Measures of β-Cell Function during the Oral Glucose Tolerance Test, Liquid Mixed-Meal Test, and Hyperglycemic Clamp Test

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Objective To evaluate clinically useful measures of β-cell function derived from the oral glucose tolerance test (OGTT) or mixed-meal (ie, Boost) tolerance test to assess insulin secretion in comparison with the gold standard, the hyperglycemic clamp (Hyper-C) test.

Study design We hypothesized that OGTT/Boost-derived measures are useful estimates of β-cell function and correlate well with insulin secretion measured by the Hyper-C test. This study was designed to assess the correlation between the ratio of the early incremental insulin/glucose responses at 15 and 30 minutes ($\Delta I_{15}/\Delta G_{15}$ and $\Delta I_{30}/\Delta G_{30}$) of the OGTT and the Boost test with insulin secretion measured during the Hyper-C test (225 mg/dL). The same indices were evaluated using C-peptide. A total of 26 children (14 males, 12 females; mean age, 9.9 ± 0.2 years; mean body mass index = 22.1 ± 1.2 kg/m^2) underwent a 2-hour Hyper-C test (225 mg/dL) and 3-hour OGTT and Boost tests with measurements of glucose, insulin, and C-peptide.

Results Correlations between Hyper-C– and OGTT-derived measures of insulin secretion were stronger for the 15-minute index than for the 30-minute index of insulin secretion and stronger for C-peptide levels than for insulin levels ($r = .7$, $P < .001$ for first-phase C-peptide vs both OGTT and Boost, $\Delta C_{15}/\Delta G_{15}$).

Conclusions In children with normal glucose tolerance, C-peptide rather than insulin level measured after 15 minutes of the OGTT or Boost test provides a reliable estimate of β-cell function that correlates well with Hyper-C–derived insulin secretion. *(J Pediatr 2008;152:618-21)*

Obesity, impaired glucose tolerance (IGT), and type 2 diabetes are characterized by insulin resistance along with impaired insulin secretion in the latter 2 entities.[1] The increasing rates of obesity,[2,3] IGT, and type 2 diabetes in children[4] call for simple, clinically obtainable measures of insulin secretion to use in the follow-up of progression from one stage to another and in the evaluation of therapeutic interventions. The oral glucose tolerance test (OGTT), recommended by the World Health Organization and the American Diabetes Association for the diagnosis of IGT and diabetes, is a frequently used clinical tool.

The present study was undertaken to evaluate whether measures of insulin release derived from the OGTT or from a mixed-meal (ie, Boost) tolerance test correlate with insulin secretion derived from the gold standard hyperglycemic clamp (Hyper-C) test in children. We hypothesized that OGTT- or Boost test–derived measures of insulin release are useful estimates of β-cell function and correlate well with insulin secretion measured by the Hyper-C test.

METHODS

The study population comprised 26 children (14 males, 12 females) ranging in age from 7 to 12 years (mean age, 9.9 ± 1.1 years). They included 15 African-American and 11 Caucasian normoglycemic children (HbA_1C, 5.2% ± 0.4%), 13 normal weight (NW) children and 13 overweight (OW) or at risk for OW children (body mass index [BMI] > 85th percentile) with a mean BMI of 22.2 ± 6.1 kg/m^2, mean body fat percentage of 27.5% ± 13.6%, and mean fat mass of 13.2 ± 10.6 kg. Some of these children were

AUC	Area under the curve	OGTT	Oral glucose tolerance test
BMI	Body mass index	OW	Overweight
IGT	Impaired glucose tolerance	RIA	Radioimmunoassay
NW	Normal weight		

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Table. Correlation of OGTT- and mixed-meal test–derived parameters of insulin secretion to first-phase insulin (1st PI) and C-peptide (1st PC) during the Hyper-C test

	$\Delta I_{15}/\Delta G_{15}$ (μU/mL per mg/dL)	$\Delta C_{15}/\Delta G_{15}$ (ng/mL per mg/dL)	$\Delta I_{30}/\Delta G_{30}$ (μU/mL per mg/dL)	$\Delta C_{30}/\Delta G_{30}$ (ng/mL per mg/dL)
OGTT				
Hyper-C				
1st PI, μU/mL	0.49 (.012)	0.57 (.003)	0.56 (.004)	0.40 (.05)
1st PC, ng/mL	0.43 (.03)	0.73 (<.001)	0.37 (.07)	0.61 (<.001)
Mixed-meal				
Hyper-C				
1st PI, μU/mL	0.67 (.001)	0.39 (.059)	0.24 (NS)	0.21 (NS)
1st PC, ng/mL	0.57 (.004)	0.73 (<.001)	0.23 (NS)	0.45 (.02)

r and *P* values are given in parentheses.
NS, Not significant.

reported previously.[5] The study design was approved by the Institutional Review Board of the University of Pittsburgh. The children were recruited through newspaper advertisements and flyers posted in the health center. All research participants and their parents or guardians gave informed consent/assent after receiving a detailed explanation of the research study. All subjects were documented to be in good health based on a thorough medical interview and physical examination. The OW and at risk for OW children were free of any associated comorbidities or syndromes linked to obesity. No None of the children was taking any medications. All of the children were determined to be in Tanner stage 1 puberty based on careful physical examination.

After an overnight fast of 10 to 12 hours, the children were studied in the Pediatric Clinical and Translational Research Center of Children's Hospital of Pittsburgh on 2 separate occasions, 1 to 2 weeks apart. Randomly, the children underwent a 3-hour OGTT (1.75 g/kg, maximum 75 g) or a Boost test (55% carbohydrate, 25% protein, and 20% fat). Blood samples were drawn at 0, 15, 30, 60, 90, 120, and 180 minutes for determination of glucose, insulin, and C-peptide levels. The children also underwent a 2-hour Hyper-C test (225 mg/dL) to measure in vivo insulin secretion.

Body composition was assessed by dual-energy x-ray absorptiometry using a Lunar absorptiometer (GE Lunar Corp, Madison, WI). Plasma glucose level was measured by the glucose oxidase method using a YSI glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin level was measured by radioimmunoassay (RIA) (Linco Research Inc, St. Charles, MO), which is 100% specific for human insulin with < 0.2% cross-reactivity with human proinsulin and no cross-reactivity with C-peptide or insulin-like growth factor (intra-assay and interassay coefficients of variation of 2.9% to 9.4% and 5.5% to 8.5%, respectively). C-peptide levels were measured by double-antibody RIA (Siemens Medical Solutions Diagnostics, Tarrytown, NY), which is 100% specific for C-peptide (intra-assay and interassay coefficients of variation of 1.7% to 8.5% and 2.2% to 8.5%, respectively).

First-phase insulin and C-peptide levels were calculated as the mean of 5 determinations from 2.5 to 12.5 minutes of the Hyper-C test. Second-phase insulin and C-peptide levels were calculated as the mean of 8 determinations from 15 to 120 minutes of the Hyper-C test, as reported previously.[6,7]

The distribution of the different variables was examined and the appropriate statistical test applied. Student's *t*-test or the Mann-Whitney test was used for 2-group comparisons. Pearson's or Spearman's correlation was used to examine bivariate relationships. The area under the curve (AUC) was calculated using the trapezoidal rule. A P value $\leq .05$ was considered statistically significant. All results are reported as mean ± standard deviation.

RESULTS

All of the children exhibited normal fasting glucose levels (82.2 ± 5.4 mg/dL). Fasting insulin level ranged from 4.7 to 39.0 μU/mL (mean, 15.0 ± 7.1 μU/mL) and fasting C-peptide level ranged from 0.5 to 2.4 ng/mL (mean, 1.4 ± 0.5 ng/mL). Glucose, insulin, and C-peptide levels peaked at 30 minutes of the OGTT and Boost tests. During the Hyper-C test, first-phase insulin levels were 114.1 ± 106.6 μU/mL and first-phase C-peptide levels were 5.0 ± 2.5 ng/mL.

The ratio of the incremental response of insulin and C-peptide to glucose at 15 and 30 minutes of the OGTT ($\Delta I_{15}/\Delta G_{15}$, $\Delta I_{30}/\Delta G_{30}$, $\Delta C_{15}/\Delta G_{15}$, $\Delta C_{30}/\Delta G_{30}$) correlated with first-phase insulin and first-phase C-peptide levels from the Hyper-C test (Table and Figure). However, the best correlate of first phase β-cell function measured during the Hyper-C test was the early 15-minutes, not the 30-minute values from the OGTT and Boost tests, $\Delta C_{15}/\Delta G_{15}$ ($r = .73$; $P < .001$) from the OGTT and $\Delta C_{15}/\Delta G_{15}$ ($r = .73$; $P < .001$) from the Boost test (Table and Figure). Similarly, the best correlate of second-phase β-cell function from the Hyper-C test was $\Delta C_{15}/\Delta G_{15}$ ($r = .71$; $P < .001$) from the OGTT and $\Delta C_{15}/\Delta G_{15}$ ($r = .76$; $P < .001$) from the Boost test.

The AUC of insulin during the OGTT was correlated more closely with second-phase ($r = .59$; $P = .002$) than with first-phase ($r = .45$; $P = .023$) insulin secretion from the Hyper-C test. Similarly, the AUC of C-peptide was correlated more closely with second-phase ($r = .61$; $P = .001$) than