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β-Cell Function, Incretin Effect, and Incretin Hormones in Obese Youth Along the Span of Glucose Tolerance From Normal to Prediabetes to Type 2 Diabetes

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PATHOPHYSIOLOGY

Using the hyperglycemic and euglycemic clamp, we demonstrated impaired β-cell function in obese youth with increasing dysglycemia. Herein we describe oral glucose tolerance test (OGTT)-modeled β-cell function and incretin effect in obese adolescents spanning the range of glucose tolerance. β-Cell function parameters were derived from established mathematical models yielding β-cell glucose sensitivity (βCGS), rate sensitivity, and insulin sensitivity in 255 obese adolescents (173 with normal glucose tolerance [NGT], 48 with impaired glucose tolerance [IGT], and 34 with type 2 diabetes [T2D]). The incretin effect was calculated as the ratio of the OGTT-βCGS to the 2-h hyperglycemic clamp-βCGS. Incretin and glucagon concentrations were measured during the OGTT. Compared with NGT, βCGS was 30 and 65% lower in youth with IGT and T2D, respectively; rate sensitivity was 40% lower in T2D. Youth with IGT or T2D had 32 and 38% reduced incretin effect compared with NGT in the face of similar changes in GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) in response to oral glucose. We conclude that glucose sensitivity deteriorates progressively in obese youth across the spectrum of glucose tolerance in association with impairment in incretin effect without reduction in GLP-1 or GIP, similar to that seen in adult dysglycemia.

A core defect in the pathogenesis of type 2 diabetes (T2D) is impaired β-cell function (1,2). In adults, longitudinal (2,3) and cross-sectional (4,5) investigations have demonstrated that β-cell function declines with increasing hyperglycemia already within the normal glucose tolerance (NGT) range and is further impaired with the onset of impaired glucose tolerance (IGT) and progression to T2D. Similarly, cross-sectional and longitudinal studies in pediatrics, using a variety of methodologies, have established that β-cell function is impaired in prediabetes, and to a worse extent in T2D (6–12), with evidence of rapid deterioration (13–15). Using the hyperglycemic clamp together with the hyperinsulinemic-euglycemic clamp, we demonstrated that β-cell function relative to insulin sensitivity was diminished in obese youth with IGT by ~40% and in T2D by ~80% compared with their NGT peers (6).

Because of the important physiological role of incretin hormones (GLP-1 and glucose-dependent insulinotropic polypeptide [GIP]) in augmenting insulin secretion, a frequent view is that GLP-1 secretion is deficient in T2D patients and, in a lesser degree, in people with prediabetes (16). However, studies in adults have yielded conflicting results showing decreased (16,17), normal

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(16,18,19), or increased (20) GLP-1 concentrations in individuals with prediabetes or T2D. Moreover, the incretin effect, defined as a higher insulin response to oral than intravenous glucose at similar prevailing glucose concentrations, is found to be markedly reduced in adults with T2D (21,22), but similar in IGT (23,24), compared with NGT. At present, pediatric data are completely lacking with respect to the incretin effect and incretin hormone secretion in youth with T2D or prediabetes. Therefore, the aims of the present investigation were as follows: *1)* to examine β-cell function, modeled from a simple 2-h oral glucose tolerance test (OGTT), in obese adolescents across the spectrum of glucose tolerance; and more importantly *2)* to assess the incretin effect and the relationship between incretin hormone response during the OGTT and β-cell function in obese adolescents with NGT, IGT, or T2D.

RESEARCH DESIGN AND METHODS

Complete data from an OGTT and a synchronized hyperglycemic clamp were available for 255 obese adolescents (173 NGT, 48 IGT, and 34 T2D), as participants in the National Institutes of Health–funded studies Childhood Insulin Resistance and Childhood Metabolic Markers of Adult Morbidity (7,25–27). All participants were pubertal (Tanner II–V) and had exogenous obesity with no clinical evidence of endocrinopathy associated with obesity except dysglycemia. Glucose tolerance and T2D were defined according to the 2003 American Diabetes Association guidelines (28). Family history for diabetes was defined as the presence of known family members with T2D in any of three generations (siblings, parents, or grandparents) (29). Adolescents with T2D were negative for GAD and insulinoma-associated protein 2 autoantibody (26), with T2D duration of <2 years except for two participants who had a 2.8- and 3.3-year duration. Youth with T2D were treated with lifestyle only (n = 7), insulin only (n = 4), metformin only (n = 16), or metformin plus insulin (n = 7). Metformin was discontinued 36 h prior to the OGTT. Patients did not receive long- or intermediate-acting insulin for 24 h prior to the OGTT. The last dose of short-acting insulin was given 6–8 h prior to the OGTT. The same applied for the hyperglycemic clamp. Participants classified as NGT or IGT were not taking any medications known to affect glucose metabolism. Some participants were reported within a different context (7,25–27). The studies were approved by our institutional review board, and parental consent and child assent were obtained prior to study participation.

All research evaluations were performed in the Pediatric Clinical and Translational Research Center (Children's Hospital of Pittsburgh, University of Pittsburgh Medical Center, Pittsburgh, PA). Body composition was evaluated with DEXA with measurement of fat-free mass (FFM), fat mass (FM), and percent body fat as described previously (7). Abdominal subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) were assessed by MRI (n = 144) or computed tomography (n = 100) at L_{4-5} intervertebral space (30,31). Eight participants (3 NGT and 5 T2D) are missing DEXA data, and 11 are missing abdominal adiposity data (6 NGT, 3 IGT, and 2 T2D) due to technical difficulties and weight exceeding the limit of measurement. Clinical characteristics of the study participants are summarized in Table 1.

OGTT

After 10–12 h of overnight fasting, participants underwent a 2-h OGTT (1.75 g/kg, maximum 75 g) (7,26). Blood samples were obtained at −15, 0, 15, 30, 60, 90, and 120 min for the measurement of glucose, insulin, C-peptide, glucagon, total GLP-1, GIP, and pancreatic polypeptide (PP).

Table 1—Clinical phenotype

	NGT	IGT	T2D	ANOVA	NGT vs. IGT	NGT vs. T2D	IGT vs. T2D
n	173	48	34				
Age (years)	14.7 ± 0.1	15.2 ± 0.3	15.1 ± 0.3	NS			
Sex (male/female) (*n*)	68/105	19/29	16/18	NS			
Race (AA/CA/Bi) (*n*)	89/78/6	16/31/1	18/16/0	NS			
Tanner (II–III/IV–V) (*n*)	24/149	6/42	3/31	NS			
FHD (no/yes)	38/135	10/36	1/32	0.04			
BMI (kg/m^2)	34.2 ± 0.5	36.5 ± 0.9	36.6 ± 1.0	0.01	0.02	0.03	NS
BMI percentile	97.4 ± 0.2	98.4 ± 0.4	99.0 ± 0.5	0.003	NS	0.01	NS
FM (kg)	39.7 ± 0.9	45.0 ± 1.8	41.2 ± 2.3	0.03	0.03	NS	NS
FFM (kg)	51.3 ± 0.8	52.8 ± 1.5	54.4 ± 1.9	NS			
Percent body fat	42.2 ± 0.5	44.8 ± 1.0	42.4 ± 1.3	NS			
VAT (cm^2)	61.5 ± 2.4	75.2 ± 4.7	86.0 ± 5.5	<0.001	0.03	<0.001	NS
SAT (cm^2)	469.5 ± 13.8	546.4 ± 26.6	546.4 ± 32.0	0.01	0.03	0.03	NS
HbA_{1c} (%)	5.4 ± 0.04	5.3 ± 0.08	6.6 ± 0.09	<0.001	NS	<0.001	<0.001

AA, African American; Bi, biracial; CA, Caucasian; FHD, family history of diabetes. Post hoc analyses using Tukey.

Hyperglycemic Clamp

Either the day after the OGTT or on a separate visit within a 1–4-week period, a 2-h hyperglycemic clamp (~225 mg/dL) was performed in a subset of 198 subjects (NGT = 122, IGT = 42, and T2D = 34) (6,7). Plasma glucose concentration was rapidly raised to 225 mg/dL with a bolus dextrose infusion and maintained at 225 mg/dL with a variable-rate infusion of 20% dextrose for 2 h (6,7,26).

Biochemical Measurements

At each sampling point, blood was collected in chilled aprotinin/EDTA tubes for insulin, C-peptide, and glucagon measurement. Dipeptidyl peptidase-4 (DPP-4) inhibitor (10 μL, catalog no. DPP4; Millipore, St. Charles, MO) was added before sampling to the aprotinin/EDTA tubes to prevent the enzymatic degradation of GLP-1 (7-36) and GLP-1 (7-37). Blood samples were immediately separated in a refrigerated centrifuge. Plasma samples were divided into aliquots and stored at −80°C until analysis. Plasma glucose was determined, at the bedside, by the glucose oxidase method using a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, OH), and plasma insulin, C-peptide, and glucagon by commercially available radioimmunoassay (Millipore), as reported by us before (26). HbA_{1c} was measured by high-performance liquid chromatography (Tosoh Medics). Total GLP-1 was measured on a microplate reader (BioTek, Winooski, VT) using a multispecies total GLP-1 ELISA Kit (catalog no. EZGLP1T-36K; Millipore). This assay specifically detects both active and inactive forms of GLP-1 (7-36 and 9-36), with no detectable cross-reactivity with GLP-2, GIP, glucagon, or oxyntomodulin. Total GIP and PP were measured on the Luminex 200 IS (Luminex, Austin, TX) using a two-plex human gut hormone MILLIPLEX Kit (catalog no. HGT-68K-02; Millipore). The antibody pairs in the panel are specific only to the desired analyte and exhibit no or negligible cross-reactivity with other analytes in the panel.

Calculations

Area under the curve (AUC) was calculated with the use of the trapezoidal method. During the OGTT, early-phase responses were calculated as the AUC for the first 30 min and late-phase responses as the AUC for the last 90 min after the glucose challenge (32). β-Cell function parameters were assessed using a mathematical model describing the relationship between insulin secretion and glucose concentrations, reported in detail by Mari and colleagues (33,34). β-Cell function parameters included β-cell glucose sensitivity (OGTT-βCGS) (in $pmol \cdot min^{-1} \cdot m^{-2} \cdot mM^{-1}$) and rate sensitivity (in $pmol \cdot m^{-2} \cdot mM^{-1}$). OGTT-βCGS reflects the ability of the β-cell to respond to changes in prevailing plasma glucose concentration at any time point during the OGTT through a dose-response function relating the two variables (34). This dose-response is modulated by a potentiation factor, which encompasses several potentiating mechanisms (release of endogenous incretin hormones, neuronal inputs, and changes in incremental plasma glucose concentration after ingestion of the glucose load), all of which increase the sensitivity of the β-cell insulin secretory response to subsequent plasma glucose concentration (34). Potentiation was quantified as the ratio between the 2-h and the baseline value and denoted as the potentiation ratio. Rate sensitivity, related to early insulin release, refers to the magnitude of the β-cell response to the rate of change in plasma glucose concentration (34). The AUC of insulin secretion during the 2-h OGTT was denoted as total insulin output (expressed in $nmol \cdot m^{-2}$). A model-based index of insulin sensitivity (oral glucose insulin sensitivity [OGIS]) was calculated using the plasma glucose and insulin concentrations measured during a standard 2-h OGTT (35); this index has been validated against the hyperinsulinemic-euglycemic clamp (36).

During the hyperglycemic clamp, insulin secretion was obtained from C-peptide levels by deconvolution (37). Acute insulin response (AIR) was calculated as the mean incremental insulin secretion between 0 and 5 min, when insulin secretion rate had fallen from the initial peak to a nadir in all subjects. Rate sensitivity was then the ratio of AIR to the corresponding glucose increment. An empirical estimate of βCGS (clamp-βCGS) was obtained as the increment in insulin secretion during the last 40 min of the clamp above basal insulin secretion, divided by the corresponding glucose increment (see the Supplementary Data for details). The clamp- and the OGTT-βCGS thus represent an average slope of the relationship between insulin secretion and glucose concentration, obtained with intravenous and oral glucose administration, respectively. Of note is that βCGS, as the mean slope of a dose-response relationship, is independent of absolute insulin secretion. As previously shown (23,24), the incretin effect is exerted not only on absolute insulin secretion but also on βCGS. In the present studies, the incretin effect was estimated as the OGTT-βCGS/clamp-βCGS ratio.

Statistical Analysis

ANOVA with Tukey post hoc correction for quantitative variables and χ^2 test for categorical variables were used to examine subject characteristics, β-cell function parameters, and early- and late-phase incretin response among the three groups. ANCOVA models were used to assess between-group differences adjusting for covariates as applicable, such as VAT or BMI. Log transformations were used to normalize the distribution for glucose, insulin, C-peptide, and GLP-1. All other variables were normally distributed. To assess the relationships between β-cell function parameters and incretin response, bivariate Pearson or Spearman correlations were applied according to data distribution. Unless otherwise stated, data are presented as mean ± SEM. Statistical significance was set at $P < 0.05$, and the statistical analyses were performed using PASW Statistics (version 20; SPSS Inc., Chicago, IL).

RESULTS

Participant Characteristics

There were no significant differences in age, sex, race, Tanner stage, FFM, or percent FM between the groups. Compared with NGT, participants with IGT and T2D had higher BMI, SAT, and VAT. As expected, HbA_{1c} was higher in the T2D group compared with NGT and IGT (Table 1).

Glucose and Hormone Responses to the OGTT

Fasting glucose and insulin concentrations increased from NGT to IGT to T2D as did 2-h plasma glucose levels (Supplementary Table 1). Two-hour insulin and C-peptide were higher in IGT compared with NGT, whereas they only tended to be lower in T2D. Among the incretin hormones, both fasting and 2-h GLP-1 were higher in IGT compared with NGT. Fasting concentrations of PP tended to be higher in IGT and T2D compared with NGT, whereas GIP showed no significant differences across groups.

In Supplementary Fig. 1, early-phase (0–30 min) insulin response (expressed as the ratio of insulin to glucose AUC) was lowest in T2D compared with NGT and IGT (2,403 ± 403 vs. 3,221 ± 179 vs. 2,963 ± 343 pmol · $mmol^{-1}$, respectively, P = 0.017); the same was true of early-phase C-peptide response (P = 0.012). In contrast, early-phase glucagon response (as the product of glucagon and glucose AUC) was highest in T2D as compared with NGT and IGT (3,005 ± 259 vs. 2,154 ± 65 vs. 2,465 ± 158 $\mu g^2 \cdot mL^{-2} \cdot min^2$, respectively, $P < 0.001$). Late-phase (30–90 min) insulin response showed the same pattern as early-phase insulin response, decreasing from NGT to T2D through IGT (14,512 ± 1,279 vs. 12,661 ± 674 vs. 10,046 ± 1,520 pmol · $mmol^{-1}$, $P < 0.001$); late-phase C-peptide response tracked with insulin ($P < 0.001$). Late-phase glucagon response increased from NGT to IGT to T2D (6,131 ± 214 vs. 7,456 ± 561 vs. 10,542 ± 1,008 $\mu g^2 \cdot mL^{-2} \cdot min^2$, $P < 0.001$) (Supplementary Fig. 1).

Incretin hormone responses are shown in Fig. 1. Because of baseline group differences in GLP-1 and PP, incremental AUCs (iAUCs), rather than total AUCs, were calculated. iAUC for GIP (2,613 ± 101, 2,611 ± 193, 2,301 ± 229 pmol · L^{-1} · h, P = 0.45) was not different among NGT, IGT, and T2D, whereas iAUC for GLP-1 (183.4 ± 25.3, 139.4 ± 48.1, 461.1 ± 57.1 pmol · L^{-1} · h, P = 0.08) and PP (510 ± 44, 597 ± 83, 752 ± 99 pmol · L^{-1} · h, P = 0.07) showed a trend. Early-phase GLP-1 iAUC was not different among the three groups (data not shown), whereas late-phase GLP-1 iAUC was significantly different among the NGT, IGT, and T2D (105.6 ± 19, 67.7 ± 36.1, 326.0 ± 42.9 pmol · L^{-1} · h, respectively, P = 0.005). Furthermore, there were no significant differences in fasting GLP-1 (14.3 ± 1.8 vs. 15.9 ± 3.3 pmol · L^{-1}, P = 0.55), 2-h GLP-1 (13.6 ± 2.5 vs. 11.3 ± 2.6 pmol · L^{-1}, P = 0.57), or GLP-1 iAUC (428.8 ± 138 vs. 528.6 ± 286 pmol · L^{-1} · h, P = 0.77) in T2D youth prescribed metformin (n = 23) versus those not prescribed metformin (n = 11). Early-phase and late-phase GIP did not differ significantly according to glucose tolerance status. Both early-phase PP iAUC (193 ± 19 vs. 273 ± 35 vs. 267 ± 42 pmol · L^{-1} · h, P = 0.06) and late-phase PP iAUC showed a trend (317 ± 32 vs. 324 ± 60 vs. 485 ± 72 pmol · L^{-1} · h, P = 0.10) among NGT, IGT, and T2D, respectively.

Insulin sensitivity, as estimated by OGIS, was significantly, and to a similar extent, impaired in IGT and T2D

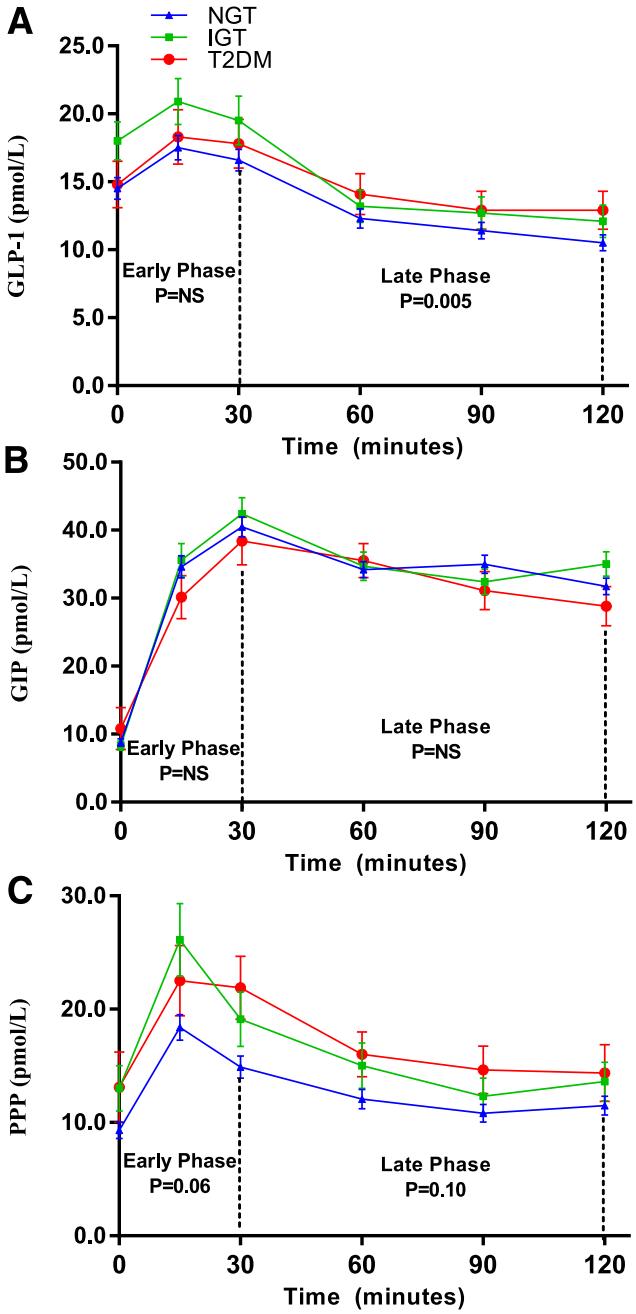

Figure 1—Incretin peptides during the OGTT; early- and late-phase responses in GLP-1 (*A*), GIP (*B*), and PP (*C*) in obese adolescents with NGT (n = 173), IGT (n = 48), and T2D (n = 34). Plots are mean ± SEM. P values are for group differences by ANOVA.

Table 2—OGTT-modeled parameters of β-cell function

	NGT	IGT	T2D	ANOVA	NGT vs. IGT	NGT vs. T2D	IGT vs. T2D
Basal insulin secretion rate (pmol · min^{-1} · m^{-2})	141 ± 5	166 ± 9	188 ± 11	<0.001	0.03	<0.001	NS
Total insulin output (nmol · m^{-2})	58 ± 2	72 ± 3	57 ± 4	0.001	0.001	NS	0.01
βCGS (pmol · min^{-1} · m^{-2} · mM^{-1})	178 ± 8	125 ± 15	64 ± 17	<0.001	0.01	<0.001	0.02
Rate sensitivity (pmol · m^{-2} · mM^{-1})	1,788 ± 100	1,608 ± 190	1,095 ± 226	0.02	NS	0.02	NS
Potentiation ratio	1.1 ± 0.03	1.1 ± 0.05	1.1 ± 0.06	NS			

Post hoc analyses using Tukey.

as compared with NGT (Supplementary Table 1), the difference remaining significant after adjusting for BMI or VAT.

β-Cell Function and Incretin Effect

During the OGTT, basal insulin secretion rate was higher in IGT and T2D compared with NGT. Total insulin output was higher in IGT compared with the other two groups (Table 2). The insulin secretion to plasma glucose dose-response functions was progressively shifted to the right and downward from NGT to IGT to T2D (Fig. 2). Consequently, βCGS was progressively lower, whereas rate sensitivity was lowest only in youth with T2D. These group differences remained statistically significant after adjusting for BMI or VAT (data not shown).

During the hyperglycemic clamp, insulin secretion rates followed the expected biphasic pattern, with an early peak followed by a second phase of increasing insulin release (Supplementary Fig. 2). AIR was markedly impaired in T2D (381 ± 119 vs. 1,968 ± 104 pmol · min^{-1} · m^{-2}, $P < 0.001$) but only marginally reduced in IGT (1,639 ± 154). Coherently with this, rate sensitivity was significantly reduced in T2D (225 ± 85 pmol · m^{-2} · mM^{-1}, $P < 0.001$, vs. 1,174 ± 54 in NGT) and slightly impaired in IGT (938 ± 92). During the second phase, insulin secretion between 80 and 120 min averaged 657 ± 25, 698 ± 43, and 255 ± 40 pmol · min^{-1} · m^{-2} in NGT, IGT, and T2D, respectively, being significantly reduced only in T2D ($P < 0.0001$) (Table 3 and Supplementary Fig. 2). Figure 3*A* depicts OGTT- and clamp-βCGS. Unlike OGTT-βCGS, where reduced βCGS was observed in both IGT and T2D compared with NGT, clamp-βCGS was similar between NGT (93 ± 4) and IGT (101 ± 6) youth but lower in T2D youth (53 ± 7 pmol · min^{-1} · m^{-2} · mM^{-1}) ($P < 0.001$ for both). Moreover, clamp-βCGS between IGT youth in the upper half (9.4–11.0 mmol/L) versus lower half (7.8–8.9 mmol/L) of the glucose concentration of the 120 min of the OGTT was not different (73.8 ± 9.6 vs. 106.7 ± 8.0 pmol · min^{-1} · m^{-2} · mM^{-1}, $P = 0.13$); however, OGTT-βCGS was significantly lower (82.2 ± 8.8 vs. 133.5 ± 8.0 pmol · min^{-1} · m^{-2} · mM^{-1}, $P = 0.008$) in IGT youth in the upper half of glucose concentration.

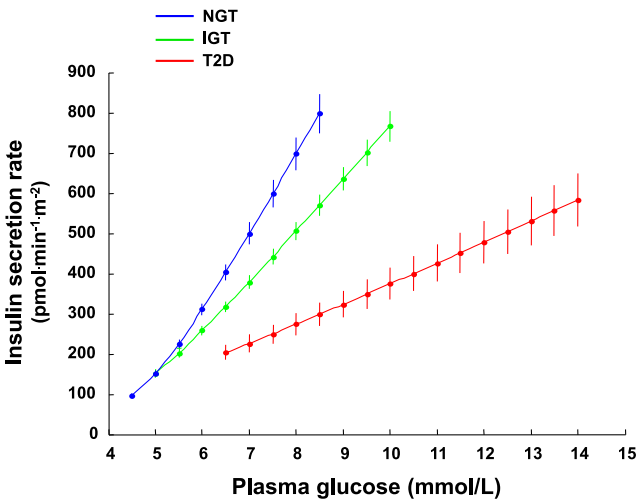

Figure 2—Dose-response of insulin secretion rates and plasma glucose concentrations in obese youth with NGT, IGT, and T2D. Plots are mean ± SEM; the mean slope of the dose-response function is βCGS.

In the whole group, OGTT-βCGS and clamp-βCGS were strongly correlated with one another ($r = 0.70$, $P < 0.0001$), but the relationship was significantly ($P < 0.02$) different across glucose tolerance status. Thus, a clamp-βCGS of 100 pmol · min^{-1} · m^{-2} · mM^{-1} predicted an OGTT-βCGS of 181 pmol · min^{-1} · m^{-2} · mM^{-1} in NGT, 122 in IGT, and 119 in T2D (Fig. 4). Consequently, the ratio OGTT-βCGS/clamp-βCGS, an estimate of the incretin effect, was significantly reduced in IGT and T2D compared with NGT, with no difference between IGT and T2D (Fig. 3*B*). Dividing T2D youth into those with short (<6 months) and those with longer disease duration (>6 months) revealed no significant difference in OGTT-βCGS/clamp-βCGS (1.2 ± 0.15 vs. 1.2 ± 0.19, $P = 0.94$).

Relationship of β-Cell Function Parameters to Glycemia and Incretin Hormones

OGTT 2-h glucose correlated negatively with OGTT-βCGS ($r = -0.41$, $P < 0.001$), rate sensitivity ($r = -0.22$, $P < 0.001$), and OGIS ($r = -0.40$, $P < 0.001$) and correlated

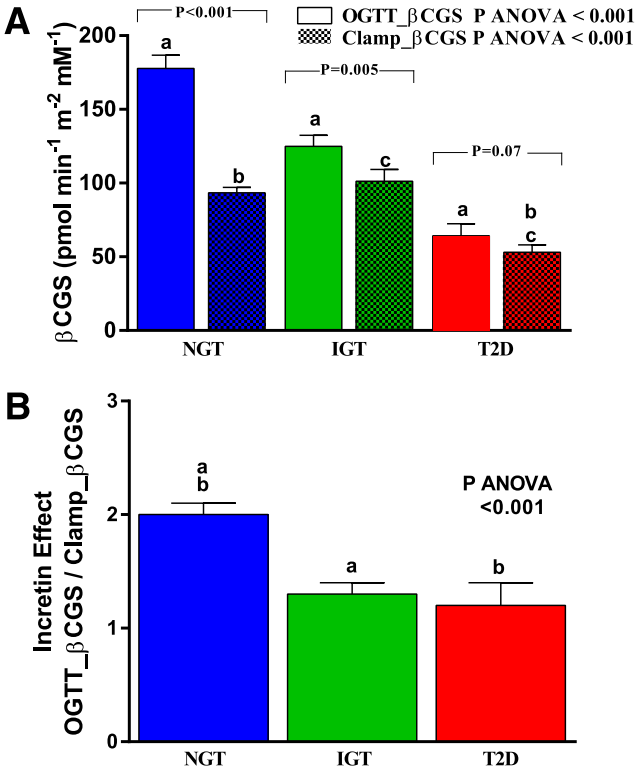

Figure 3—βCGS (*A*) and incretin effect (*B*) in obese adolescents with NGT (n = 173), IGT (n = 48), and T2D (n = 34). Differences among the three groups within each of the OGTT-βCGS and clamp-βCGS were analyzed with ANOVA. Tukey post hoc test for significant (P < 0.05) differences between any two groups is indicated with the same letter. Paired Student t test P values between the OGTT-βCGS and clamp-βCGS are shown above the bar graphs.

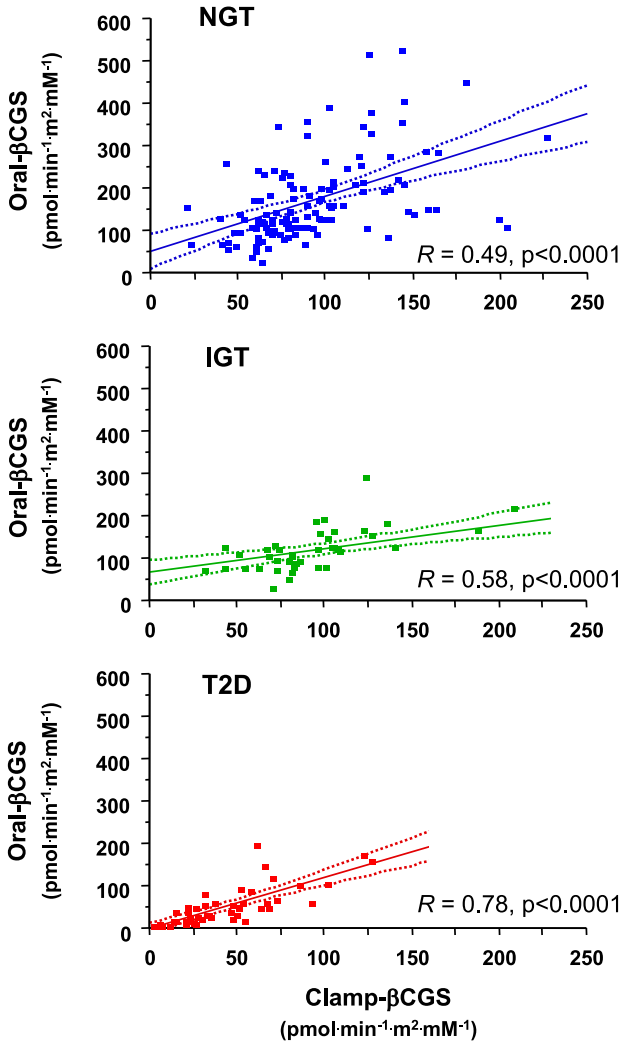

Figure 4—Relationship between βCGS and oral and intravenous glucose stimulation in the three study groups. The full lines are the best fits, and the dotted lines are their 95% CIs.

positively with 2-h GLP-1 (r = 0.22, P < 0.001) and GLP-1 AUC (r = 0.18, P = 0.005). Multiple linear regression analyses models assessing the independent effects of age, sex, race, VAT, insulin sensitivity, clamp-βCGS, incretin effect, OGTT-βCGS, and rate sensitivity are presented in Table 4. Insulin sensitivity, incretin effect, clamp-βCGS, and rate sensitivity explained 69% of variance in OGTT glucose AUC and 44% of the variance in OGTT 2-h glucose concentration. Including family history of diabetes into the model made no significant independent contribution.

DISCUSSION

The current study adds to the scarce literature in pediatric T2D by *1*) providing novel information on the incretin effect and incretin concentrations during the OGTT in obese adolescents across the spectrum of glucose tolerance from NGT to IGT to T2D, and *2*) further documenting the abnormalities in β-cell function and insulin sensitivity in these obese adolescents. The key finding of the current studies is that youth with IGT or T2D have a reduced incretin effect compared with their NGT peers without reductions in GLP-1 and GIP concentrations. In these youth, the incretin effect is an important determinant of the glycemic response to oral glucose.

β-Cell function is a key determinant of T2D (1,2) as it declines with the onset of IGT and progression to T2D in adults (2–5). In the current study, we demonstrate that βCGS was 30 and 65% lower in IGT and T2D, respectively, compared with NGT, with a 40% lower rate sensitivity in those with T2D. These results are in agreement with protocols involving intravenous glucose administration in both adults (2,3,5) and youth (6–12), and with OGTT-modeled data in adults (38–40). In particular, compared with obese NGT, βCGS was 40 and 85% lower in IGT and T2D adults, respectively, and rate sensitivity was significantly lower in T2D, consistent with the current data in youth. In another study (39), total insulin output was significantly higher in adults with impaired glucose regulation compared with NGT, whereas βCGS was 42% lower. A decline in βCGS, but not rate sensitivity, was also reported in Mexican American adults with IGT compared with NGT (40).

Table 3—Insulin secretion, βCGS, and incretin effect using different time interval of the hyperglycemic clamp

	80–120 min	Ratio to NGT	20–120 min	Ratio to NGT
Insulin secretion (pmol · min^{-1} · m^{-2})				
NGT	657 ± 25	1	566 ± 20	1
IGT	698 ± 43	1.06	578 ± 34	1.02
T2D	255 ± 40	0.39	218 ± 31	0.39
βCGS (pmol · min^{-1} · m^{-2} · mM^{-1})				
NGT	93 ± 4	1	79 ± 5	1
IGT	101 ± 6	1.09	82 ± 3	1.04
T2D	42 ± 6	0.45	35 ± 5	0.44
Incretin effect				
NGT	1.97 ± 0.08	1	2.27 ± 0.09	1
IGT	1.33 ± 0.14	0.68	1.61 ± 0.15	0.71
T2D	1.15 ± 0.13	0.58	1.38 ± 0.14	0.61

In the current study, the finding that IGT was associated with reduced βCGS when the measure is derived from the OGTT, but not from the clamp, is of particular interest and suggests that in prediabetes, impairment in incretin effect may precede defective β-cell secretory response to intravenous glucose. In more advanced stages of dysglycemia, such as T2D, both incretin effect and β-cell function appear to be impaired since βCGS derived from either the clamp or the OGTT is abnormal. The temporal sequence with which these metabolic abnormalities develop relative to one another during the different stages of dysglycemia remains uncertain. Although the mechanisms underlying the reduced β-cell response to oral glucose are undefined, recent investigations suggest that chronic exposure to higher glucose concentrations may downregulate GIP receptor expression (41,42). Whereas there were no significant differences in clamp-βCGS between the IGT youth in the upper versus the lower half of glucose concentration, OGTT-βCGS was significantly lower in IGT youth in the upper half of glucose concentration, consistent with the above proposed mechanism. On the other hand, the lack of difference in the clamp-βCGS may be due to the fact that insulin sensitivity is not accounted for. When we compare clamp-βCGS between NGT and IGT with OGIS as a covariate, we do not see significant differences (96.3 ± 3.9 vs. 90.7 ± 6.8 pmol · min^{-1} · m^{-2} · mM^{-1}, P = 1.50). This implies that insulin sensitivity may play a role in clamp-βCGS and that IGT may indeed have a relative impairment of β-cell function compared with NGT. However, such cross-sectional findings must be interpreted with caution, because many individuals with IGT may never develop diabetes, and their metabolic characteristics may well differ from those who do.

GLP-1 and GIP have been shown to be increased (20,43,44), decreased (16,17,45–47), or normal (16,18,19,

Table 4—Significant independent predictors of glucose AUC and 2-h glucose of the OGTT

	Partial r	Model r^2	Change in r^2	P
Dependent variable: OGTT AUC				
Model 1				
Insulin sensitivity	−0.72	0.27	0.27	<0.001
Incretin effect	−0.65	0.43	0.17	<0.001
Clamp-βCGS	−0.54	0.63	0.20	<0.001
Rate sensitivity	−0.39	0.69	0.06	<0.001
Model 2				
Insulin sensitivity	−0.69	0.29	0.29	<0.001
OGTT-βCGS	−0.65	0.57	0.28	<0.001
Rate sensitivity	−0.40	0.64	0.07	<0.001
Dependent variable: 2-h glucose				
Model 1				
Insulin sensitivity	−0.50	0.14	0.14	<0.001
Incretin effect	−0.46	0.41	0.13	<0.001
Clamp-βCGS	−0.42	0.28	0.13	<0.001
Rate sensitivity	−0.23	0.44	0.03	<0.001
Model 2				
Insulin sensitivity	−0.50	0.36	0.17	<0.001
OGTT-βCGS	−0.48	0.19	0.19	<0.001
Rate sensitivity	−0.27	0.41	0.05	<0.001

Incretin effect = OGTT-βCGS/clamp-βCGS.

24,46–49) in adults with T2D or IGT. A detailed meta-analysis by Calanna et al. (48,49) published very recently further supports no reduction in GLP-1 and GIP in adults with T2D. To our knowledge, there are no published incretin data in youth comparing NGT to IGT to T2D. In the current study, incretin hormone concentrations in response to the oral glucose load were not different between NGT, IGT, and T2D and were unlikely to explain the impaired incretin effect in IGT and T2D. However, it should be considered that the circulating concentrations of total GLP-1 and total GIP only partially reflect the activity of incretin hormones (50), which work through the intact forms only, and may also function independent of circulating levels. Furthermore, metformin has been shown to increase GLP-1 secretion in vitro (51) and could have masked a reduction in GLP-1 secretion in these subjects. However, we did not observe any significant differences in fasting GLP-1, 2-h GLP-1, or GLP-1 iAUC in T2D youth prescribed metformin versus those not prescribed metformin. With respect to the temporal pattern of incretin hormone response, in the current study, GLP-1 concentrations demonstrated an initial rise followed by a decline ~30 min after the glucose load. As shown by a recent systematic review by Nauck et al. (16), GLP-1 response may increase and then slowly decline, with a biphasic pattern or a monophasic pattern. Our results, however, are only relevant to a glucose load as the incretin response to a mixed meal may be different. Additional investigations in pediatrics are much needed to enhance knowledge with respect to the interplay of incretin hormones, their effect, and β-cell function in the evolution of prediabetes and T2D. Also of pathophysiological, clinical, and therapeutic relevance is the current finding of an augmented glucagon response in both IGT and T2D youth in the face of higher plasma glucose concentrations. This relative hyperglucagonemia, a correlate of β-cell dysfunction, further augments glucose dysregulation. Similar observations of α-cell upregulation were made in obese insulin-resistant youth with NGT or IGT (52).

With regard to the incretin effect, the novel finding is that the IGT and T2D youth both demonstrated a significantly reduced incretin effect, by 32 and 38% respectively, compared with their NGT peers. Since glucose levels during the clamp markedly exceeded the glucose levels during OGTT in IGT and NGT, the incretin effect may have been underestimated using this measure. In the current study, we used the ratio of the βCGS derived from the OGTT to the βCGS from the hyperglycemic clamp because absolute insulin secretion is dependent on the glucose concentrations, which were not matched. Although this index has not been validated against the gold standard isoglycemic protocol, glucose sensitivity is the most sensitive parameter accounting for glucose levels as its use to calculate the incretin effect from the isoglycemic method has been shown to be consistent with the more classical parameter obtained from insulin secretion (23). Furthermore, glucose sensitivity was consistent within individuals in the two tests (Supplementary Fig. 4) and was always higher with the OGTT than the clamp (by 95 ± 10% in NGT, 33 ± 8% in IGT, and 15 ± 10% in T2D). More importantly, the OGTT-βCGS/clamp-βCGS ratio retrieves the quantitative reduction in incretin effect (30–40%) that has been previously reported (21–23) and more recently confirmed (53) with the use of the OGTT and the isoglycemic protocol in adults with T2D. Our data in youth also confirm the quantitative contribution of the incretin effect to glucose tolerance (indexed by the 2-h plasma glucose concentration on the OGTT) (Table 4). In fact, glucose sensitivity could be estimated empirically from the ratio of incremental insulin secretion to incremental glucose levels (as shown in the Supplementary Data).

In our subjects, the incretin defect was not associated with a decrease in GLP-1 release in response to oral glucose (Fig. 1), which resonates with findings in adults, as reviewed by Nauck et al. (16), and suggests β-cell resistance to GLP-1 action on the β-cell (54). Furthermore, there is now mounting evidence that the incretin defect of adult T2D is not a consequence of chronic hyperglycemia, as initially argued (21), but a constitutive feature of T2D given that antihyperglycemic treatment is not associated with any improvement in incretin effect (53,55,56). Furthermore, recently Knop et al. (57) described reduced incretin effect in obese adults with NGT compared with healthy, lean NGT adults. These data shed light on a progressive defect that may even precede any underlying glucose dysregulation. Despite our limitation of not having a healthy, nonobese control group, our finding of an incretin defect in youths with IGT compared with obese NGT lends support to the postulate that an incretin defect may be an early, inherent part of T2D pathogenesis. Whether an incretin defect is present in youth with simple obesity and no dysglycemia remains to be determined.

In conclusion, OGTT-based βCGS declines progressively in obese youth across the spectrum of glucose tolerance from NGT to IGT to T2D. This is associated with a clear deficit in incretin effect with no evidence of decreased circulating concentrations of incretin hormones.

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