Rapid Deterioration of Insulin Secretion in Obese Adolescents Preceding the Onset of Type 2 Diabetes

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Objective To identify pathophysiologic changes that lead to the onset of type 2 diabetes (T2DM) in adolescents.
Study design Obese adolescents with normal glucose tolerance (n = 41) were studied longitudinally over the course of 4 years with serial measure of the acute insulin response to glucose (AIR_g) as well as proinsulin (PI) concentrations. Insulin resistance was estimated with the homeostatic model assessment of insulin resistance (HOMA-IR), the disposition index (DI) computed as $AIR_g \times 1/HOMA\text{-}IR$, and intravenous glucose tolerance estimated as the glucose disappearance constant.
Results Four adolescents developed diabetes mellitus (DM) during the study, and the rest of the cohort remained nondiabetic. Baseline PI exceeded the IQR of the nondiabetic group in 3 of 4 subjects with DM, and all had >85% reduction from baseline AIR_g, and DI, within 6 months of diagnosis. All the subjects with DM gained weight over the course of the study, but these changes paralleled those for the nondiabetic group. HOMA-IR increased substantially in 1 of the subjects with DM at the time of diagnosis but was comparable with baseline in the other 3. The DI and glucose disappearance constant of the subjects with DM was less than the 10th percentile of the nondiabetic group before and after diagnosis.
Conclusion Conversion from normal glucose tolerance to T2DM in adolescents can occur rapidly, and the onset of T2DM is heralded by a substantial decrease in AIR_g and DI, as well as increased release of PI. These results support loss of β-cell function as the proximate step in the development of T2DM in this age group. *(J Pediatr 2015;166:672-8)*.

Type 2 diabetes (T2DM) has become an increasingly common problem in young people during the past 2 decades.[1,2] The increasing prevalence of what had previously been thought of as a disease of adults has accompanied the general increase in obesity among children and adolescents. Similar to adults with T2DM, affected youth seem to progress through a prediabetic state marked by insulin resistance and impaired insulin secretion.[3] In fact, the inability of islet β cells to compensate for insulin resistance appears to be a core pathogenic mechanism underlying diabetes in patients of all ages.[2,4,5]

The development of T2DM in adults is a progressive process that manifests over years and perhaps decades.[6-8] Cross-sectional studies in adults have demonstrated that at progressive levels of increased fasting glucose, or impaired oral glucose tolerance, β-cell function is decreased,[9] and future risk of diabetes can be predicted well before any clinical manifestations arise.[10,11] Similar findings have been collected in adolescents showing differences in insulin resistance and insulin secretion across stages from normal glucose tolerance (NGT) to prediabetes.[3,12,13] Although it is clear that adolescents who develop T2DM are obese and insulin resistant and also have abnormalities in β-cell function, the pathophysiology behind the transition from prediabetes to diabetes is not well understood. Moreover, there is only limited information available about the pace of progression in young people, which is presumably more rapid than in adults to account for the development of clinical disease over the short period of adolescence.[2]

Although much more common than in the past, the incidence of T2DM in adolescents is still sufficiently low that prospective studies to identify pathophysiologic changes leading to disease have been impractical. In this paper, we describe the development of T2DM in 4 adolescents during the course of a 4-year longitudinal study of insulin secretion in obesity. Our goal was to identify the key changes involved in the transition from NGT to diabetes, and our results support a rapid decrease in β-cell function as the key feature in this progression.

AIR_g	Acute insulin response to glucose	HOMA-IR	Homeostatic model assessment of insulin resistance
BMI	Body mass index		
DI	Disposition index	NGT	Normal glucose tolerance
DM	Diabetes mellitus	OGTT	Oral glucose tolerance test
HbA_{1c}	Hemoglobin A1c	PI	Proinsulin
HOMA	Homeostatic model assessment	PI/I	Proinsulin to insulin ratio
		T2DM	Type 2 diabetes

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Methods

The study population consisted of 41 obese adolescents with NGT who comprised a control population for a longitudinal study of β-cell function in adolescents with T2DM. Inclusion criteria consisted of onset of puberty (≥ Tanner II breast development for girls, ≥4 mL of testicular volume for boys), a body mass index (BMI) greater than the 99th percentile for age and sex, and normal findings on a 75-g oral glucose tolerance test (OGTT) within 3 months of study initiation. Individuals were excluded if they had active concurrent illnesses, secondary diabetes, or were pregnant. Five of the subjects who did not develop diabetes, and one who eventually developed diabetes mellitus (DM; diabetic subject 2, or DM2), were taking metformin as treatment for insulin resistance at the time of their entry into the study; control subjects taking metformin were excluded from the analysis, and the subject who developed DM was included. During the course of observation, 4 subjects developed T2DM, leaving 32 adolescents in the nondiabetic group at the conclusion of the 4-year study. DM2 continued on metformin after she was diagnosed and a second subject, DM1, was started on metformin after a diagnosis of diabetes. Before enrollment, written informed consent/assent was obtained from all subjects or their parents (for those <18 years of age) according to the guidelines of the Cincinnati Children's Hospital and the University of Cincinnati College of Medicine Institutional Review Boards.

After an overnight fast, subjects were admitted to the Clinical and Translational Research Center at the Cincinnati Children's Hospital Medical Center. Body weight was measured with a digital scale to the nearest 0.1 kg, and height was measured with a wall-mounted stadiometer to the 0.1 cm at each visit. Indwelling venous catheters were placed in both forearms for infusion of test substances and blood sampling. The arm designated for blood drawing was wrapped in a heating pad to maintain stable blood flow. Subjects received an intravenous bolus of dextrose (0.3 g/kg, maximum dose 40 g) and blood was sampled at 2, 4, 6, 8, 10, 12, 15, 18, 21, 25, and 30 minutes thereafter. Blood samples were immediately placed on ice and centrifuged to separate plasma within an hour of collection. Plasma was stored at −80°C until processing. After the initial visit, subjects returned at 6 months, 1, 2, 3, and 4 years.

Glucose measurements were made at the time of study on whole blood samples via a YSI glucose analyzer (Yellow Springs Instrument, Yellow Springs, Ohio). Plasma insulin was measured by radioimmunoassay as previously described.[14] This assay uses a guinea pig anti-insulin serum that does not distinguish between proinsulin (PI) and fully processed insulin. PI was measured via a commercial radioimmunoassay kit (Millipore/Linco, Inc, St. Louis, Missouri). Hemoglobin A_{1c} (HbA_{1c}) was determined by a modification of a high-performance liquid-chromatography method with an Alliance 2690/2695 HPLC (Waters Corporation, Milford, Massachusetts) and a PolyCAT A column (PolyLC, Inc, Columbia, Maryland), and results reported as percent of total hemoglobin.

Statistical Analyses

Fasting glucose and insulin levels were calculated as the mean of 2 samples taken before glucose administration. Glucose disappearance during the intravenous glucose tolerance test was estimated as the glucose disappearance constant by calculating the slope of the ln[glucose] from 10 to 30 minutes. The acute insulin response to glucose (AIR_g) was computed as the area under the curve over baseline of insulin concentrations at 2, 4, 6, 8, and 10 minutes after the intravenous administration of glucose using Riemann sums based on the midpoint of each interval. Fasting PI to insulin ratios (PI/I) were calculated for each subject before the glucose bolus by dividing the mean of 2 PI measurements by the mean of 2 insulin measurements. Stimulated PI/I ratios were calculated using the mean PI value and mean of insulin value from +4 min and +10 min after glucose infusion. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the online Oxford calculator (www.dtu.ox.ac.uk/homa). The disposition index (DI) was defined as the product of insulin secretion (AIR_g) and insulin sensitivity (1/HOMA-IR). To validate this combination of variables as representative of the hyperbolic relationship previously established between insulin secretion and insulin sensitivity, ln[AIR_g] was plotted against ln[1/HOMA-IR] for the 32 subjects who did not develop diabetes and analyzed as previously described.[15] This relationship was linear, with a slope of -1.14 ± 0.19 that had 95% confidence limits of −0.94 and −1.35, a range that includes −1 the theoretical value for 2 variables related by a rectangular hyperbola. Thus, in this data set, the relationship of ln[AIR_g] and ln[1/HOMA-IR] is hyperbolic, supporting this combination in the computation of DI.

Data were analyzed using SAS v. 9.3 (SAS Institute, Cary, North Carolina). Continuous data were summarized as median and IQR for the nondiabetic group, and individual patients with DM were evaluated as being within or outside the nondiabetic IQR. Categorical data were summarized as frequency counts. All analyses were conducted considering the subjects without diabetes as a normative group and the 4 subjects who developed diabetes individually (DM1, DM2, DM3, and DM4).

Results

Clinical characteristics, fasting glucose and hormone measures, and results of β-cell function testing at the initial study visit for those who did (DM) and did not develop diabetes are presented in **Table I**. Five adolescents without diabetes were placed on metformin for insulin resistance before the study period and were excluded from analysis. The remaining subjects without diabetes (n = 32) had a median age of 14.4 years (IQR 12.6-15.7), BMI of 35.3 (IQR 29.9-41.6), 44% were male, and had a racial/ethnic distribution of 63% black, 31% non-Hispanic white, and 6% other. The 5 excluded from analysis controls who did not develop diabetes were predominantly female, had greater BMI, and lower AIR_g and DI than the controls not on metformin.