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Indices of Insulin Secretion during a Liquid Mixed Meal Test in Patients with Diabetes

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Abstract

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

Study design—27 youth with Ab[−] and 15 youth with Ab⁺ clinically diagnosed T2DM had 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 Ab⁺ vs. Ab[−] clinically-diagnosed-T2DM. After controlling for BMI, $\Delta I_{30}/\Delta G_{30}$, $\Delta C_{30}/\Delta G_{30}$, $AUC_{C-peptide}/AUC_{glucose}$, and $AUC_{Insulin}/AUC_{glucose}$ were ($p<0.05$) lower in Ab⁺ vs. Ab[−]. Sensitivity indices were significantly higher in Ab⁺ vs. Ab[−]. Oral DI, $1/I_F \times \Delta I_{30}/\Delta G_{30}$ (0.04 ± 0.02 vs. 0.12 ± 0.02 mg/dl^{−1}, $p=0.005$) and $1/C_F \times \Delta C_{30}/\Delta G_{30}$ (0.02 ± 0.009 vs. 0.05 ± 0.006 mg/dl^{−1}, $p=0.018$) were lower in patients with Ab⁺ vs. Ab[−]. ROC 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 to identify youth with Ab⁺.

Conclusion—During a liquid mixed meal test, indices of β -cell function were lower and insulin sensitivity higher in Ab⁺ vs. Ab[−] clinically-diagnosed-T2DM with high sensitivity and specificity for fasting and stimulated C-peptide as markers of antibody positive status. Indices of insulin

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secretion during this standardized mixed meal test could be utilized to assess β -cell function in therapeutic trials of β -cell restoration in youth with T2DM.

Keywords

Insulin sensitivity; Insulin secretion; Disposition index; Mixed meal test; Youth type 2 diabetes; Pancreatic autoantibody

The increasing prevalence of obesity in children has resulted in the surge of type 2 diabetes mellitus (T2DM) in youth (1). Further, the childhood obesity epidemic has translated to increasing obesity in youth with type 1 diabetes mellitus (T1DM) (2–4), thus making the clinical distinction between obese youth with autoimmune T1DM and T2DM difficult. The SEARCH for Diabetes in Youth study (3) revealed that ~ 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, it was reported that the progression to insulin use was significantly higher in those with islet cell autoantibodies compared with those without (60% vs. 33%) (10). However, neither antibody status nor HLA markers were able to predict the clinical course (8). Initiation of insulin therapy is largely a clinician-driven decision and may not always reflect β -cell function (10). Therefore, an easily applicable standardized test to measure and follow β -cell function in youth with diabetes is desirable. Previously, we demonstrated that OGTT-derived indices of insulin sensitivity and secretion are not sensitive measures to distinguish metabolic/pathophysiological differences between youth with a clinical diagnosis of T2DM who have evidence of pancreatic autoimmunity (Ab^+) vs. those with negative autoantibodies (Ab^-) (11) in contrast to the clamp studies (12). Liquid mixed meal tolerance tests are frequently utilized to evaluate β -cell function in and in pediatrics (13–16). In individuals with T1DM, the mixed meal test is highly reproducible, well tolerated, and elicits significant C-peptide response (16). Further, the mixed meal stimulus results in greater β -cell response and higher estimates of insulin secretion compared with the OGTT in adults with 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-derived insulin secretion measures (20). Therefore, in this study we investigated indices of insulin secretion and sensitivity during the mixed meal test, between youth with Ab^- vs. Ab^+ clinically diagnosed T2DM. We hypothesized that differences in insulin secretion between patients with Ab^+ vs. Ab^- will be detected, and liquid mixed meal derived cut-off values for insulin secretion indices can help distinguish individuals as Ab^+ vs. Ab^- with adequate sensitivity and specificity.

METHODS

Forty-two 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 (CHP). Islet cell antibody screening, with the NIDDK harmonization assay, revealed 27 with negative antibodies (Ab^-) and 15 with one or two positive antibodies (Ab^+). Some of these youth were reported before (11, 12, 22), 31 who participated in the OGTT study (11), and 32 who participated in the 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 Institutional Review Board and consents and assents obtained prior to the investigation. Glutamic acid decarboxylase 65 kDa autoantibody (GAD65 Ab) and insulinoma associated

protein 2 autoantibody (IA2) were measured using the NIDDK standardized assay as before (11).

Study procedures were performed at the Pediatric Clinical and Translational Research Center (PCTRC) at CHP. After a 10 to 12 hour overnight fast, participants ingested 237 ml high protein Boost-HP (Nestle) consisting of 33 g carbohydrate, 15 g protein and 6 g fat, (%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. Metformin was discontinued 36 hours prior to the mixed meal test. Patients did not receive long or intermediate acting insulin for 24 hours prior to the mixed meal. The last dose of short acting insulin was given 6 to 8 hours prior to the mixed meal. Body composition was determined by dual energy x-ray absorptiometry (DEXA). Subcutaneous abdominal adipose tissue (SAT) and visceral adipose tissue (VAT) were examined by a single slice CT scan at L4-L5 as described (22). Plasma glucose was measured with a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, Ohio). Insulin and C-peptide were measured using radioimmunoassay as before (22). HbA1c was measured by high performance liquid chromatography (Tosoh Medics, Inc. 1998) (22).

The homeostasis model assessment for insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), $1/\text{fasting insulin } (1/I_F)$, fasting glucose to insulin ratio (G_F/I_F), $1/\text{fasting C-peptide } (1/C_F)$ and fasting glucose to C-peptide (G_F/C_F) were calculated (11). We previously demonstrated that these fasting indices of insulin sensitivity correlate well with the hyperinsulinemic euglycemic clamp-measured insulin sensitivity in obese adolescents with normal or impaired glucose regulation (23). Also, fasting C-peptide correlates well with clamp derived insulin sensitivity in youth with T2DM ($r = -0.6$, $p = 0.003$, unpublished data).

The insulinogenic index ($\Delta I_{30}/\Delta G_{30}$), and C-peptide-derived 30 minutes index ($\Delta C_{30}/\Delta G_{30}$), fasting C-peptide to glucose ratio (C_F/G_F) and the homeostasis assessment model of insulin secretion HOMA $\% \beta$ were calculated as before (11). The area under the curve (AUC) for glucose, insulin and C-peptide were calculated using the trapezoidal rule (11). Measures of the oral disposition index (oDI) from the mixed meal were calculated as $1/I_F \times \Delta I_{30}/\Delta G_{30}$, $G_F/I_F \times \Delta I_{30}/\Delta G_{30}$, (24), $1/C_F \times \Delta C_{30}/\Delta G_{30}$, and $G_F/C_F \times \Delta C_{30}/\Delta G_{30}$ (24, 25).

Statistical analyses

Comparison of continuous variables between the two groups (Ab^+ and Ab^-) was performed using t-test or Mann Whitney U test. Univariate analysis of variance was used to compare the 2 groups for variables of interest adjusting for BMI as a covariate. Repeated measures analysis with time as a factor and BMI as a covariate was used to compare mixed meal derived data. Categorical variables were compared using the χ^2 analysis. Receiver-operating characteristic (ROC) curves were developed for mixed meal-derived insulin secretion indices to evaluate the ability of each index to differentiate β -cell function between Ab^+ and Ab^- clinically-diagnosed-T2DM. A p value of 0.05 was considered statistically significant. All values are reported as mean $\pm$ SEM. Power analysis indicated that a sample size of 16 subjects per group was needed to detect 40% difference in C-peptide area under the curve (AUC) with a power of 0.8 or 14 subjects per group are needed to detect 50% difference in C-peptide AUC with a power of 0.9.

RESULTS

The clinical and physical characteristics of the study participants are summarized in Table I. There were no significant differences in age, sex, Tanner stage or ethnicity between the 2

groups. The patients with Ab⁺ had significantly lower BMI, waist circumference, and VAT compared with the subjects with Ab⁻. Diabetes duration and HbA1c at the time of the study were comparable between patients with Ab⁺ and Ab⁻. More patients with Ab⁻ were on metformin and lifestyle intervention and less were receiving insulin compared with patients with Ab⁺.

Fasting and mixed meal glucose, insulin, and C-peptide

After adjusting for BMI, fasting glucose and insulin were not significantly different between patients with Ab⁻ and Ab⁺. However, fasting C-peptide was lower in patients with Ab⁺ vs. Ab⁻ (Table II and Figure 1, B). In repeated measures analysis of covariance with BMI as the covariate, glucose levels during the mixed meal test were not different between the 2 groups (Figure 1, A). However, C-peptide concentrations and the C-peptide/glucose ratio during the liquid mixed meal were significantly lower in Ab⁺ compared with patients with Ab⁻ (Figure 1, B and C). The difference between the two groups in insulin levels and in the ratio of insulin to glucose trended towards significance (Figure 1, D and E). The glucose AUC was similar between the two groups (Figure 1, F). However, C-peptide AUC (934.6 ± 63.2 vs. 509.5 ± 87.8 ng/ml.min, $p=0.001$) and C-peptide AUC relative to glucose AUC (0.059 ± 0.004 vs. 0.028 ± 0.006 ng/ml per mg/dl, $p<0.001$) were ~50% lower in the Ab⁺ compared with the Ab⁻ group (Figure 1, G and H). The significant difference in unadjusted insulin AUC changed after adjusting for BMI (Figure 1, I). However, insulin AUC relative to glucose AUC was 40% lower in Ab⁺ compared with the patients with Ab⁻, and remained significant after adjusting for BMI ($p=0.03$) (Figure 1, J).

Liquid mixed meal-derived indices

Insulin-based indices of insulin sensitivity (G_F/I_F), $1/I_F$, HOMA-IR and QUICKI were not different between patients with Ab⁻ and Ab⁺. However, C-peptide-based indices, G_F/C_F and $1/C_F$ were significantly higher in the Ab⁺ group (Table II).

HOMA-% β was not different between the 2 groups (Table II). However, fasting C-peptide, C-peptide/glucose ratio, 30 minute insulinogenic index ($\Delta I_{30}/\Delta G_{30}$) and $\Delta C_{30}/\Delta G_{30}$ were significantly lower in patients with Ab⁺ vs. Ab⁻, and remained so after adjusting for BMI differences between the two groups (Table II). Figure 2 shows the ROC curves for various insulin secretion indices as markers of Ab⁺ status. The greatest area under the ROC curves were $\Delta C_{30}/\Delta G_{30}$ (0.895), C-peptide AUC/glucose AUC (0.89), followed by C_F/G_F (0.87) and fasting C-peptide (0.86). For fasting C-peptide, a concentration less than 3.2 ng/ml showed 87% sensitivity and 74% specificity, and for $\Delta C_{30}/\Delta G_{30}$ a value below 0.075 ng/ml per mg/dl showed 93% sensitivity and 80% specificity to detect Ab⁺ status. Lower values for the ROC_{AUC} were obtained for insulin-derived indices $\Delta I_{30}/\Delta G_{30}$ and insulin AUC/glucose AUC (0.81 and 0.83) (not shown in the Figure) with the lowest for HOMA % β (0.6).

β -cell function relative to insulin sensitivity, oDI derived from fasting indices of insulin sensitivity and mixed meal measures of insulin secretion, was significantly lower in the Ab⁺ group compared with the Ab⁻ group before and after adjusting for BMI (Table II).

DISCUSSION

The present findings of lower insulin secretion in youth with clinically diagnosed Ab⁺ vs. Ab⁻ T2DM are consistent with the only other pediatric study of mixed meal-derived insulin secretion (6). Those investigators showed lower insulin and C-peptide levels at 90 minutes, and lower incremental response of C-peptide and insulin during the first 30 minutes after the mixed meal in Ab⁺ vs. Ab⁻. However they did not account for differences in HbA1c

(8.7 ± 2.5 vs. 7.8 ± 2.1 , $p=0.003$) and weight between the 2 groups, and BMI was not available and not adjusted for (6). Our current findings are also consistent with those from the “Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY)” study which demonstrated lower fasting C-peptide levels in 118 youth with Ab^+ compared with 1088 youth with Ab^- clinically diagnosed to have T2DM (5). Lower fasting C-peptide levels were also described in Ab^+ vs. Ab^- young adults (15–34 years old) (7). Lastly, our present observations using the liquid mixed meal are in line with our previous findings using the hyperglycemic clamp, where first phase insulin secretion was 35–40% lower in youth with Ab^+ vs. those with Ab^- (12). The hyperglycemic clamp evaluates the insulin response to intravenously administered sustained hyperglycemia (~ 225 mg/dl) and is considered the gold standard to evaluate β -cell function because the glycemic stimulus is uniform across individuals unlike orally administered glucose which results in different plasma glucose concentrations in different individuals. Using the OGTT, only the fasting C-peptide and C-peptide AUC to glucose AUC were significantly lower in youth with Ab^+ vs. those with Ab^- (11), and with the mixed meal test all secretion indices except for HOMA-% β were significantly lower in youth with Ab^+ vs. those with Ab^- . The difference between the results utilizing the OGTT vs. the mixed meal test in this current study may be explained by higher β -cell sensitivity to a mixed meal stimulus as opposed to the glucose only stimulus of the OGTT (17). This may be due to the effect of different nutrients in the mixed meal including protein and fat in inducing insulin secretion through different mechanisms (19). Some of these differences in insulin response to different stimuli may be mediated by incretin hormone levels (26) which were not measured in the current study.

Similar to our previous OGTT study (11), fasting indices of insulin sensitivity (with the exception of C-peptide based indices) were not different between youth with Ab^+ vs. those with Ab^- . Similarly, other investigators did not find significant differences in HOMA-IR in individuals with Ab^+ vs. those with Ab^- (6). However, with the hyperinsulinemic-euglycemic clamp, there were significant differences in *in-vivo* insulin sensitivity, higher in youth with Ab^+ vs. those with Ab^- (12). This is because the clamp, a much more sensitive tool where both glycemia and insulinemia are experimentally controlled, measures peripheral insulin sensitivity and fasting indices may mostly reflect hepatic sensitivity. The insulin sensitivity of the youth with Ab^+ is lower than is typically seen in youth with T1DM (27) likely related to the higher BMI in our study population.

β -cell function relative to insulin sensitivity, calculated as the oral disposition index, was significantly lower in youth with Ab^+ vs. those with Ab^- . This contrasts with our clamp observations of similar DI in the two groups (12) likely because of the euglycemic clamp-measured differences in *in-vivo* insulin sensitivity discussed above. Unlike the OGTT or the mixed meal, the clamp method is the gold standard for measuring *in vivo* insulin sensitivity, but it remains a research tool which is difficult to apply on a wide scale in children. An additional explanation for the failure to detect differences in insulin sensitivity indices from fasting measures is that, despite severe impairment in first-phase insulin secretion in youth with T2DM, fasting insulin concentrations are elevated, most likely reflective of decreased insulin clearance (28).

Lastly, the ROC analyses demonstrate that the mixed meal-derived C-peptide indices have acceptable discriminatory value in evaluating β -cell function in Ab^+ vs. Ab^- clinically-diagnosed-T2DM and provide reliable cutoffs with adequate sensitivity and specificity. Our study supports the use of the mixed meal tolerance test in clinical trials evaluating beta cell function in youth with Ab^+ or Ab^- T2DM, because its application is more feasible than the clamp, and it provided data consistent with the more sensitive clamp studies. Further, its repeated application over time is possible without imposing much patient burden, which makes it suitable for the longitudinal follow-up of β -cell function. One limitation of this

study is the relatively small number of youth with positive autoantibodies with inadequate statistical power to evaluate insulin secretion measures in patients positive for single vs. double antibodies. However, in our clamp investigations, the latter group had significantly greater impairment in insulin secretion than the former group suggesting a dose-effect phenomenon (12). Insulin antibodies were not measured in this study given that some patients were receiving or may have received exogenous insulin. As such, we cannot completely exclude that some individuals with negative autoantibodies may be positive for insulin autoantibody. Future studies should consider obtaining insulin antibodies prior to initiation of insulin. In addition, the newly available zinc transporter 8 autoantibody may provide a more comprehensive evaluation of the pancreatic autoimmunity status. Also, racial/ethnic differences in insulin response to the mixed meal may be important. This will need to be addressed in future larger studies.

The liquid mixed meal test, a standardized and frequently utilized research tool, could be a useful means in assessing β -cell function in youth with diabetes, with or without antibodies, and in the longitudinal follow-up of therapeutic interventions designed to preserve and/or restore β -cell function in these youth.

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Figure 1.

A–E: Glucose, C-peptide, C-peptide/glucose ratio, insulin, and insulin/glucose, respectively, during the mixed meal in patients with clinically diagnosed Ab⁻ (blank triangles) vs. Ab⁺ (filled triangles) T2DM; p-values are for repeated measures analysis with BMI as a covariate. F–J: AUC for glucose, C-peptide, C-peptide/glucose, insulin, insulin/glucose, respectively, during the mixed meal test in patients with clinically diagnosed Ab⁻ (blank bars) vs Ab⁺ (filled bars) T2DM. P-values adjusted for BMI.

Figure 2.

ROC curves of mixed meal-derived C-peptide indices in youth with clinically diagnosed Ab⁺ and Ab⁻ T2DM. The ROC_{AUC} for each index is included in parentheses. The AUC for HOMA % β is shown as the lowest ROC curve.

Table1

Participants' Characteristics.

	Clinically diagnosed T2DM		<i>P-value</i>
	Ab⁻ n=27	Ab⁺ n=15	
Age (years)	15.2±0.4	14.5±0.6	ns
Sex (Male/Female) *	11/16	7/8	ns
Ethnicity (AA/W) *	15/12	5/10	ns
Tanner (n) *			ns
<i>II-III</i>	2	3	
<i>IV-V</i>	25	12	
BMI (Kg/m ²)	36.5±1.0	30.0±2.0	0.008
Waist circumference (cm)	107.7±2.8	92.1±4.1	0.002
Percent Body Fat (%)	42.2±1.4	38.4±2.1	ns
VAT (cm ²)	88.4±8.1	47.1±5.7	0.001
SAT (cm ²)	551.1±28.5	411.7±56.6	0.02
HbA1c (%)	6.5±0.2	6.2±0.3	ns
Diabetes duration (months)	8.9±2.0	5.0±1.0	ns
Antibody Titers [§]			
GAD (DK units/ml)	0.8±0.3	548.3±161.6	<0.001
IA2 (DK units/ml)	0.1± 0.1	288.3±76.8	<0.001
Treatment modality n (%) *			0.01
<i>Lifestyle</i>	7 (26%)	2 (13.4%)	
<i>Insulin</i>	2 (7%)	6 (40%)	
<i>Metformin</i>	11 (41%)	1 (6.6%)	
<i>Insulin & Metformin</i>	7 (26%)	6 (40%)	

Chi-square analysis for categorical variables and t-test or Mann Whitney U test for quantitative variables.

* Chi-square analysis. AA: African American, W: White; Data are mean ± standard error. Body composition data was missing for 4 subjects in the Ab⁻ group who were above the weight limit of the DEXA machine and VAT data was not available for 1 Ab⁻ and 1 Ab⁺ youth due to technical difficulty.

[§]The cutoff values for antibody positivity are 33 DK units/ml for GAD 65 and 5 DK units/ml for IA2 (11).

Table 2

Fasting and mixed meal-derived surrogate indices of insulin sensitivity and secretion in youth with clinically-diagnosed Ab⁻ vs. Ab⁺ T2DM.

	Clinically diagnosed T2DM		<i>Adjusted P-value</i>
	Ab⁻	Ab⁺	
	n=27	n=15	
G _F (mg/dl)	109.7±4.8	117.6±6.6	ns
I _F (μU/ml)	43.0±6.9	37.1±9.5	ns
C _F (ng/ml)	4.1±0.3	2.4±0.5	0.009
Fasting indices of insulin sensitivity			
HOMA-IR	12.3±2.4	11.4±3.3	ns
QUICKI	0.28±0.005	0.29±0.006	ns
G _F /I _F (mg/dl per μU/ml)	3.8±0.4	4.4±0.6	ns
1/ I _F (μU/ml) ⁻¹	0.037±0.004	0.038±0.006	ns
G _F /C _F (mg/dl per ng/ml)	31.9±3.0	53.1±4.2	0.001
1/ C _F (ng/ml) ⁻¹	0.30±0.03	0.47±0.04	0.005
Fasting and liquid mixed meal-derived indices of insulin secretion			
HOMA % β	391.7±60.2	260.4±82.0	ns
C _F /G _F (ng/ml per mg/dl)	0.038±0.003	0.020±0.004	0.003
Δ I ₃₀ /ΔG ₃₀ (μU/ml per mg/dl)	4.2±0.7	1.6±0.9	0.04
ΔC ₃₀ /ΔG ₃₀ (ng/ml per mg/dl)	0.16±0.02	0.05±0.03	0.001
Liquid mixed meal-derived Disposition Indices			
G _F /I _F × ΔI ₃₀ /ΔG ₃₀	12.3±1.5	4.8±2.0	0.007
1/I _F × ΔI ₃₀ /ΔG ₃₀ (mg/dl) ⁻¹	0.12±0.02	0.04±0.02	0.005
G _F /C _F × ΔC ₃₀ /ΔG ₃₀	4.9±0.6	2.3±0.8	0.014
1/C _F × ΔC ₃₀ /ΔG ₃₀ (mg/dl) ⁻¹	0.05±0.006	0.02±0.009	0.018

Data are means adjusted for BMI± standard error.