β-Cell Function Across the Spectrum of Glucose Tolerance in Obese Youth

Ram Weiss,[1] Sonia Caprio,[1] Maddalena Trombetta,[2] Sara E. Taksali,[1] William V. Tamborlane,[1,3] and Riccardo Bonadonna[2]

The profile of insulin secretion and the role of proinsulin processing across the spectrum of glucose tolerance in obese youth have not been studied. The aims of this study were to define the role of insulin secretion and proinsulin processing in glucose regulation in obese youth. We performed hyperglycemic clamps to assess insulin secretion, applying a model of glucose-stimulated insulin secretion to the glucose and C-peptide concentration data. Thirty obese youth with normal glucose tolerance (NGT), 22 with impaired glucose tolerance (IGT), and 10 with type 2 diabetes were studied. The three groups had comparable anthropometric measures and insulin sensitivity. The glucose sensitivity of first-phase secretion showed a significant stepwise decline from NGT to IGT and from IGT to type 2 diabetes. The glucose sensitivity of second-phase secretion was similar in NGT and IGT subjects yet was significantly lower in subjects with type 2 diabetes. Proinsulin-to-insulin ratios were comparable during first- and second-phase secretion between subjects with NGT and IGT and were significantly increased in type 2 diabetes. Obese youth with IGT have a significant defect in first-phase insulin secretion, while a defect in second-phase secretion and proinsulin processing is specific for type 2 diabetes in this age-group. *Diabetes* 54: 1735–1743, 2005

The unabated rise in the prevalence of childhood obesity (1) has been accompanied by the emergence of impaired glucose tolerance (IGT) as well as type 2 diabetes (2) in childhood. In contrast to the vast literature on the pathogenesis of IGT and type 2 diabetes in adults, little is known about the underlying mechanisms implicated in the development of these disorders of glucose metabolism in youth.

The insulin response of a healthy β-cell to a square-wave glucose infusion is biphasic in nature (3). From IGT through the course of type 2 diabetes, abnormalities in the biphasic nature of insulin release have been found in adults (4–6). Indeed, an altered acute-phase insulin release in response to glucose marks the early stages of decline in β-cell function (7). Recent studies reported that in obese adolescents, the hallmark of IGT is insulin resistance, with no apparent decline in first-phase insulin release (8,9). However, in obese adolescents with type 2 diabetes, impaired β-cell function was clearly evident (10). Thus, the findings in children with IGT are in contrast with most evidence obtained in adults with IGT. This apparent discrepancy may be due to several factors: *1*) age-specific pathophysiology of glucose regulation; *2*) lack of statistical power of the studies performed in obese adolescents, owing to small sample sizes; and *3*) confounding effects exerted by the inverse relationship between insulin sensitivity and insulin secretion (11–14). The latter factor may be of special relevance because the β-cell adapts very efficiently to changing insulin resistance (13,15). Furthermore, if β-cell function is assessed using data based on insulin concentration, the picture might be confounded further. Indeed, insulin concentration is determined by both β-cell secretion and insulin clearance by the liver, both of which change homeostatically in response to variations in insulin sensitivity (15,16). Therein lies the rationale to use models of β-cell response to glucose based on C-peptide concentration (12,17–19), which is cleared by the kidney and less affected by changes in insulin sensitivity, and thus does not suffer from the limitations of insulin concentration. Application of this modeling strategy has demonstrated that even in the adult with normal glucose regulation, first-phase insulin secretion linearly decreases with worsening of glucose homeostasis, whereas second-phase secretion is inversely related to insulin sensitivity (12).

The aim of this study was to define the role of insulin secretion in glucose regulation of the obese adolescent. We performed hyperglycemic clamps to assess insulin sensitivity and secretion, the latter by applying a model of glucose-stimulated insulin secretion to the glucose and C-peptide concentration data, based on the most recent modeling tools (12,18–20). We endeavored to answer the following questions in the obese adolescent: *1*) Does first-phase secretion steadily worsen hand-in-hand with glucose tolerance? *2*) Is second-phase insulin secretion inversely related to insulin sensitivity? *3*) If so, and taking into account the inverse relationship between second-

From the [1]Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut; the [2]Department of Biomedical & Surgical Sciences, Section of Endocrinology & Metabolic Diseases, University of Verona and Azienda Ospedaliera di Verona, Verona, Italy; and the [3]General Clinical Research Center of the Yale University School of Medicine, New Haven, Connecticut.

Address correspondence and reprint requests to Dr. Ram Weiss, Department of Pediatrics, Yale University School of Medicine, 333 Cedar St., P.O. Box 208064, New Haven, CT, 06520. E-mail: ram.weiss@yale.edu.

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IGT, impaired glucose tolerance; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test.

phase secretion and insulin sensitivity, is second-phase impaired in parallel with the alterations in glucose tolerance?

Another postulated early defect in β-cell function is in proinsulin processing. Proinsulin-to-insulin conversion is a highly efficient process in the β-cell (21). An increased proinsulin-to-insulin ratio is suggestive of an abnormality of proinsulin processing and has been found in some studies in adults with IGT and type 2 diabetes (22), while not in others (23). Elevated proinsulin levels predict the development of diabetes in adults (24), and the proinsulin-to-insulin ratio is strongly correlated with first-phase insulin secretion (25). Whether a proinsulin processing defect is present in the obese adolescent with IGT is unknown. To gain insights into the role of altered proinsulin processing in the development of IGT and type 2 diabetes in obese adolescents, we measured proinsulin levels and calculated the proinsulin-to-insulin ratios at fasting and in response to an acute hyperglycemia.

RESEARCH DESIGN AND METHODS

Subjects were recruited from a multiethnic cohort of obese children and adolescents drawn from the Pediatric Obesity Clinic at Yale. All participants underwent a standard oral glucose tolerance test (OGTT) as described (26). Thereafter, subjects were asked to volunteer for performing the hyperglycemic clamp. Eligible subjects with normal glucose tolerance (NGT) or IGT had to be 8–20 years of age, on no medications that may alter glucose metabolism, and otherwise healthy. Subjects with type 2 diabetes were within 6 months of diagnosis, and none had positive autoimmune markers of type 1 diabetes (GAD-65, ICA-512, and IA). All underwent a complete physical examination and detailed medical history. All had a BMI *Z* score >2 for age and sex. IGT and type 2 diabetes were defined according to the American Diabetes Association guidelines (27).

A total of 62 subjects were studied: 30 had NGT, 22 had IGT, and 10 had type 2 diabetes. Of these, 22 subjects have been described in a previous publication (8). Participants were instructed by a dietitian to follow a diet of at least 250 g of carbohydrates per day for 3 days before the study and to refrain from physical activity. Subjects with type 2 diabetes were instructed to stop metformin 3 days before the study. Subjects on insulin were instructed to withhold medium-acting insulin (NPH or Lente) for 36 h before the study and to use only an insulin analog during that time, up to 12 h before the study.

Metabolic studies: hyperglycemic clamp. Hyperglycemic clamps were performed as previously described (8). To quantify insulin secretion, plasma glucose was rapidly raised to 11.1 mmol/l by infusing 20% dextrose at variable rates and was kept at that level for 120 min (8,28). Samples were drawn at 2, 4, 6, 8, and 10 min and every 10 min afterward for glucose, insulin, and C-peptide concentrations. Proinsulin was measured at 2, 4, 6, 8, and 10 min and every 30 min thereafter.

Analytical methods. Plasma glucose was determined with a YSI 2700 Analyzer (Yellow Springs Instruments). Plasma lipid levels were determined with an AutoAnalyzer (Roche-Hitachi 747-200). Plasma free fatty acids were assayed by a colorimetric method. Plasma insulin was measured with a radioimmunoassay (Linco, St. Charles, MO), which has <1% cross-reactivity with C-peptide and proinsulin. Plasma C-peptide levels were determined with an assay made by Diagnostic Product (Los Angeles, CA). Total proinsulin was measured with another radioimmunoassay kit (Linco), which has no cross-reactivity with insulin and a detection limit of 0.15 pmol. The intra-assay variation was 4.5% for insulin and 5.9% for C-peptide, and the interassay variation was 10% for insulin and 11% for C-peptide.

Analysis of the hyperglycemic clamp data. Insulin sensitivity (S_i) during the hyperglycemic clamp was computed as the ratio of the glucose clearance (computed as the ratio of glucose infusion rate divided by the average glucose concentration) between 60 and 120 min of the clamp normalized by body surface area and divided by the average insulin concentration during the same time interval (S_i units are $ml \cdot min^{-1} \cdot m^{-2}$ per pmol/l). Incremental first-phase concentration of insulin and C-peptide was calculated as the mean value in 2, 4, 6, 8, and 10 min minus the fasting level. Mean second-phase concentration of insulin and C-peptide was calculated as the mean value between 60 and 120 min.

The analysis of the glucose and C-peptide curves during the hyperglycemic clamp follows the general strategy proposed by several laboratories (12,19,20) with some slight modifications. Briefly, insulin secretion during the hyperglycemic clamp is the sum of three components: *1*) basal (postabsorptive) insulin secretion rate; *2*) insulin secretion in response to the rate of increase in plasma glucose ("dynamic" secretion component [12,19–20]), known as first-phase insulin secretion; *3*) insulin secretion in response to the actual glucose levels above the postabsorptive glucose concentration ("static" or "proportional" secretion component [12,19–20]), known as second-phase insulin secretion.

A complete description of the modeling strategy can be found in the APPENDIX. Parameters were estimated by implementing this minimal model of C-peptide secretion in the SAAM-II 1.1.2 software (SAAM Institute, Seattle, WA). Numerical values of the unknown parameters were estimated by using nonlinear least squares. Weights were chosen optimally, i.e., equal to the inverse of the variance of the measurement errors, which were assumed to be additive, uncorrelated, with zero mean, and a constant coefficient of variation (CV) of 13%.

The main outputs of this model are:

- Glucose sensitivity of first-phase secretion (dynamic secretion component): expressed as the amount of insulin secreted in response to an increase rate of 1 mmol/l in glucose concentration between time 0′ and 1′ of the study (units: $pmol \cdot m^{-2} \cdot [mmol \cdot l^{-1} \cdot min^{-1}]^{-1}$). The CVs of glucose sensitivity of first-phase secretion were 8.8 ± 1.1, 10.3 ± 1.2, and 18.5 ± 4.0% in adolescents with NGT, IGT, and type 2 diabetes, respectively.
- Glucose sensitivity of second-phase secretion (static secretion component): expressed as the steady-state insulin secretion rate in response to a step increase in glucose concentration of 1 mmol/l (units: $pmol \cdot min^{-1} \cdot m^{-2}$ per mmol/l). The CVs of glucose sensitivity of second-phase secretion were 9.6 ± 1.4, 8.9 ± 0.8, and 7.9 ± 1.3% in adolescents with NGT, IGT, and type 2 diabetes, respectively.

Analysis of proinsulin secretion during the hyperglycemic clamp. Proinsulin and insulin are present together within secretory granules of the β-cell, and their concentration ratio provides an estimate of proinsulin processing. Both have distinct patterns of elimination, thus the ratio of proinsulin to insulin in plasma provides a reliable surrogate of the ratio within the granules of the β-cell only following acute stimulation of insulin secretion, when plasma concentration are least affected by differences in kinetics of elimination. The proinsulin-to-insulin ratio was thus calculated at the trough following the acute stimulation, i.e., as the mean level of 4 and 6 min. As an index of proinsulin secretion during the second phase, we divided the mean proinsulin level during 30–120 min of the study by the total amount of insulin secreted during the second phase (pmol per 120′ per BSA).

Statistical analysis. Baseline clinical data are presented as means ± SD. All data are presented as means ± SEM, except for the weighted residuals of C-peptide, which are presented as means ± SD. All between-group comparisons were run first by MANOVA. When statistical significance was found, for some variables, only two individual contrasts were performed, i.e., IGT vs. NGT and type 2 diabetes vs. IGT; in all other cases, individual comparisons were performed by applying the Bonferroni's correction. Simple correlations were sought by computing Pearson's *r* correlation coefficients. Nonlinear regression analyses were carried out by the sequential quadratic programming method with bootstrap estimates of the standard errors of the parameters. On the basis of the results obtained in the NGT group (see below), this study had a 80% likelihood of detecting a difference between NGT and IGT of 39% in glucose sensitivity of first-phase secretion, of 25% in glucose sensitivity of second-phase secretion, and of 32% in insulin sensitivity at $P < 0.05$. Statistical significance was declared at $P < 0.05$. All statistics were computed with the SPSS 10.1 software.

RESULTS

Anthropometric and fasting biochemical parameters. Sex distribution was comparable among the three groups (Table 1). Subjects with type 2 diabetes were slightly older ($P < 0.02$), but with a similar degree of obesity, as reflected by BMI and percentage of body fat.

Fasting glucose levels were slightly higher in subjects with type 2 diabetes ($P = 0.07$) (Table 2). Fasting insulin and C-peptide concentrations did not differ significantly among the groups. Proinsulin and the proinsulin-to-insulin ratio at fasting were comparable among the groups, as well as triglyceride and free fatty acid levels. HbA_{1c} was significantly higher in subjects with type 2 diabetes.

Pancreatic hormones and insulin sensitivity. Plasma glucose levels were raised to a hyperglycemic plateau of

TABLE 1
Demographic and anthropometric features of the study subjects

	NGT	IGT	Type 2 diabetes	*P* value
n (M/F)	30 (12/18)	22 (10/12)	10 (4/6)	0.92
Ethnicity (AA/C/H)	6/8/16	7/9/6	5/3/2	0.144
Age (years)	13.8 ± 0.5	13.1 ± 0.6	15.0 ± 0.8	<0.05
Height (cm)	160 ± 2	159 ± 2	166 ± 4	0.25
Weight (kg)	99.6 ± 4	95 ± 6	106 ± 6	0.47
BMI (kg/m^2)	38.0 ± 1.1	37.2 ± 1.6	38.3 ± 1.3	0.78
Percent body fat	41.3 ± 1.1	41.3 ± 1.4	38.7 ± 1.9	0.27

Data are means ± SE. AA, African American; C, Caucasian; H, Hispanic.

11.1 mmol/l in all three groups (Fig. 1). There were some minor, albeit statistically significant, differences in plasma glucose concentrations between type 2 diabetic and nondiabetic subjects at 40 and 50 min. All groups demonstrated a biphasic pattern of insulin/C-peptide secretion, yet of different magnitudes. Plasma C-peptide concentrations mirrored insulin concentrations in the contrasts between type 2 diabetic and nondiabetic adolescents. Mean incremental first-phase insulin concentrations were not different between NGT and IGT subjects (118 ± 69 vs. 94 ± 59 μU/ml, $P = 0.46$) yet were lower in subjects with type 2 diabetes (48 ± 34 μU/ml) compared with subjects with NGT ($P = 0.013$). Mean incremental first-phase C-peptide concentrations were greater in NGT compared with IGT subjects (1,404 ± 665 vs. 960 ± 435 pmol/l, $P = 0.015$) and versus subjects with type 2 diabetes (590 ± 377 pmol/l, $P = 0.001$).

Mean second-phase insulin concentration was comparable among the participants, (262 ± 142, 312 ± 284, and 129 ± 73 μU/ml for NGT, IGT, and type 2 diabetes, respectively), while mean second-phase C-peptide concentrations were greater in subjects with NGT compared with subjects with type 2 diabetes (4,174 ± 1,079 vs. 2,709 ± 1,190 pmol/l, $P = 0.007$) but not different than subjects with IGT (4,145 ± 1,357 pmol/l, $P = 0.99$).

Insulin sensitivity was not statistically different among the three groups ($P = 0.17$), although there was a trend ($P < 0.06$) for lower values in the IGT subjects (0.094 ± 0.014) than in NGT (0.119 ± 0.013) and in type 2 diabetic (0.143 ± 0.024) subjects.

Model-derived parameters. The model fit to the C-peptide data were fairly good in all three groups, as shown by the weighted residuals (Table 3). Glucose sensitivity of first-phase insulin secretion was lower in IGT than in NGT adolescents ($P < 0.01$) and even lower in type 2 diabetic than in IGT subjects ($P < 0.01$) (Fig. 2). Glucose sensitivity of second-phase insulin secretion was similar in NGT and in IGT participants but lower in type 2 diabetic patients than in either nondiabetic group ($P = 0.004$) (Fig. 2).

Glucose sensitivities of first- and second-phase insulin secretion were positively correlated to each other in the pooled dataset ($P = 0.002$) (Table 4). Glucose sensitivity of first-phase insulin secretion was not correlated with insulin sensitivity but was negatively correlated to both fasting (Table 4) and 2-h ($r = -0.39$, $P < 0.01$) plasma glucose.

There was no correlation between fasting plasma glucose and glucose sensitivity of second-phase insulin secretion in the pooled dataset (Table 4). However, in the subjects with NGT, there was a positive correlation between these two parameters ($r = 0.42$; $P = 0.02$). Glucose sensitivity of second-phase insulin secretion was negatively correlated with insulin sensitivity in the pooled dataset ($P < 0.0001$) (Table 4). This negative correlation was statistically significant in the adolescents with NGT ($r = -0.42$, $P < 0.03$) and in those with IGT ($r = -0.63$, $P = 0.002$) (Fig. 3). In the children with type 2 diabetes ($n = 10$), the correlation between insulin sensitivity and glucose sensitivity of second-phase insulin secretion ($r = -0.52$) was not significant. Glucose sensitivity of first- and second-phase secretion correlated with the OGTT-derived insulinogenic index ($r = 0.31$, $P = 0.02$ and $r = 0.38$, $P = 0.005$, respectively).

In the pooled dataset, a power function was the best descriptor of the inverse relationship between insulin sensitivity and glucose sensitivity of second-phase secretion ($P < 0.01$), with a similar curve for the adolescents with either NGT or IGT and a separate curve, significantly ($P < 0.05$) shifted to the left, for the adolescents with type 2 diabetes (Fig. 4). Thus, for any degree of insulin sensitivity, glucose sensitivity of second-phase insulin secretion was significantly less in type 2 diabetic than in either IGT or NGT adolescents (Fig. 4). The exponent (−0.367) of this

TABLE 2
Fasting biochemical values

	NGT	IGT	Type 2 diabetes	*P* value
Glucose (mmol/l)	5.27 ± 0.09	5.32 ± 0.09	5.71 ± 0.21	0.07
Insulin (mU/l)	33.2 ± 2.4	40.0 ± 3.5	36.8 ± 3.6	0.36
C-peptide (nmol/l)	1.12 ± 0.07	1.33 ± 0.08	1.11 ± 0.02	0.23
HbA_{1c} (%)	5.2 ± 0.3	5.4 ± 0.4	7.1 ± 1.6*	*P* <0.001
Proinsulin (pmol/l)	26 ± 2	34 ± 5	33 ± 7	0.22
Proinsulin-to-insulin ratio	0.80 ± 0.31	0.78 ± 0.30	1.07 ± 0.64	0.17
Triglicerides (mg/dl)	98 ± 8	119 ± 13	128 ± 27	0.25
Free fatty acids	545 ± 222	528 ± 125	480 ± 111	0.62

Data are means ± SE.

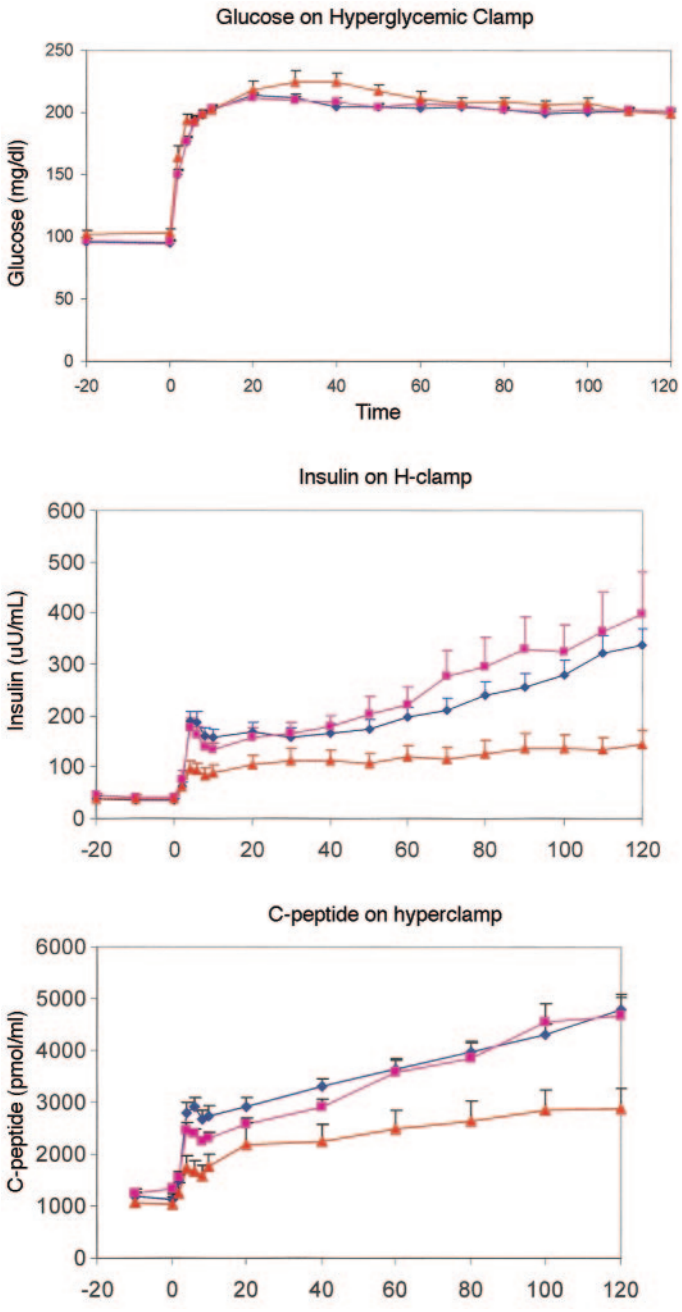

FIG. 1. Glucose, insulin, and C-peptide during the hyperglycemic clamp. ♦, NGT; ■, IGT; ▲, type 2 diabetes (T2DM). *A*: *$P < 0.05$ and **$P < 0.01$ for type 2 diabetes vs. IGT. *B*: †$P = 0.01$ for 4, 6, and 8 min; *$P < 0.05$ and **$P < 0.01$ for diabetes vs. IGT. *C*: †$P < 0.05$ for IGT vs. NGT for 6 and 8 min and $P < 0.01$ for type 2 diabetes vs. IGT for 4 and 6 min; *$P < 0.05$ and **$P < 0.01$ for type 2 diabetes vs. IGT.

power function was significantly different from −1 (95% CI −0.50 to 0.10).

Proinsulin responses. Proinsulin levels were similar between NGT and IGT subjects, with diabetic subjects tending to lower their levels along the study (Fig. 5). In contrast, the proinsulin-to-insulin ratio during the study, at the trough of the first phase (4–6 min) and at the last 30 min of the clamp was significantly higher in diabetic subjects compared with both other groups, while it was similar in NGT and IGT subjects. The proinsulin-to-insulin ratio during the second phase was 0.15 ± 0.01 in NGT, 0.15 ± 0.01 in IGT, and 0.24 ± 0.04 pmol · l^{-1} · $pmol^{-1}$ per 120′ × BSA in diabetic subjects ($P = 0.004$ vs. IGT or NGT). The proinsulin-to-insulin ratio negatively correlated with first-phase sensitivity of the β-cell ($r = -0.57$, $P < 0.001$).

TABLE 3
Weighted residuals (i.e., difference between measured and model predicted C-peptide concentration divided by the SD of C-peptide measurement) of model fit to plasma C-peptide experimental data of the hyperglycemic clamps performed in NGT, IGT, and type 2 diabetic adolescents

	C-peptide weighted residuals		
Time	NGT ($n = 30$)	IGT ($n = 22$)	DM2 ($n = 10$)
2	−0.34 ± 0.36	−0.06 ± 0.44	−0.08 ± 0.11
4	0.41 ± 0.38	0.31 ± 0.38	0.28 ± 0.58
6	0.18 ± 0.47	−0.04 ± 0.34	0.14 ± 0.55
8	−0.37 ± 0.52	−0.41 ± 0.33	−0.45 ± 0.34
10	−0.16 ± 0.63	−0.07 ± 0.39	0.10 ± 0.57
20	0.50 ± 0.61	0.58 ± 0.61	0.32 ± 0.37
40	0.15 ± 0.75	−0.39 ± 0.75	−0.18 ± 0.65
60	−0.30 ± 0.59	−0.05 ± 0.66	−0.21 ± 0.50
80	−0.20 ± 0.70	0.04 ± 0.66	−0.10 ± 0.83
100	−0.02 ± 0.65	0.19 ± 0.57	0.34 ± 0.42
120	0.58 ± 0.80	0.19 ± 0.65	0.23 ± 0.77

Data are means ± SD. The weighted residuals are a quantitative assessment of the goodness-of-fit of the models to the data: a theorically perfect fit should generate weighted residuals with mean of 0 and SD of 1.

DISCUSSION

In this study, we performed a relatively large number of hyperglycemic clamps in order to study the role of β-cell function in the alterations of glucose regulation in the obese adolescent. State-of-the-art modeling of insulin secretion was applied to the hyperglycemic clamp studies (12,18). The importance of using C-peptide for assessment of insulin secretion is illustrated by the statistically significant differences in plasma C-peptide levels between NGT and IGT adolescents at early time points of the clamp, which were not mirrored by plasma insulin concentrations.

First-phase insulin secretion. Our data demonstrate that in the passage from NGT to IGT to type 2 diabetes, the obese adolescent experiences a fall in first-phase insulin secretion. For each change in glucose tolerance, the decrement of first-phase sensitivity to glucose was approximately the same (~1,500 pmol/m · (mmol · l^{-1} · min^{-1})$^{-1}$, or one-third of the average value in the obese adolescent with NGT. Since there were no significant differences in insulin sensitivity among the three groups, the best correlate of glucose tolerance class was first-phase insulin secretion, in close agreement with a wealth of data obtained in adults (12). This does not mean that insulin resistance is not important in the pathophysiology of hyperglycemia. Rather, in the complex interplay between the body tissue demands for insulin and the capability of β-cells to cope with them, cross-sectional studies of groups homogeneous for insulin sensitivity are bound to highlight the fact that β-cell performance declines with raising levels of glucose. The truly relevant findings of studies such as ours are not whether β-cell function is impaired when glycemia rises, but at which stage of glucose regulation and which facets of β-cell function fail.

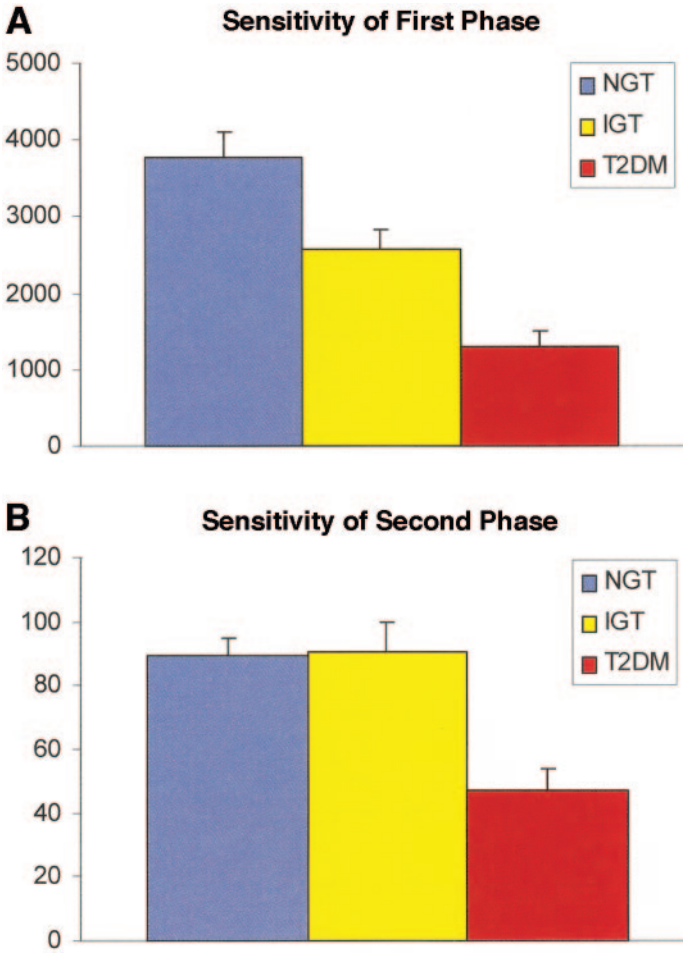

FIG. 2. Glucose sensitivity of the first- and second-phase insulin response in NGT, IGT, and diabetic subjects. *A*: Glucose sensitivity of β-cell first-phase secretion in adolescents with NGT (blue), IGT (yellow), and type 2 diabetes (T2DM) (red). *B*: Glucose sensitivity of β-cell second-phase secretion in adolescents with NGT (blue), IGT (yellow), and type 2 diabetes (red). $P < 0.01$ for IGT vs. NGT; $P < 0.02$ for type 2 diabetes vs. IGT.

The seemingly comparable insulin sensitivity among the groups is at variance with our previous study, where we clearly found greater insulin resistance in obese adolescents with IGT compared with obese adolescents with NGT (8). Insulin sensitivity measured using the hyperglycemic clamp is the result of insulin action on peripheral tissues and of mass action of glucose or glucose effectiveness. The latter may be totally preserved, making it difficult to bring out defects in insulin sensitivity in groups of insulin-resistant subjects using this technique. Furthermore, the spectrum of insulin sensitivity values in obese children and adolescents with NGT is very broad and overlaps with the distribution of insulin sensitivity in youngsters with IGT (29). The cross-sectional nature of the study prevents us from indicating the time course of development of insulin resistance and secretion defects. There may be inherent genetic factors unique for subjects prone to develop altered glucose metabolism that emerge in the context of severe obesity-related insulin resistance.

Importantly, the profile of plasma insulin concentration showed a much milder decline, which was not statistically significant, implying a change in insulin clearance that attempts to compensate for the decline in β-cell secretion. Indeed, this phenomenon has been described in dogs fed a high-lipid diet (15) and in first-degree relatives of patients with type 2 diabetes after a 4-day intravenous lipid infusion (30). Thus, in children insulin clearance may undergo adaptive changes to maintain glucose homeostasis and should be taken into account when insulin concentration is used to assess β-cell secretory function.

The relevance of the decline of first-phase insulin secretion to glucose regulation in obese children, albeit being a rather common finding, is still not entirely understood. In adults, acute loss of first-phase insulin secretion, achieved experimentally by somatostatin infusion, blunts and delays the inhibition of endogenous glucose production (31). In several studies, insulin levels at early time points of a meal, which do not necessarily reflect first-phase insulin secretion, are strongly related to glucose tolerance, assessed as glucose concentration at 120 min (32,33). Using OGTTs in obese youth and the insulinogenic index as a surrogate for the acute insulin response, we showed that increased 2-h plasma glucose levels within the range of NGT are associated with a specific impairment of β-cell responsiveness distinct from the deterioration of insulin sensitivity (34). In Pima Indians, individuals who progress from NGT to type 2 diabetes display a prominent and progressive fall in the acute insulin response during an intravenous glucose tolerance test (35). More recently, it has been shown that the acute insulin response to intravenous glucose is strongly related to (presumed) β-cell mass in patients undergoing islet transplantation (36). Evidence has been provided that a loss of β-cell mass characterizes type 2 diabetes, presumably as a result of accelerated apoptosis (37). Thus, the progressive decrease in first-phase insulin secretion as glucose regulation worsens, seen in the present study, may be due in part and be proportional to a decline in β-cell mass in obese adolescents, the latter representing a critical pathogenetic step in the onset of altered glucose metabolism. In such a case,

TABLE 4
Correlation matrix in the whole dataset ($n = 62$)

	Fasting glucose	Fasting insulin	Fasting C-peptide	S_i	First-phase glucose sensitivity
Fasting glucose	1				
Fasting insulin	0.32*	1			
Fasting C-peptide	0.28†	0.70‡	1		
S_i	−0.14	−0.67‡	−0.59‡	1	
First-phase glucose sensitivity	−0.31†	0.04	0.04	−0.19	1
Second-phase glucose sensitivity	0.07	0.30†	0.33*	−0.53‡	0.39*

*$P < 0.01$; †$P < 0.05$; ‡$P < 0.001$.

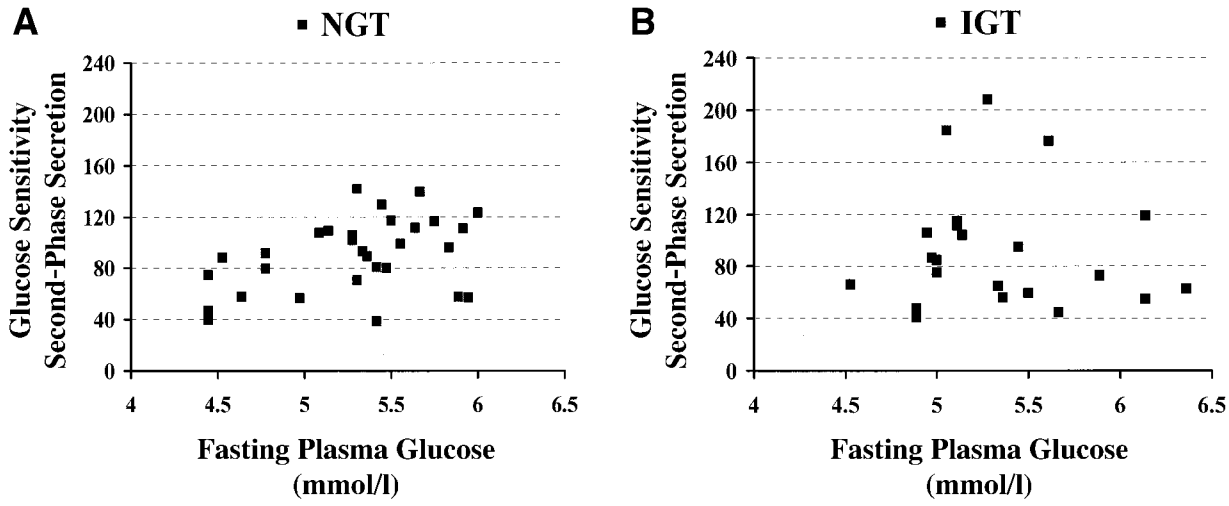

FIG. 3. Relation of fasting plasma glucose and the β-cell glucose sensitivity of second-phase insulin secretion in subjects with NGT (*A*) and IGT (*B*). Scatterplot of glucose sensitivity of β-cell second-phase secretion (*y* axis) vs. fasting plasma glucose in adolescents with NGT (*A*), IGT (*B*).

first-phase insulin secretion may represent an important target and a key index to monitor the effects of therapy.

Second-phase insulin secretion. Our results revealed that, in close agreement with the adult data (12), an inverse curvilinear relationship exists between insulin sensitivity, as assessed by the hyperglycemic clamp, and glucose sensitivity of second-phase secretion in the obese adolescent. This relationship is best described by a power function whose exponent is significantly different from −1. Of note, insulin sensitivity, assessed by the hyperglycemic clamp, reflects peripheral insulin sensitivity because under these experimental conditions endogenous glucose production is neglible (38). Thus, in childhood, the β-cell burden to compensate for insulin resistance grows disproportionately larger when insulin resistance worsens. Other implications of the inverse curvilinear relation between insulin sensitivity and insulin secretion have been discussed by Kahn et al. (39) and by Ferrannini et al. (40).

The relationship between second-phase insulin secretion and insulin sensitivity is not grossly altered in the adolescent with IGT but is disrupted in type 2 diabetes. In contrast, in adults, most data show that the second phase of insulin secretion is also altered in IGT, even when prevailing insulin sensitivity/resistance is taken into account. It is possible, but still unproven, that our finding is age specific and reflects peculiarities of the pathophysiology of IGT related to adolescence.

Since second-phase secretion is still normal in IGT, the defect of the second phase seen in the adolescents with type 2 diabetes is an impairment specific to this age range. This does not mean that the defect of the first phase is less influential than the defect of the second phase in causing hyperglycemia, yet simply recognizes that the decline of

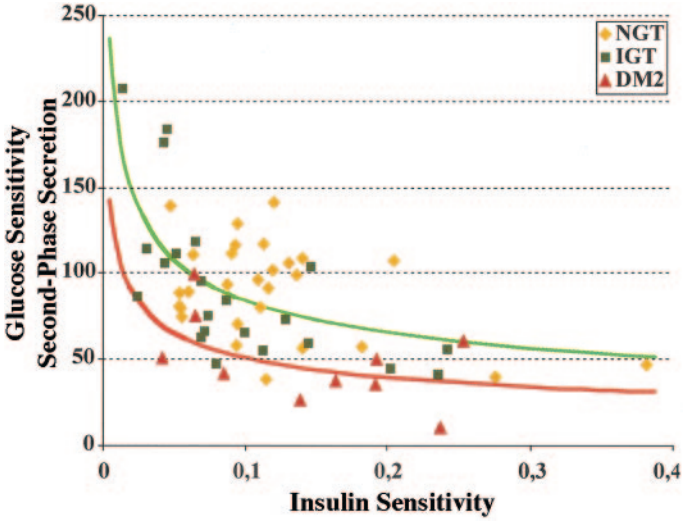

FIG. 4. Relation of insulin sensitivity and the β-cell glucose sensitivity of second-phase secretion. Scatterplot of glucose sensitivity of β-cell second-phase secretion ($\sigma 2^{nd}$; *y* axis) vs. insulin sensitivity (S_i; *x* axis) in adolescents with NGT (♦), IGT (■), and type 2 diabetes (T2DM) (▲). The two curves are generated by the following equation found by nonlinear regression analysis, as described in RESEARCH DESIGN AND METHODS: $\sigma 2^{nd} = A \times (1 + ds \times C) \times IS^B$, where ds = 0 in NGT and IGT subjects and ds = 1 in type 2 diabetes subjects, IS = insulin sensitivity, A = 36.7 (95% CI 26.3–66.1), B = −0.37 (−0.49 to −0.10), and C = −0.40 (−0.57 to −0.25).

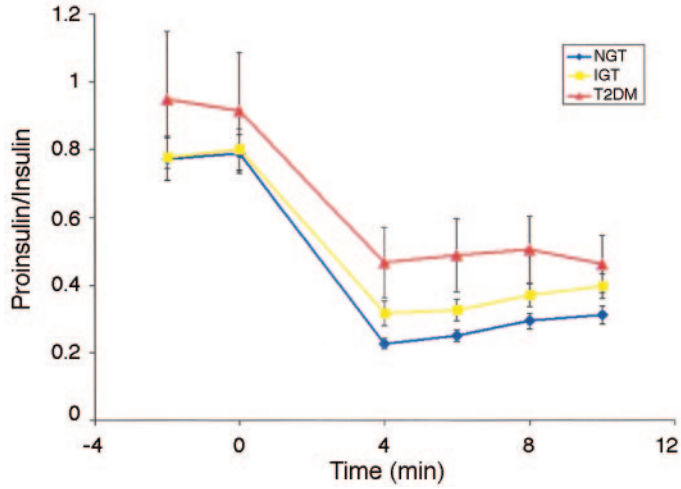

FIG. 5. The proinsulin-to-insulin ratio during acute-phase insulin secretion. Proinsulin-to-insulin ratio during the hyperglycemic clamp. $P < 0.001$ and $P = 0.01$ for 4 min, $P = 0.001$ and $P = 0.04$ for 6 min for type 2 diabetes (red) vs. NGT (blue) and IGT (yellow).

the first phase accompanies the increase in ambient glucose levels across the whole range of glycemia, whereas the defect in the second phase is required for the development of type 2 diabetes. Importantly, the relationship between second-phase secretion and insulin sensitivity, although still detectable in the children with diabetes, is clearly shifted to the left. Thus, we speculate that loss of the capability of β-cell second-phase secretion to compensate for insulin resistance is a pathophysiological fingerprint of type 2 diabetes in childhood. In a nutshell, the defect of the first phase is the most sensitive index of both nondiabetic and diabetic hyperglycemia, but the disruption of the second phase is a specific hallmark of type 2 diabetes.

The positive correlation between fasting plasma glucose and glucose sensitivity of second-phase insulin secretion observed in the NGT subjects is reminiscent of the positive effect that chronic mild hyperglycemia may exert on β-cell function in humans (41). Since this relationship was not present in the children with IGT, it may be a clue for the presence of a subtle defect of second-phase insulin secretion in IGT. However, in our opinion, this observation has only a hypothesis-generating value and it needs to be replicated in other datasets.

Proinsulin processing. In the present study, we found an increased proinsulin-to-insulin ratio after acute stimulation of insulin secretion only in the presence of overt diabetes. In contrast, in the obese adolescents with IGT these ratios were not increased. These findings are at variance with some data reported in adults with IGT (42) yet in agreement with others (23). It is conceivable that in the adolescent, unlike in the adult, increased proinsulin-to-insulin ratios are dependent on more prolonged hyperglycemia, and thus defects in proinsulin processing do not precede the onset of overt diabetes. Another factor may be due to the young age of our subjects, as it has been suggested that the relative proinsulin content increases with age (43) and that deterioration of proinsulin processing is age dependent.

In summary, when compared with obese adolescents with similar insulin resistance, those with IGT and those with type 2 diabetes display a progressive loss of glucose sensitivity of β-cell first-phase secretion. In contrast, the homeostatic loop between insulin sensitivity and glucose sensitivity of β-cell second-phase secretion is apparently preserved in IGT but disrupted in type 2 diabetes. This is accompanied by alterations in the proinsulin-to-insulin processing, as reflected by an increase in the proinsulin-to-insulin secretion ratio. Thus, IGT is characterized by an alteration of first-phase secretion, and several β-cell defects concur to cause diabetes in childhood. The exact quantitative relevance for glucose homeostasis, pathophysiologic primacy, time sequence, and molecular basis of these abnormalities remains to be determined.

APPENDIX

The analysis of the glucose and C-peptide curves during the hyperglycemic clamps follows the general strategy used by Toffolo et al. (18) and by Mari et al. (19) with some modifications. Both these research groups have introduced the concept that the so-called first-phase insulin secretion can be modeled as a response of the β-cell to the rate of increase in glucose concentration, when the latter is >0. To this we added a time-dependent fading of this form of stimulus on insulin secretion, which was described by Toschi et al. in humans (44). It has been known for a long time that the so-called second-phase insulin secretion can be described as a β-cell response to glucose concentration above a glucose threshold (14,45). Herein are the equations describing our model of glucose induced insulin secretion during a hyperglycmeic clamp:

$$dcp_1(t)/dt = ISR(t) + cp_{two} \times k_{12} - (k_{01} + k_{21}) \times cp_1$$

where ISR = insulin secretion rate, cp_1 = C-peptide mass in the accessible compartment, cp_2 = C-peptide mass in the remote compartment, k_{12} and k_{21} = rate constants between the two C-peptide compartments, and k_{01} = rate constant of the irreversible loss of C-peptide from the accessible compartment.

$$ISR(t) = BSR + SR1^{st}(t) + SR2^{nd}(t)$$

where BSR = basal insulin secretion rate, $SR1^{st}$ = first-phase insulin secretion rate, and $SR2^{nd}$ = second-phase insulin secretion rate.

$$BSR = CP_b \times V_1 \times k_{01}$$

where CP_b is basal (pretest) C-peptide concentration and V_1 is the volume of the accessible compartment of C-peptide.

$$SR1^{st(t) = X1^{st}}(t) \times \tau^{-1}$$

$$dX1^{st}(t)/dt = \sigma 1^{st} \times \{[dG(t)/dt]/[\log(1.1 + t)]\} - X1^{st}(t) \times t^{-1} \text{ if } dG(t)/dt > 0$$

$$dX1^{st}(t)/dt = -X1^{st}(t) \times t^{-1} \text{ if } dG(t)/dt \leq 0$$

where $\sigma 1^{st}$ = glucose sensitivity of first-phase insulin secretion, G(t) = plasma glucose concentration, $X1^{st}(t)$ = C-peptide mass made available for first-phase insulin secretion, τ = time constant of first-phase insulin secretion, and the term $\log(1.1 + t)$ accomodates the time-associated decline of σ1st documented in humans during a hyperglycemic stimulus. Finally, the response $SR1^{st}(t)$ to the rate of increase of glucose is detected at the sampling site after a pure time delay (distinct from τ), which is another unknown parameter estimated by the model.

$$SR2^{nd}(t) = X2^{nd}(t) \times \delta^{-1}$$

$$dX2^{nd}(t)/dt = \sigma 2^{nd} \times [G(t) - \theta] - X2^{nd}(t) \times \tau^{-1}$$

where $\sigma 2^{nd}$ = glucose sensitivity of second-phase insulin secretion, $X2^{nd}(t)$ = C-peptide mass made available for first-phase insulin secretion, θ = glucose threshold above which β-cell responds with second-phase insulin secretion to plasma glucose concentration, and δ = time constant of second-phase insulin secretion.

Please note that in previous reports (12,45), $\sigma 2^{nd}$ was presented as β. The reasons to change the abbreviation are to mark the use of a somewhat different model and to highlight the conceptual similarity between $\sigma 1^{st}$ and $\sigma 2^{nd}$. Both of them are "glucose sensitivities" of the β-cell to the rate of increase in glucose concentration and to glucose concentration itself, respectively. C-peptide kinetic parameters were computed according to the equations by Van Cauter et al. (17). CP_b and θ were assumed to be equal to

pretest C-peptide and glucose concentration, respectively. This model was implemented in the SAAM 1.2 software. The unknown parameters estimated by the model were: $\sigma 1^{st}$ = glucose sensitivity of first-phase insulin secretion, τ = time constant of first-phase insulin secretion, $\sigma 2^{nd}$ = glucose sensitivity of second-phase insulin secretion, and δ = time constant of second-phase insulin secretion.

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