughs ($P = .001$ test for trend). The subjects on cyclosporine also had lower CIR₁₂₀ than those off calcineurin inhibitors or those with tacrolimus trough $\leq 6 \mu\text{g}/\text{mL}$ (Figure 3, A). Those on cyclosporine were significantly farther from transplant (median 17.1, IQR 11.7-19.1 years) than those on low-trough tacrolimus (median 10.9, IQR 7.0-14.5 years, $P = .01$).

Lower CIR₆₀ and CIR₁₂₀ was not seen in subjects with tacrolimus troughs 3-6 $\mu\text{g}/\text{mL}$ on the day of study visit, compared with those with tacrolimus troughs $<3 \mu\text{g}/\text{mL}$ (Figure 4; available at www.jpeds.com) CIR suppression was not seen when subjects were grouped by the mean of their 3 most recent tacrolimus troughs before study visit (<3 or off calcineurin inhibitors, 3-6, $>6 \mu\text{g}/\text{mL}$), even though tacrolimus trough at study visit and mean of 3 most recent were highly correlated ($r = 0.56$, $P < .001$). (data not shown).

Of all subjects on tacrolimus, 50 were ≥ 6 years from liver transplant, and 14 were <6 years from transplant. When stratified by years since transplant, those ≥ 6 years from transplant with tacrolimus troughs $>6 \mu\text{g}/\text{mL}$ had the lowest CIRs (Figure 3, B). CIR suppression was not seen in children with

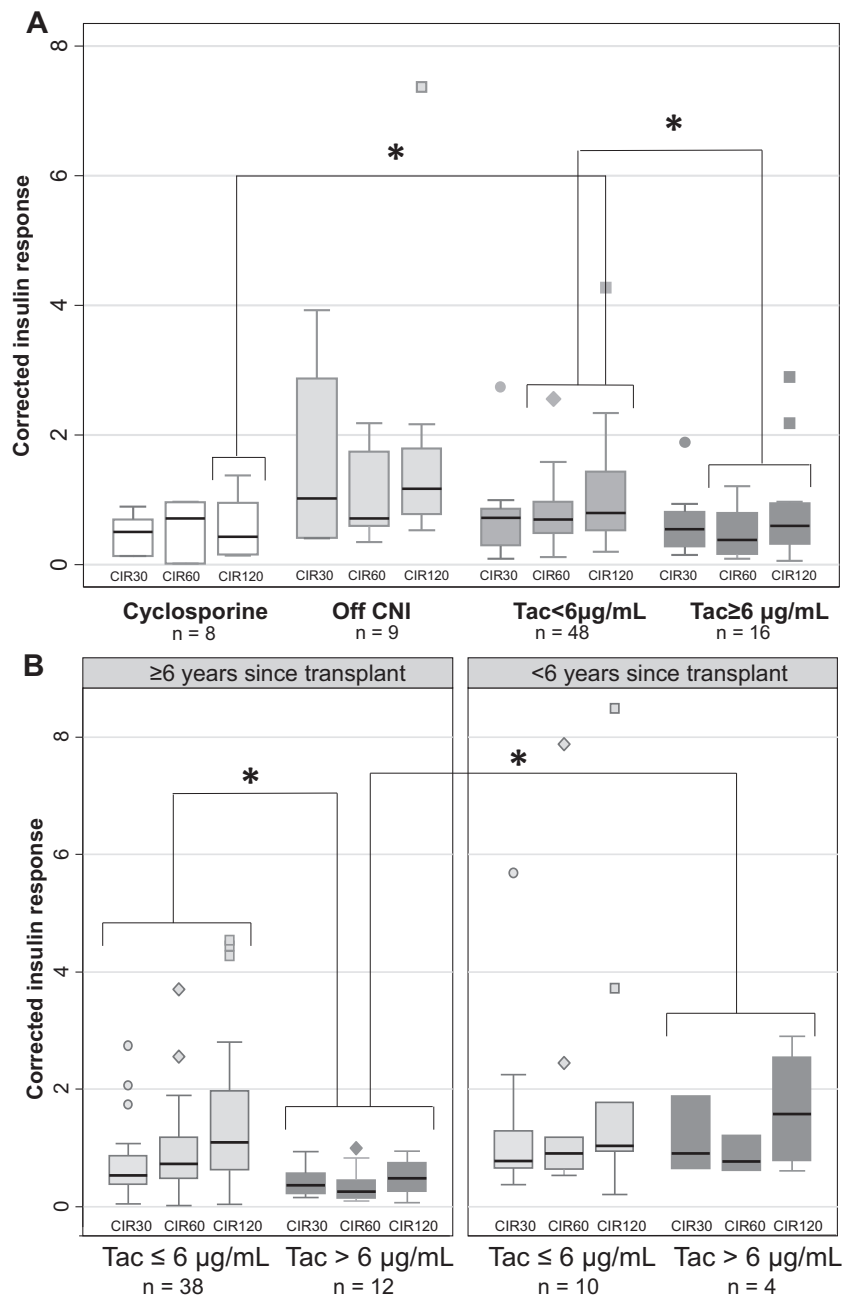


Figure 3. CIR during OGTT **A**, by calcineurin inhibitor at time of study visit. Greater tacrolimus trough on the day of study visit is associated with lower CIR₆₀ and CIR₁₂₀. **B**, by tacrolimus trough at study visit and number of years since first liver transplant. CIR suppression at 60 and 120 minutes was most marked in subjects with greater tacrolimus troughs and farther out from transplant. **P* < .05 for CIR difference at timepoint between indicated groups. *CNI*, calcineurin inhibitor; *Tac*, tacrolimus.

tacrolimus troughs >6 μg/mL that were less than 6 years since transplant, although only 4 subjects were in this category (Figure 3, B).

There were no significant differences by tacrolimus trough ≤6 vs >6 μg/mL in fasting estimates of β-cell function (HOMA of β-cell function), or in measures of insulin sensitivity (HOMA-IR, WB-ISI) (data not shown).

Overweight/Obese Subjects Increase Insulin Secretion to Maintain Glucose Tolerance

We next examined the impact of overweight/obesity on CIR. IGT was seen in 30% of the normal weight and 30% of the overweight/obese subjects (*P* = .99). The overweight/obese subjects that remained NGT had greater CIR₆₀ and CIR₁₂₀ than the normal-weight subjects with NGT, and they were able to

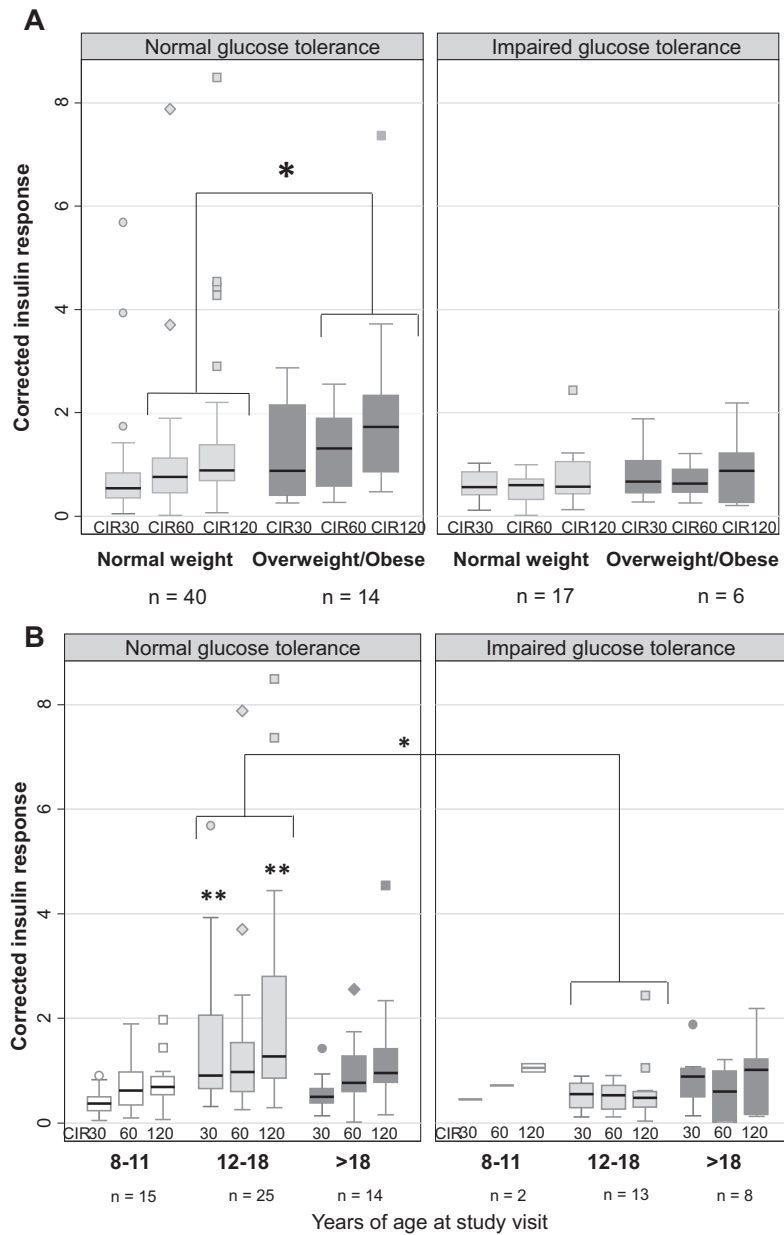


Figure 5. CIR during OGTT, by glucose tolerance and **A**, by weight status. Overweight/obese children who were NGT had greater CIR₆₀ and CIR₁₂₀ than normal-weight children with NGT. Within the overweight/obese group, CIR was not significantly different between the NGT and IGT at any time point; **B**, by age group. Adolescents 12-18 years of age at study visit had greater CIR₃₀ than both children 8-11 and adults >18, and greater CIR₁₂₀ than the children. Adolescents who remained NGT had greater CIR and increased their CIR during the OGTT. There was no difference at any time point between the NGT and IGT groups in the other age categories. **P* < .05 for CIR difference at time point between indicated groups. ***P* < .05 for difference between NGT adolescents and NGT in other age groups.

increase their CIR during the OGTT (*P* = .09 test for trend) (Figure 5, A). The overweight/obese subjects with IGT had a blunted CIR response (*P* = .86 test for trend). Among the overweight/obese subjects, CIRs were not significantly greater in the NGT than the IGT group, although sample size was small (Figure 5, A).

As expected, the overweight/obese patients (*n* = 21) were more insulin resistant than normal-weight subjects (*n* = 60), as reflected by greater HOMA-IR (median 3.33, IQR 2.24-5.23, vs normal weight median 2.23, IQR 1.50-3.07, *P* = .003) and lower WB-ISI (median 2.65, IQR 2.40-2.92, vs normal weight median 4.38, IQR 2.89-5.83, *P* = .004). In summary, overweight/obese

subjects who could increase their CIR during the OGTT, demonstrating hypersecretion of insulin, were able to overcome their insulin resistance and maintain NGT (Figure 5, A).

Adolescents and Pubertal Subjects Increase Insulin Secretion to Maintain Glucose Tolerance

Age at visit and Tanner stage were examined as confounders of CIR, because adolescence and Tanner stage 2-4 are associated with insulin resistance. Adolescents who remained NGT had greater CIR than adolescents with IGT at every time point. Subjects with IGT in all age groups failed to increase CIR during the OGTT ($P > .10$ test-for-trend for all 3 age groups) (Figure 5, B). The same pattern was seen when subjects were stratified by Tanner stage, with subjects of Tanner stage 2-4 with NGT generating greater CIR₆₀ and CIR₁₂₀ than subjects with Tanner stage 2-4 with IGT (Figure 6; available at www.jpeds.com).

Within the NGT group, adolescents had significantly greater CIR₃₀ than children 8-11 years and adults older than 18 years and greater CIR₁₂₀ than the younger group (Figure 5, B). The greater CIRs that adolescents needed to maintain NGT represents insulin resistance. Adolescents had greater HOMA-IR (median 2.57, IQR 2.19-3.86, $P = .01$) than children (HOMA-IR median 1.67, IQR 1.15-2.50 or adults (HOMA-IR median 2.26, IQR 1.63-3.67).

Calcineurin Inhibitor Exposure Associated with Insulin Secretion, after Controlling for Other Variables

In multivariable analysis controlling for years since transplant, overweight/obesity, and adolescent age, CIR₁₂₀ was significantly lower in those with tacrolimus trough $>6 \mu\text{g/mL}$ at study visit (-0.71 , $P = .01$) and those on cyclosporine (-0.67 , $P = .009$) than in those with tacrolimus trough $\leq 6 \mu\text{g/mL}$ (Table IV; available at www.jpeds.com). Adolescence also was associated with significantly greater CIR ($+0.55$, $P = .02$). The model confirmed that CIR increased during the OGTT (Table IV). Significant predictors did not change if time since transplant was modeled as a continuous variable (years). Sensitivity analysis excluding overweight/obesity and years since transplant, and excluding those on cyclosporine, did not change significant predictors (data not shown). Neither tacrolimus trough as a continuous variable nor tacrolimus trough $\leq 3 \mu\text{g/mL}$ vs $3-6 \mu\text{g/mL}$ was associated significantly with CIR in this multivariable model. Mean of 3 most recent tacrolimus troughs also was not a significant predictor of CIR, as a binary or continuous variable (data not shown).

Rejection Before Study Visit as a Proxy for Immunosuppression Exposure

We also examined retransplant, biopsy-proven acute rejection episodes, and chronic rejection as proxies for immunosuppression exposure. Six subjects had undergone liver retransplant, all within 6 months of first transplant; only 1 had IGT. Of those with IGT, 48% had ≥ 2 acute rejection episodes since transplant, compared with 26% of those with NGT ($P = .06$). Glucose tolerance status was not associated with timing of acute rejection (<6 months vs ≥ 6 months post-transplant)

or having the most recent biopsy-proven acute rejection episode within the last 5 years (data not shown). Only 6% of the cohort had chronic rejection; it was not associated with IGT.

IGT and Hyperglycemia during Acute Rejection after the Study Visit

Nine subjects had an acute rejection episode during follow-up after their study visit. Of the 3 with IGT at study visit, 2 (67%) developed hyperglycemia during treatment for the rejection, 1 during methylprednisolone pulse and 1 with increased tacrolimus. Of the 6 without IGT at study visit, only 1 (17%) had hyperglycemia during the rejection episode; this subject was overweight/obese with insulin resistance (HOMA-IR 5.60) ($P = .22$, Fisher exact test).

Discussion

Prediabetes was common in this cohort of pediatric recipients of liver transplant, seen in 44% of those studied, despite steroid-free immunosuppression and long-term stability with good graft function in the majority. IGT was the most common abnormality of glucose tolerance, seen in 27% of participants. In contrast, the prevalence of prediabetes in US adolescents is 16%, with only 3.4% IGT.²⁶ Although IGT and diabetes have been a focus of long-term care in adults after liver transplant, sensitive screening and proactive prevention strategies have not been emphasized for the pediatric population. Our analysis of OGTT, done systematically for research in this population, suggests that the potential contribution of prediabetes and diabetes to long-term morbidity has been underestimated for children.

IGT was not detectable via fasting laboratory measures or HbA1c, which are the currently recommended methods of screening for prediabetes in pediatric recipients of liver transplant.²⁷ It was not easily predictable from demographics, age, or time since transplant. Neither HOMA-IR, which is used widely but does not evaluate β -cell secretion, nor HOMA percent β -cell function, which uses fasting measures to estimate β -cell secretion in the "resting" state, were accurate measures of IGT in our cohort.

IGT in our cohort was characterized by insulin hyposecretion, particularly late in the glucose tolerance test. Tacrolimus trough $>6 \mu\text{g/mL}$ appeared to suppress insulin secretion, as evidenced by lower CIR in those with greater tacrolimus troughs. Given that subjects with low troughs at study visit had been exposed to greater levels in the past, closer to their transplant, our data also suggest that the insulin hyposecretion may be reversible. In *in vitro* studies, calcineurin inhibitors decrease insulin gene transcription in a time and dose-dependent but reversible manner—leading to decreased insulin synthesis and secretion.^{12,28} In rats, tacrolimus treatment decreased pancreatic islet size and induced islet apoptosis, but hyperglycemia was reversed and insulin secretion normalized when tacrolimus was stopped.^{29,30} Of note, these rats did have dose-dependent worsening of hyperglycemia; insulin levels increased at the greatest tacrolimus doses. In humans, tacrolimus-induced reduction in insulin secretion, with

dose-dependence and reversibility, has been demonstrated in adult recipients of transplant but never in a cohort with such extended post-transplant follow-up.^{13,31}

The association between greater tacrolimus trough and lower insulin secretion was seen particularly in those with ≥ 6 years of calcineurin inhibitor exposure. The children on cyclosporine also had relatively low CIR₁₂₀; these children had more years of calcineurin inhibitor exposure. These associations may represent chronic pancreatic β -cell damage caused by chronic exposure, or possibly an increased vulnerability to calcineurin-inhibitor effects at critical periods in development. The fact that overall prevalence of prediabetes is so unexpectedly high in our population also suggests an impact of chronic exposure. Interestingly, studies in neonatal mice and in islets from human children demonstrate that calcineurin inhibition impairs β -cell maturation and proliferation.^{32,33} Clarifying whether exposure to calcineurin inhibitors in infancy or early childhood reduces later β -cell mass is an important topic for prospective studies.

Also notable were the greater CIRs seen in the few subjects not on calcineurin inhibitors, despite the fact that all were ≥ 6 years from transplant. This further supports the idea that calcineurin inhibitor-induced suppression of insulin secretion may be reversed to some extent, even long-term after transplant. It also may indicate that these children tolerated lower doses of calcineurin inhibitor when they were on it, which is why they were good candidates for immunosuppression withdrawal. In addition, we were not able to find a consistent, significant association between CIR and chronic calcineurin inhibitor exposure, as measured by years since transplant or mean of recent tacrolimus exposure, in multivariable models. Further study is needed to determine whether this lack of association is an artifact of small sample size or incomplete data on previous tacrolimus exposure, or truly represents a reversible phenomenon.

Subjects in our cohort who were at risk for IGT because of insulin resistance, including the overweight/obese and adolescents, maintained NGT if they could increase CIR during their OGTT. Those who were IGT could not increase their CIR during the OGTT; without sufficient insulin to overcome their insulin resistance, they were hyperglycemic. Of note, the majority of our cohort had a family history of diabetes and many were Hispanic, which is associated with insulin resistance in the general population.

OGTT has been recommended for post-transplant diabetes and prediabetes screening in recent expert consensus guidelines in adults⁵ and has been suggested recently for children as well.^{3,34} Our analysis supports OGTT as a valuable, and feasible, tool for identifying children at risk of developing clinical diabetes with stressors like corticosteroids, increased calcineurin inhibitor dosing, or other events like significant weight gain or puberty.

Conversely, fasting laboratory values and HbA1c were inadequate screens for IGT in our cohort. A relatively low sensitivity of HbA1c for prediabetes, and low concordance with OGTT, has been demonstrated in nontransplanted cohorts of adolescents, particularly the overweight/obese. The cause of this

is not understood entirely.^{35,36} HbA1c also can be falsely low in patients with chronic liver disease, anemia, increased red blood cell turnover, or a history of aspirin use.^{25,37} Most circulating tacrolimus is bound to erythrocyte membranes; however, its impact on membrane permeability and whether it might impact diffusion of glucose across the membrane to bind hemoglobin does not appear to be known. Finally, a recent study of adolescents with continuous glucose monitoring suggested the HbA1c was a reasonable measure of average blood sugar but that OGTT was better correlated with glucose variability and hyperglycemic extremes.³⁸ Normal HbA1c with abnormal OGTT or fasting blood sugar in our cohort may thus reflect their β -cell dysfunction. Both chronic blood sugar elevations and variability have been linked to morbidity in diabetics.

In children with rejection episodes after their study visit, hyperglycemia was seen in 66% of those with IGT and only 17% of those with NGT. This finding suggests that pediatric recipients of liver transplant with IGT are vulnerable to clinically significant hyperglycemia when stressed by corticosteroids or increased tacrolimus. Early markers of later risk are important to identify, so that we can use preventive strategies.

Recent immunosuppression withdrawal trials show that calcineurin inhibitors can be minimized, and often stopped, in these children and young adults.¹⁴⁻¹⁶ Long-term, prospective studies are needed to differentiate temporary insulin suppression from permanent β -cell damage. Identifying times—either post-transplant or age-related—and exposure levels associated with irreversible damage and clinical hyperglycemia might allow us to target reductions in calcineurin inhibitor dosing to critical periods.

Our sample size limited full elucidation of IGT risk factors. We relied on time since transplant and rejection episodes as proxies for cumulative calcineurin-inhibitor exposure. The lack of association that we found between these variables and CIR or IGT may be a consequence of sample size. We did not measure C-peptide, which estimates production of new insulin. Also, we evaluated patients at only one time point in this initial study. Larger, prospective studies, with tracked changes in immunosuppression and repeated evaluation by OGTT, will be needed to definitively determine the impact of calcineurin inhibitor dosing and minimization. Larger studies, including patients already diagnosed with diabetes and those with both IFG and IGT, might also help elucidate the role of clinical characteristics known to increase risk in non-transplant populations—including race/ethnicity, age, and family history.

In summary, IGT is common after pediatric liver transplant but requires OGTT for detection. It is characterized by impaired insulin secretion. The impaired insulin secretion seen in our cohort was associated with current but not past tacrolimus levels, suggesting that the impairment might be reversible with minimized calcineurin inhibitor exposure. Longitudinal studies are needed to understand the long-term clinical significance of these findings. Prospectively followed cohorts will be crucial to investigate the development of glucose intolerance, rate of progression, and whether these processes might be prevented with early intervention.⁵ ■

Subjects reported as off of immunosuppression were weaned during the Immunosuppression Withdrawal trials (ITN029ST, 1U01AI100807); no data from that trial were used in the present analysis, but we much appreciate the investigators' cooperation with our efforts.

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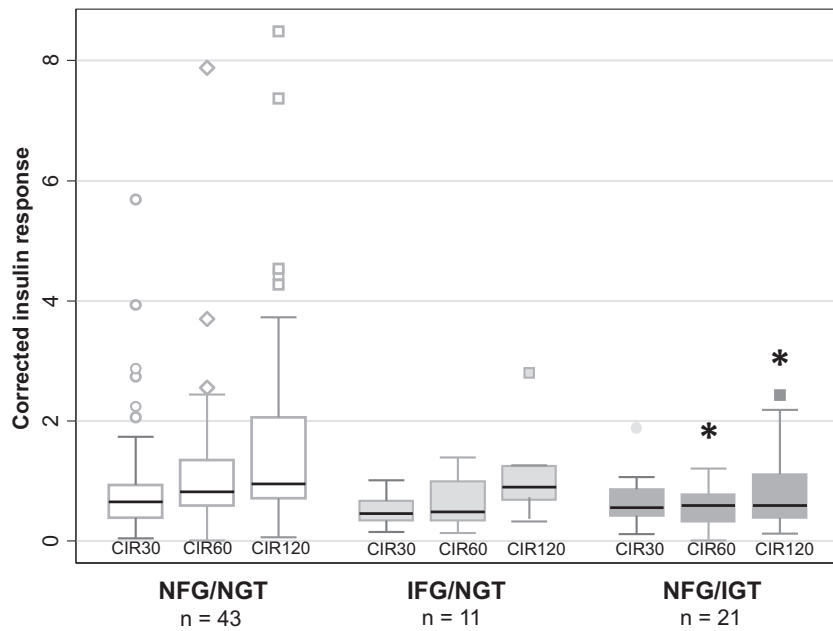


Figure 1. CIR at 30, 60, and 120 minutes following an oral glucose load in recipients of pediatric liver transplant, by glucose tolerance status. Total n = 75; 2 subjects with IFG and IGT excluded from this analysis.

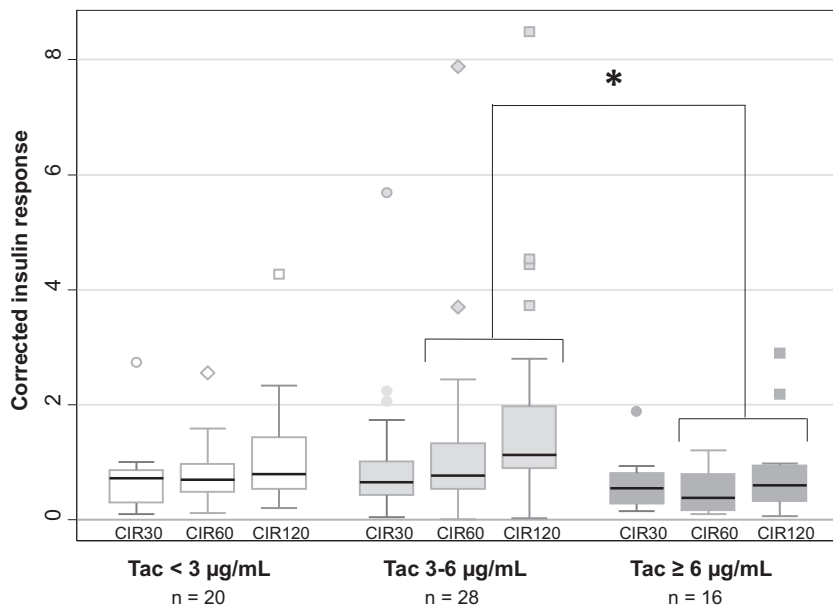


Figure 4. CIR at 30, 60, and 120 minutes after an oral glucose load (OGTT) in recipients of pediatric liver transplant, by categories of tacrolimus trough on the day of study visit. Only participants on tacrolimus at study visit included in this graph (n = 64). CIR₆₀ and CIR₁₂₀ were significantly higher in those with tacrolimus trough 3-6 µg/mL than those with tacrolimus ≥6 µg/mL.

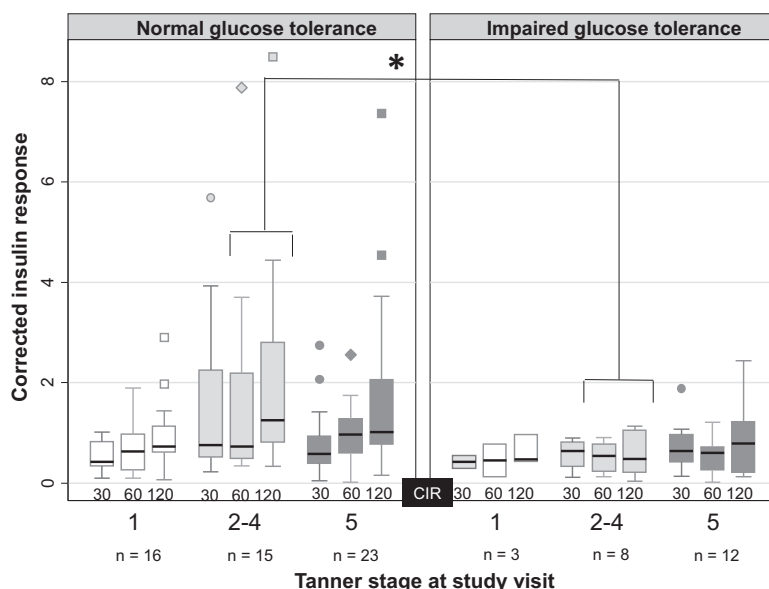


Figure 6. CIR at 30, 60, and 120 minutes after an oral glucose load (OGTT) in recipients of pediatric liver transplant, by Tanner stage at study visit. In pubertal subjects—Tanner stage 2-4 at study visit, CIR₆₀ and CIR₁₂₀ were significantly lower in those with IGT than NGT.

Table I. Glucose regulation measures—calculation and interpretation*

Measures	Calculation	Abnormal value	Authors	Year
Fasting glucose	–	≥100 mg/dL	American Diabetes Association ⁴	2012
2-h glucose	–	≥140 mg/dL		
Stimulated measures				
CIR	$(Ins_x * 100) / (Gluc_x (Gluc_x - 70))$	Not determined	Sluiter et al ²¹	1976
WB-ISI	$\frac{10,000}{\sqrt{(Gluc_0 \times Ins_0)(Gluc_{mean} \times Ins_{mean})}}$	Not determined	Matsuda and DeFronzo ²²	1999
Fasting measures				
HOMA-IR	$(Gluc_0 * Ins_0) / 405$	≥3.16	Keskin et al ²³	2005
HOMA percent β-cell function	$(360 Ins_0) / (Gluc_0 - 63)$	~100% (no defined “normal range”)	Wallace et al ²⁴	2004

*Glucose measured in mg/dL, insulin measured in mU/L.

Table III. Measures of glucose tolerance in pediatric recipients of liver transplant, by glucose tolerance group

Measurements	All (n = 81)*	NGT, NFG (NFG/NGT; n = 43)	IGT, NFG (IGT; n = 21)	P (IGT vs NFG/NGT)	Impaired fasting glucose, NGT (IFG, NGT; n = 11)	P (IFG vs NFG/NGT)
Fasting glucose, mg/dL	92 (87-96)	90 (86-93)	91 (87-93)	1.00	102 (100-105)	<.001
Fasting insulin, mU/L	10.6 (7.6-15.0)	10.2 (7.6-15.2)	10.6 (7.0-11.7)	.97	13.4 (9.1-17.8)	.25
HOMA-IR	2.4 (1.7-3.4)	2.3 (1.7-3.4)	2.3 (1.6-2.6)	.97	3.6 (2.2-4.5)	.05
HOMA percent β -cell function	130.8 (105.0-195.4)	146.8 (106.4-214.5)	136.3 (111.4-169.0)	.86	108.0 (88.5-167.4)	.23
HbA1c, %	4.9 (4.6-5.2)	4.9 (4.4-5.2)	4.9 (4.8-5.0)	.93	4.8 (4.7-5.0)	.40
Peak glucose, mg/dL	159 (144-180)	151 (127-162)	180 (173-186)	<.001	172 (152-200)	.008
Peak insulin, mU/L	102.6 (61.6-163.3)	89.1 (55.2-158.1)	149.1 (100.0-188.1)	.02	110.5 (67.5-170.6)	.58
Time of peak glucose, min [†]	n = 72	n = 38	n = 20	<.001	n = 11	.46
30	46%	66%	0%		55%	
60	40%	29%	60%		45%	
120	14%	5%	40%		0	
Time of peak insulin, min [†]				<.001		.94
30	44%	53%	16%		55%	
60	29%	34%	16%		36%	
120	26%	13%	68%		9%	
WB-ISI [‡]	3.71 (2.64-5.14)	4.38 (2.77-6.37)	3.11 (2.54-4.69)	.21	3.31 (2.57-4.32)	.25

IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NFG, normal fasting glucose; NGT, normal glucose tolerance.

*Data represent proportion or median, IQR. McNemar χ^2 test for categorical variables, Kruskal-Wallis for continuous variables. Subgroup analysis (n = 75) excludes 2 with IFG + IGT, and 4 with incomplete OGTT data.

[†]Participants with incomplete OGTT such that peak could not be determined were excluded. N in each column indicates number of included subjects for that calculation.

[‡]WB-ISI calculated on n = 68 with no missing OGTT values, including 38 NFG/NGT, 9 IFG, 19 IGT, and 2 IFG/IGT.

Table IV. Predictors of CIR, multivariable analysis

Predictors	Difference in CIR from reference group (coefficient)	95% CI for CIR difference	P
Calcineurin inhibitor at study visit			
Tacrolimus, trough $\leq 6 \mu\text{g/mL}$	REF	REF	REF
Tacrolimus, trough $> 6 \mu\text{g/mL}$	-0.71	-1.26, -0.15	.01
Cyclosporine, any trough	-0.67	-1.18, -0.17	.009
No calcineurin inhibitor	0.59	-0.78, 1.95	.40
Adolescent (12-18 y at study visit)	0.55	0.11, 0.99	.02
Timepoint during OGTT			
CIR ₁₂₀	REF	REF	REF
CIR ₆₀	-0.47	-0.79, -0.15	.004
CIR ₃₀	-0.69	-1.04, -0.33	<.001
<6 y since transplant	0.72	-0.19, 1.64	.12
Overweight/obese	0.23	-0.27, 0.73	.37