Primary Defects in β-Cell Function Further Exacerbated by Worsening of Insulin Resistance Mark the Development of Impaired Glucose Tolerance in Obese Adolescents

ANNA M.G. CALI, MD[1]
CHIARA DALLA MAN, PHD[2]
CLAUDIO COBELLI, PHD[2]
JAMES DZIURA, PHD[3]
AISHA SEYAL, MPH[1]
MELISSA SHAW[1]
KARIN ALLEN, RN[3]
SHU CHEN, PHD[3]
SONIA CAPRIO, MD[1]

OBJECTIVE — Impaired glucose tolerance (IGT) is a pre-diabetic state of increasing prevalence among obese adolescents. The purpose of this study was to determine the natural history of progression from normal glucose tolerance (NGT) to IGT in obese adolescents.

RESEARCH DESIGN AND METHODS — We determined the evolution of β-cell function, insulin sensitivity (S_I), and glucose tolerance in a multiethnic group of 60 obese adolescents over the course of approximately 30 months. Each subject underwent three serial 3-h oral glucose tolerance tests. Dynamic, static, and total β-cell responsivity (Φ_d, Φ_s, and Φ_{tot}, respectively) and S_i were assessed by oral C-peptide and glucose minimal models. The disposition index (DI), which adjusts insulin secretion for S_i, was calculated.

RESULTS — At baseline, all 60 subjects had NGT. Seventy-seven percent (46 subjects) maintained NGT over the three testing periods (nonprogressors), whereas 23% (14 subjects) developed IGT over time (progressors). At baseline, percent fat and BMI *Z* score were comparable between the groups. Fasting plasma glucose, 2-h glucose, glucose area under the curve at 180 min, and Φ_d were significantly different between the two groups at baseline, whereas S_i was comparable between the two groups. Over time, although S_i remained unchanged in nonprogressors, it steadily worsened by ~45% ($P > 0.04$) in progressors. β-Cell responsivity decreased by 20% in progressors, whereas it remained stable in nonprogressors. The DI showed a progressive decline in progressors compared with a modest improvement in nonprogressors ($P = 0.02$).

CONCLUSIONS — Obese adolescents who progress to IGT may manifest primary defects in β-cell function. In addition, progressive decline in S_i further aggravates β-cell function, contributing to the worsening of glucose intolerance.

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Understanding the underlying putative metabolic defects leading to the development of type 2 diabetes requires studies that focus on the earliest stages of the disease before the onset of any alterations in glucose tolerance. In adults, type 2 diabetes is the final stage in the progression of the disease (1–3), characterized by a progressive worsening in both insulin resistance and secretion (4–7). Whether a similar profile also occurs in youth developing type 2 diabetes is unknown. Much of the understanding of type 2 diabetes in youth originates from cross-sectional studies performed in obese adolescents with overt disease (8) or with impaired glucose tolerance (IGT) (9,10). One longitudinal study in obese adolescents with IGT at baseline indicated that over a period of 23 months, 45% reverted to normal glucose tolerance (NGT), 30% maintained IGT, and 25% developed type 2 diabetes(11). Thus, youth with IGT are at high risk for developing type 2 diabetes because of the presence of both insulin resistance and β-cell dysfunction.

To assess the metabolic sequence of events that might be implicated in the transition from NGT to IGT, we performed serial oral glucose tolerance tests (OGTTs) along with anthropometric measures in a group of obese adolescents over a period of approximately 3 years. Using the oral minimal model (OMM) (12,13), we determined β-cell responsivity (Φ), insulin sensitivity (S_i), and disposition index (DI) and thus have repeated measures of both insulin secretion and insulin action before and during the evolution of IGT in obese adolescents. In a longitudinal study, we tested the hypothesis that preexisting β-cell dysfunction, further exacerbated by a progressive worsening in S_i, characterizes the onset of IGT in childhood obesity.

RESEARCH DESIGN AND METHODS

— The Yale Pathophysiology of Type 2 Diabetes in Obese Youth Study is a long-term project aimed at examining early alterations in glucose metabolism in obese children and adolescents (14,15). The study protocol was approved by the Human Investigations Committee of the Yale School of Medicine. Written parental consent and child assent were obtained before the study. The subjects were recruited from our Pediatric Obesity Clinic. To be eligible for the study, subjects had to be obese

From the [1]Department of Pediatrics, Yale School of Medicine, New Haven, Connecticut; the [2]Department of Information Engineering, University of Padua, Padua, Italy; and the [3]Yale Center for Clinical Investigation, Yale School of Medicine, New Haven, Connecticut.
Corresponding author: Sonia Caprio, sonia.caprio@yale.edu.
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(>95th percentile for age and sex) and were excluded from this analysis if they were using medications that may affect glucose metabolism. Participants were followed biannually as outpatients by the clinical staff and received only general standard nutritional guidance and recommendations for physical activity. No apparent differences in adherence to these recommendations emerged between subjects. The subjects included in this analysis were chosen based on having three repeated OGTTs. In our longitudinal follow-up study, the OGTT was initially repeated on an annual basis. In the subsequent years, however, due to budgetary cuts the protocol was modified and the OGTT was repeated every 18 to 24 months. This time interval is based on our previous study suggesting that changes in categories of glucose tolerance in obese adolescents are likely to occur over a relatively short period of time (~23 months) (11).

For this report, we analyzed data from 60 obese adolescents (21 male and 39 female) from whom we currently have three serial OGTTs and whose potential changes in glucose tolerance over an average of ~30 months we were thus able to longitudinally assess. All 60 subjects had NGT at baseline. Of these, remarkably, 46 maintained the same status during the second and third OGTTs (nonprogressors). In contrast, 14 progressed to IGT at the third OGTT, whereas at the second OGTT only 4 had already progressed to IGT. The other 10 were still of NGT, albeit at much higher levels of glucose than at the first OGTT. None of these subjects had progressed to IGT and converted to NGT, at least in this particular study.

OGTT

All subjects were invited to the Yale Center for Clinical Investigation for an OGTT at 8 A.M. following an overnight fast, as previously reported (9). Baseline blood samples were obtained from subjects with the use of an indwelling venous line for measurement of levels of glucose, insulin, C-peptide, lipid profile, free fatty acids, adiponectin, interleukin-6, and leptin. An OGTT was then performed with the administration of glucose at 1.75 g/kg body wt (maximum dose 75 g); blood samples were obtained at 0 min and every 30 min thereafter for 180 min for the measurement of plasma glucose, insulin, and C-peptide.

Anthropometric measurements

Total body composition was performed using a Tanita Scale (Bioimpedance) each time the subject came for the repeated OGTT. Body weight was measured with a digital scale to the nearest 0.1 kg, and height was measured in triplicate with a wall-mounted stadiometer at each visit.

Assessment of S_i: oral glucose minimal model

S_i was estimated from plasma glucose and insulin concentrations measured during the 3-h OGTT using the oral glucose minimal model (12,13). S_i measures the overall effect of insulin on stimulating glucose disposal and inhibiting glucose production. This index has been validated against the euglycemic clamp, showing a correlation of 0.81 ($P = 0.001$) (16).

Assessment of β-cell function: oral C-peptide minimal model

β-Cell responsivity indexes were estimated from plasma glucose and C-peptide concentrations measured during the OGTT by using the oral C-peptide minimal model (13,17,18). Reproducibility of β-cell responsivity (dynamic [Φ_d] and static [Φ_s]) and S_I from the OMM is between 20 and 30% (18), which is very similar to that of the intravenous glucose tolerance test. The model assumes that insulin secretion is made up of two components. A dynamic component is likely to represent secretion of promptly releasable insulin and is proportional to the rate of glucose concentration through a parameter, Φ_d, that defines the dynamic responsivity index. The static component derives from provision of new insulin to the releasable pool and is proportional to delayed glucose through parameter Φ_s. From Φ_d and Φ_s, one can also calculate a single overall β-cell responsivity index. Finally, the DI is obtained by taking the product of Φ and S_I ($DI = \Phi \times S_I$) (18).

Measurements of C-peptide and glucose levels at 10 and 20 min of the OGTT are critical for the modeling of β-cell function (18). In the present study, however, these early times were missing for some of the subjects, so we used all nine points obtained during the 180 min of the OGTT. To test whether there is any difference between indexes calculated with and without the early times (10 and 20 min), we examined a subset of 188 subjects from our cohort who actually underwent a nine-sample 180-min OGTT (including samples at 10 and 20 min). This allowed us to compare results obtained from a seven-sample 180-min OGTT (without minutes 10 and 20) with results obtained from a nine-sample 180-min OGTT. We found that reliable estimates of both S_i ($r = 0.93$, $P < 0.000$) and, after appropriate smoothing of glucose data, total β-cell responsivity (Φ_{tot}) ($r = 0.99$, $P < 000$) can be obtained from seven-sample experiments, whereas Φ_s ($r = 0.93$; $P < 000$) and Φ_d ($r = 0.75$; P 0.001) (albeit well correlated) were 8% underestimated and 30% overestimated, respectively.

Statistical analyses

Demographic and anthropometric characteristics at baseline were compared using Fisher's exact and *t* tests. Variables with positively skewed distributions were log transformed to conform to distributional assumptions required for statistical inference. Linear trajectories from repeated measures of outcomes over time were estimated by random effects regression (19). Each outcome was regressed on fixed factors of time (continuous), group (progressors vs. nonprogressors), age at first visit (continuous), sex, race (Caucasian, African American, or Hispanic), and first-degree family history of type 2 diabetes (yes/no). To evaluate whether rates of changes in outcomes varied by group, an interaction of group by time was included. Between-subject variability in values of outcomes and rates of change at 0 min was permitted by the inclusion of random effects for intercept and time, which were also allowed to covary. Rates of change for log-transformed variables imply a multiplicative model, and exponentiated regression coefficients therefore represent the percent change in the outcome for a 1-month change in time. For ease of interpretation, percent changes over time are expressed for the approximate average time of follow-up, which was 30 months. Models were fit using PROC MIXED in SAS v 9.1 (SAS, Cary, NC). *P* values <0.05 were used as thresholds for significance.

Analytical methods

Plasma glucose levels were measured using the YSI 2700 STAT analyzer (Yellow Springs Instruments), and lipid levels were measured using an autoanalyzer (model 747-200; Roche-Hitachi). Plasma insulin was measured with a radioimmunoassay (RIA) (Linco, St. Charles, MO) that has <1% cross-reactivity with C-peptide and proinsulin. Plasma C-peptide levels were determined with an assay by

Diagnostic Products (Los Angeles, CA). The intra-assay variation was 5.4% for insulin and 11.6% for C-peptide, and the interassay variation was 6.2% for insulin and 8.47% for C-peptide. Plasma adiponectin levels were measured by a double antibody-antibody RIA from Linco by our research laboratory. The intra- and interassay coefficients of variation were 7.1 and 9.5%, respectively. Plasma leptin levels were measured using an RIA from Linco. The intra- and interassay coefficients of variation were 6.5 and 8.0%, respectively.

RESULTS

Differences in anthropometric and metabolic phenotypes at baseline between nonprogressors and progressors

From the original cohort, 46 subjects (77%) were found to have kept their NGT status at each of the three serial OGTTs and thus are classified as nonprogressors. In contrast, 14 subjects (23%) developed IGT by the third OGTT and are classified as progressors. The two groups at baseline had similar age, sex, ethnicity distribution, Tanner stage of development, BMI, and BMI *Z* score (Table 1). Percent total fat tended to be higher in progressors ($P = 0.07$, Table 1). Whereas systolic blood pressure was similar in the two groups, diastolic blood pressure was higher in progressors ($P = 0.049$).

No significant differences were found in the level of proinsulin, adiponectin, interleukin-6, HDL cholesterol, and triglycerides. Leptin was slightly higher in progressors ($P = 0.02$) (Table 2). At baseline, S_i was comparable in the two groups (Table 2); in contrast, Φ_d was significantly lower in progressors than in nonprogressors ($P = 0.04$). No differences were noted for Φ_s and Φ_{tot}. The DI tended to be lower in progressors ($P = 0.14$). A1C was slightly higher in progressors ($P = 0.049$).

Glucose, insulin, and C-peptide responses

Figure A1 (available in an online appendix at http://dx.doi.org/10.2237/dc08-1274) compares the changes in glucose, insulin, and C-peptide responses in the two groups at each of the three OGTTs. The median (interquartile range) time for follow-up between the first and second OGTT was 15.3 months (12.0–18.4) and 16.5 months (14.5–26.3) for the nonprogressors and progressors, respectively, and between the second and third OGTT was 30.0 months (24.7–36.9) and 29.8 months (26.8–42.2), respectively. At baseline (first OGTT), fasting plasma glucose levels ($P = 0.002$) and glucose area under the curve (AUC) ($P < 0.05$) were significantly higher in progressors, whereas fasting insulin and C-peptide, as well as the insulin and C-peptide AUCs, were not significantly different between the two groups. The plasma glucose responses at the second and third OGTTs were significantly greater at all times in progressors than in the nonprogressors. The insulin and C-peptide levels and AUCs were slightly higher at the third OGTT in progressors than in the nonprogressors.

Table 1—*Demographic and anthropometric characteristics of the study participants at baseline*

	Nonprogressors	Progressors	*P*
Sex (%)			0.53
Male	15 (32.6)	6 (42.9)	
Female	31 (67.4)	8 (57.1)	
Race (%)			0.69
Caucasian	18 (39.1)	4 (28.6)	
African American	13 (28.3)	4 (28.6)	
Hispanic	15 (32.6)	6 (42.8)	
Family history of obesity	43 (96)	12 (86)	0.24
Family history of type 2 diabetes	16 (36)	4 (29)	0.75
Tanner stage	2–3	2–3	0.84
Age (years)	12.3 ± 3.1	12.3 ± 3.5	0.97
Height (m)	155.6 ± 14.2	153 ± 18.7	0.64
Weight (kg)	87.4 ± 27.9	85.2 ± 30.8	0.81
BMI (kg/m^2)	35.1 ± 7.3	34.9 ± 6.2	0.91
BMI *Z* score	2.45 ± 0.34	2.53 ± 0.33	0.45
Percent fat	43.9 ± 6.2	48.7 ± 7.5	0.07
Systolic blood pressure (mmHg)	119.8 ± 12.7	123 ± 11.3	0.46
Diastolic blood pressure (mmHg)	67.6 ± 11.0	74.1 ± 9.3	0.045*

Data are *n* (%) or means ± SD. *$P < 0.05$.

Anthropometric changes

Trajectories for BMI, BMI *Z* score, and percent total fat across the follow-up period were analyzed to compare the rates of

Table 2—*Metabolic parameters of the study participants at baseline*

	Nonprogressors	Progressors	*P*
Fasting plasma glucose (mmol/l)	4.92 ± 0.05	5.27 ± 0.11	0.002
2-h glucose (mmol/l)	6.2 ± 0.12	6.5 ± 0.28	0.27
Fasting insulin (pmol/l)	183.0 (130.5–229.5)	195.0 (138.0–229.5)	0.89
Proinsulin (pmol/l)	20 (14.0–32.0)	22 (13.0–25.5)	0.56
A1C (%)	5.3 ± 0.3	5.5 ± 0.3	0.049
S_I	3.8 (1.99–9.05)	4.5 (2.1–4.5)	0.82
β-Cell responsivity (Φ)			
Φ_s	68.9 (52.9–85.5)	62.5 (48.8–89.1)	0.95
Φ_d	2,514.7 (1,488.6–3,618.8)	2,001.6 (1,295.0–2,276.4)	0.04
Φ_{tot}	106.7 (79.1–139.8)	87.9 (77.8–119.8)	0.35
DI	612.5 (406.2–1,348.1)	545.8 (368.7–1,147.7)	0.66
Adipokines			
Leptin (ng/ml)	25 (17.0–41.0)	31 (28.3–43.8)	0.02
Adiponectin (μg/ml)	6.8 (4.0–9.6)	6.9 (5.6–8.8)	0.43
IL-6 (pg/ml)	1.89 (1.1–3.1)	3.1 (1.5–4.5)	0.21
Lipids			
HDL cholesterol (mmol/l)	1.05 (0.83–1.18)	1.03 (0.83–1.22)	0.96
Triglycerides (mmol/l)	1.03 (0.77–1.7)	1.13 (0.80–1.39)	0.76

Data are means ± SD or median (interquartile range).

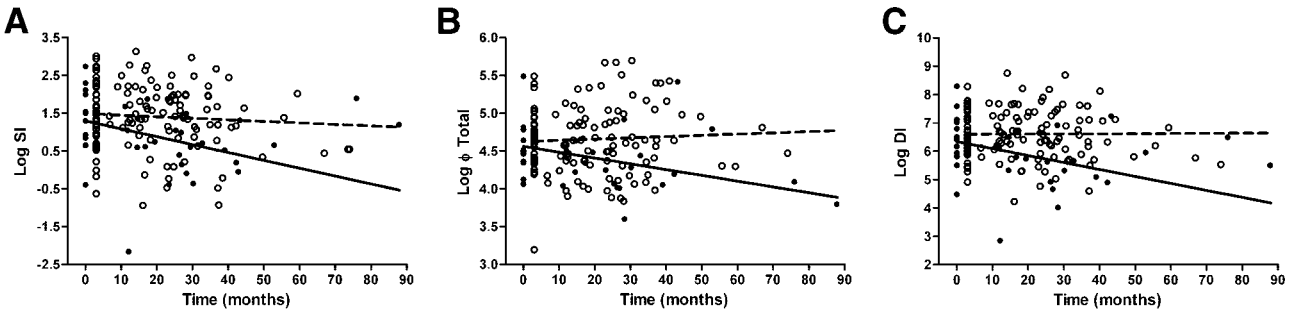

Figure 1— *S_I (A), Φ_{tot} (B), and DI (C) trajectories during the three serial OGTTs in progressors (straight line) and nonprogressors (dashed line).*

change between the nonprogressors and progressors (online appendix Figure A2). When adjusted for age, sex, race, and family history of type 2 diabetes or obesity, BMI increased significantly in both groups at a rate of 0.07 kg/m^2 per month ($P < 0.001$). Of note, the trajectories for BMI, BMI *Z* score, and percent total fat were not different over time between the two groups ($P = 0.30$, 0.77, and 0.14 for BMI, BMI *Z* score, and percent fat, respectively).

Changes in S_I, Φ, and DI

Figure 1 compares the trajectories during the three serial tests for S_I, Φ, and DI. Data were adjusted for age, sex, race, and family history of type 2 diabetes and/or obesity. Further analysis did not reveal sex to be a significant modifier of any of the three trajectories. Nevertheless, such an interaction cannot be ruled out because our sample size was not sufficient to detect such a relation.

S_I trajectories showed marked differences over time between the two groups. Despite similar S_I at baseline (Table 2, $P = 0.62$), in progressors, S_I deteriorated over time at a rate of −0.02 log units per month, which translates to a 45% reduction over 30 months compared with the rather stable S_I over 30 months in the nonprogressors ($P = 0.04$ for rate of change between nonprogressors and progressors). Likewise, β-cell function, measured by Φ, decreased by 0.008 log units per month, or a total of 20% over 30 months in progressors, whereas it remained relatively stable in the nonprogressors (an increase of 5% over 30 months, $P = 0.09$ for rate of change between the nonprogressors and progressors). Of note, Φ was on average 25% higher in the nonprogressors across the follow-up period ($P = 0.04$). When the appropriateness of insulin secretion for the prevailing level of S_I was considered, the DI showed a progressive decline in progressors of 0.02 log units per month, which translated to a 52% reduction over 30 months compared with an average 1.3% improvement over 30 months in nonprogressors ($P = 0.02$ for rate of change between the nonprogressors and progressors).

Predictors of IGT

In an additional multivariable mixed-model analysis, S_I and Φ were associated with 2-h glucose changes from baseline while age at first visit, sex, race/ethnicity, BMI *Z* score, and family history of either obesity or type 2 diabetes were controlled for. Each 10-unit decrease in S_i was associated with an 11 mg/dl increase in 2-h glucose ($P = 0.04$), and each 100-unit decrease in Φ was associated with a 15 mg/dl increase in 2-h glucose ($P < 0.001$).

CONCLUSIONS — In the present study, glucose, insulin, and C-peptide plasma concentrations were measured longitudinally during three serial OGTTs and analyzed with the OMM to assess both sensitivity and secretion of insulin. Based on these measures, we provide novel information about how glucose levels change in relation to changing β-cell function and S_i in a multiethnic group of obese adolescents at high risk for type 2 diabetes. The rationale for studying IGT is based on its emergence as a relatively common complication of adolescent obesity, as well as its transitional nature as a pre-diabetic state that may fuel the development of type 2 diabetes in youth.

At baseline, the two groups had comparable age and pubertal stage of development and a seemingly similar degree of obesity. Small but significant differences were noted in fasting glucose and 2-h glucose AUC levels, which at baseline were higher in progressors. Of note, S_I was similar in both groups. However, β-cell function, as indicated by Φ_d (18), was significantly lower in progressors than in nonprogressors. Thus, those who progressed to IGT had relatively worse β-cell function at baseline. The present study suggests that an early defect in β-cell function may underlie the development of IGT in obese youth and possibly type 2 diabetes.

One could raise the point that a possible reason for differences in β-cell responsivity is inaccuracy because Φ_d was estimated with only a seven-sample OGTT. However, our analyses tended to exclude systematic overestimation of Φ_d that could have affected the study's conclusion. In fact, the subset of subjects used to assess the reliability of Φ_d, Φ_s, and Φ_{tot} estimated without the 10- and 20-min samples contained both progressors and nonprogressors, and analysis showed the same 30% average overestimation of Φ_d in both groups. Given the good correlation between the estimates for samples both including and excluding time 10 and 20, a 30% overestimation of Φ_d should likely reflect only on the parameter values reported in the two groups—not on the difference assessed at baseline.

During the 30 months of follow-up, S_I and β-cell responsivity significantly decreased in progressors, whereas it did not change in nonprogressors. Progressors had a 20% reduction in β-cell function from baseline value by the third evaluation. Interestingly, S_I showed markedly different trajectories in the two groups during the entire study. S_I declined markedly in progressors, who had values that were 45% lower than baseline by the third OGTT after all the subjects had transitioned to IGT. In contrast, for those who retained NGT status, S_I remained remarkably stable. When considering the interaction between changes in insulin secretion and S_I, we found that the trajectory for the DI in progressors mirrored the clear pattern of gradual decline in both S_I and secretion. Thus, the initial fragility of

β-cell function became even more pronounced with the progressive worsening of insulin resistance in the obese adolescents who developed IGT.

Evidence that declines in β-cell function, S_I, and DI (20) are critical determinants of deteriorating glucose tolerance is consistent with findings from adult studies in Pima Indians (21), the Insulin Resistance Atherosclerosis Study (1,22), and a study in Hispanic women (23). The mechanism underlying the progressive decline in β-cell function is not fully understood. It may be related to a genetic predisposition compounded by environmental factors such as increased caloric intake and the development of obesity. Kahn et al. (24) showed that the development of central adiposity was associated with loss of β-cell function, suggesting that changes in central or visceral fat–derived factors may predispose high-risk individuals to β-cell dysfunction. Recently, Goran et al. (25) reported, in a very interesting paper, progressive S_I deterioration and an increase in visceral fat content in obese Hispanic children with persistent pre-diabetes.

Surprisingly, adiponectin levels did not reflect the changes in S_I that we observed in progressors. We have no explanation for the lack of changes in this adipokine; perhaps it is because we measured only total adiponectin and not its active high–molecular-weight form.

Limitations of the current study are its relatively short period of follow-up time, its small sample size, and the fact that we used subjects drawn from a pediatric obesity clinic. On the other hand, strengths include its use of three consecutive serial OGTTs and determination of insulin sensitivity and secretion using the OMM during the subjects' transition from NGT to IGT.

In conclusion, obese adolescents who progress to IGT manifest primary defects in β-cell function. In addition, progressive decline in S_i further aggravates β-cell function, contributing to the worsening of glucose intolerance. This longitudinal study suggests that prevention of type 2 diabetes in obese youth should start very early, targeting both insulin resistance and β-cell dysfunction.

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