Indices of Insulin Secretion during a Liquid Mixed-Meal Test in Obese Youth with Diabetes

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Objective To compare indices of insulin secretion, insulin sensitivity (IS), and oral disposition index (oDI) during the liquid mixed-meal test in obese youth with clinically diagnosed type 2 diabetes mellitus (T2DM) and negative autoantibodies (Ab^-) versus those with T2DM and positive autoantibodies (Ab^+) to examine whether differences in β-cell function can be detected between the 2 groups.

Study design Twenty-seven youth with Ab^- and 15 youth with Ab^+ clinically diagnosed T2DM underwent a mixed-meal test (Boost; 55% carbohydrate, 25% protein, and 20% fat). Fasting and mixed-meal–derived insulin and C-peptide indices of IS, secretion (30-minute insulinogenic [$\Delta I_{30}/\Delta G_{30}$] and C-peptide [$\Delta C_{30}/\Delta G_{30}$]), and oDI were calculated.

Results Indices of insulin secretion were ~40%-50% lower in patients with Ab^+ T2DM compared with those with Ab^- T2DM. After controlling for body mass index, $\Delta I_{30}/\Delta G_{30}$, $\Delta C_{30}/\Delta G_{30}$, C-peptide area under the curve (AUC)/glucose AUC, and insulin AUC/glucose AUC were significantly ($P < .05$) lower in the Ab^+ group compared with the Ab^- group. Sensitivity indices were significantly higher in the Ab^+ group. The oDI, 1/fasting insulin $\times$ $\Delta I_{30}/\Delta G_{30}$ (0.04 ± 0.02 vs 0.12 ± 0.02 mg/dL^{-1}; $P = .005$), and 1/fasting C-peptide $\times$ $\Delta C_{30}/\Delta G_{30}$ (0.02 ± 0.009 vs 0.05 ± 0.006 mg/dL^{-1}; $P = .018$) were lower in the Ab^+ group. Receiver operating characteristic curve analyses revealed that fasting C-peptide <3.2 ng/mL had 87% sensitivity and 74% specificity and $\Delta C_{30}/\Delta G_{30}$ <0.075 ng/mL per mg/dL had 93% sensitivity and 80% specificity for identifying youth with Ab^+ T2DM.

Conclusion During a liquid mixed-meal test, indices of β-cell function were lower and IS was higher in patients with Ab^+ T2DM versus those with Ab^- T2DM, with high sensitivity and specificity for fasting and stimulated C-peptide as markers of Ab^+ status. Indices of insulin secretion during this standardized mixed-meal test could be used to assess β-cell function in therapeutic trials of β-cell restoration in youth with T2DM. *(J Pediatr 2013;162:924-9)*.

The increasing prevalence of obesity in children has resulted in a surge in the incidence of youth with type 2 diabetes mellitus (T2DM).[1] In addition, the childhood obesity epidemic has translated to increasing obesity in youth with type 1 diabetes mellitus (T1DM),[2-4] thus complicating the clinical distinction between obese youth with autoimmune T1DM and T2DM. According to the SEARCH for Diabetes in Youth study, ~12% of youth with T1DM are obese and 22% are overweight.[3] On the other hand, up to 10% of youth clinically diagnosed with T2DM have circulating islet cell autoantibodies, suggesting that these are obese youth with autoimmune T1DM.[5,6] In adults with T2DM, the presence of islet cell autoimmunity predicts a more aggressive disease course[7,8] and the need for insulin therapy.[8,9] In youth with T2DM, the progression to insulin use is significantly greater in those with islet cell autoantibodies compared with those without (60% vs 33%).[10] Neither antibody (Ab) status nor human leukocyte antigen marker status is able to predict the clinical course, however.[8] Initiation of insulin therapy is largely a clinician-driven decision and might not always reflect β-cell function.[10] Thus, a readily applicable standardized test to measure and follow β-cell function in youth with diabetes is desirable.

We previously demonstrated that oral glucose tolerance test (OGTT)-derived indices of insulin sensitivity (IS) and insulin secretion are insufficiently sensitive to distinguish metabolic/pathopysiologic differences between youth with a clinical diagnosis of T2DM with evidence of pancreatic autoimmunity (Ab^+) versus those with negative autoantibodies (Ab^-),[11] in contrast to clamp studies.[12] Liquid

$\Delta C_{30}/\Delta G_{30}$	C-peptide–derived 30-minute index	HOMA-IR	Homeostasis model assessment of insulin resistance
$\Delta I_{30}/\Delta G_{30}$	Insulinogenic 30-minute index	I_F	Fasting insulin
Ab	Antibody	IS	Insulin sensitivity
AUC	Area under the curve	oDI	Oral disposition index
BMI	Body mass index	OGTT	Oral glucose tolerance test
C_F	Fasting C-peptide	ROC	Receiver operating characteristic
G_F	Fasting glucose		
HbA1c	Hemoglobin A1c	T1DM	Type 1 diabetes mellitus
HOMA-%β	Homeostasis model assessment of insulin secretion	T2DM	Type 2 diabetes mellitus

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mixed-meal tolerance tests are frequently used to evaluate β-cell function in adults and in pediatric patients.[13-16] In patients with T1DM, the mixed-meal test is highly reproducible and well tolerated, and elicits a significant C-peptide response.[16] Furthermore, the mixed-meal stimulus results in greater β-cell response and higher estimated insulin secretion compared with the OGTT in adults with both normal and impaired glucose regulation.[17-19] In normoglycemic prepubertal normal-weight and overweight children, indices of insulin secretion from the liquid mixed-meal correlated with clamp test–derived insulin secretion measures.[20] Consequently, in the present study we investigated indices of insulin secretion and sensitivity during the mixed-meal test in youth with clinically diagnosed Ab^- and Ab^+ T2DM. We hypothesized that differences in insulin secretion between the Ab^+ and Ab^- groups would be detected, and that liquid mixed-meal–derived cutoff values for insulin secretion indices can help differentiate Ab^+ and Ab^- T2DM with adequate sensitivity and specificity.

Methods

A total of 42 obese adolescents with a clinical diagnosis of T2DM based on the American Diabetes Association diagnostic criteria[21] were recruited from the Diabetes Center at the Children's Hospital of Pittsburgh. Islet cell Ab screening with the National Institute of Diabetes and Digestive and Kidney Diseases harmonization assay identified 27 patients with negative antibodies (Ab^- group) and 15 with 1 or 2 positive antibodies (Ab^+ group). Some of these youth had been reported previously, including 31 who participated in an OGTT study[11] and 32 who participated in clamp studies.[12,22] All study participants were pubertal according to Tanner stage criteria. The treatment modalities at the time of the study and the characteristics of the study population are summarized in Table I. All studies were approved by the University of Pittsburgh's Institutional Review Board, and appropriate consents and assents were obtained before the start of the investigation. Glutamic acid decarboxylase 65-kDa autoantibody and insulinoma-associated protein 2 autoantibody levels were measured using the National Institute of Diabetes and Digestive and Kidney Diseases standardized assay as described previously.[11]

Study procedures were performed at the Pediatric Clinical and Translational Research Center at Children's Hospital of Pittsburgh. After a 10- to 12-hour overnight fast, participants ingested 237 mL of high-protein Boost-HP (Nestle, Vevey, Switzerland), consisting of 33 g of carbohydrate, 15 g of protein, and 6 g of fat (percent calories: 55% carbohydrate, 25% protein, and 20% fat). Blood samples were obtained at −15, 0, 15, 30, 60, 90, and 120 minutes for determination of glucose, insulin, and C-peptide levels. Metformin was discontinued 36 hours before the mixed-meal test. No patient received long-acting or intermediate-acting insulin for 24 hours before the mixed-meal test. The last dose of short-acting insulin was given 6-8 hours before the mixed-meal test. Body composition was determined by dual-energy X-ray absorptiometry analysis. Subcutaneous abdominal adipose tissue and visceral adipose tissue were evaluated by a single-slice computed tomography scan at L4-L5 as described previously.[22] Plasma glucose level was measured with

Table I. Participant characteristics

	Clinically diagnosed T2DM		
	Ab^- (n = 27)	Ab^+ (n = 15)	*P* value
Age, years, mean ± SE	15.2 ± 0.4	14.5 ± 0.6	NS
Sex, male/female, n*	11/16	7/8	NS
Ethnicity, African American/Caucasian, n*	15/12	5/10	NS
Tanner stage, n*			NS
II-III	2	3	
IV-V	25	12	
BMI, kg/m^2, mean ± SE	36.5 ± 1.0	30.0 ± 2.0	.008
Waist circumference, cm, mean ± SE	107.7 ± 2.8	92.1 ± 4.1	.002
Percent body fat, mean ± SE	42.2 ± 1.4	38.4 ± 2.1	NS
Visceral adipose tissue, cm^2, mean ± SE	88.4 ± 8.1	47.1 ± 5.7	.001
Subcutaneous abdominal adipose tissue, cm^2, mean ± SE	551.1 ± 28.5	411.7 ± 56.6	.02
HbA1c, %, mean ± SE	6.5 ± 0.2	6.2 ± 0.3	NS
Diabetes duration, months, mean ± SE	8.9 ± 2.0	5.0 ± 1.0	NS
Ab titers†			
GAD65, DK units/mL	0.8 ± 0.3	548.3 ± 161.6	<.001
IA2, DK units/mL	0.1 ± 0.1	288.3 ± 76.8	<.001
Treatment modality, n (%)*			.01
Lifestyle	7 (26)	2 (13.4)	
Insulin	2 (7)	6 (40)	
Metformin	11 (41)	1 (6.6)	
Insulin and metformin	7 (26)	6 (40)	

DK, NIDDK assay units; *GAD65*, Glutamic acid decarboxylase 65-kDa; *IA2*, Insulinoma-associated protein 2; *NS*, not significant.
Values are derived from χ^2 analysis for categorical variables and the *t* test or Mann Whitney *U* test for quantitative variables.
*χ^2 analysis. Body composition data were missing for 4 subjects in the Ab^- group who were above the weight limit of the dual-energy X-ray absorptiometry machine, and visceral adipose tissue data were not available for 1 Ab^- youth and 1 Ab^+ youth owing to technical difficulties.
†The cutoff values for Ab positivity were 33 DK units/mL for GAD65 and 5 DK units/mL for IA2.[11]