Puberty Is Associated with a Rising Hemoglobin A1c, Even in Youth with Normal Weight

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Our objective was to explore the longitudinal trajectory of hemoglobin A1c (HbA1c) in well-characterized youth (n = 84) with normal weight and obesity during puberty. HbA1c rose from early puberty to Tanner stage 5, even in healthy, normal weight youth, revealing important implications for defining normal glycemia and prediabetes in adolescents. *(J Pediatr 2021;230:244-7).*

With the increase in pediatric obesity, has come a parallel rise in youth-onset type 2 diabetes (T2D) incidence, with devistating consequences for those affected.[1] However, even among those with risk factors, pediatric T2D incidence remains relatively low, hampering our ability to understand factors influencing progression to T2D. Large studies would be required to longitudinally define youth-specific predictors of T2D, including defining glycemic cut-points (ie, prediabetes) predicting progression. More fundamentally, little is known about the glycemic trajectory in adolescence and the impact of physiologic transient pubertal changes in insulin sensitivity and secretion. In a large cross-sectional study of 6th to 8th graders (HEALTHY Study), we demonstrated that the hemoglobin A1c (HbA1c) normal distribution extends into the prediabetes range in healthy, normal weight adolescents.[2] In fact, 2% of lean youth in HEALTHY had a prediabetes-range HbA1c; the highest among African American youth.

The Health Influences of Puberty (HIP) Study was designed to assess changes in insulin sensitivity and secretion in youth with normal weight and obesity as they progressed from early puberty to Tanner stage 5 (T5). HIP demonstrated that, though youth with obesity were substantially more insulin resistant during puberty, their compensatory insulin response, estimated by disposition index (DI), was similar to normal weight youth.[3] However, in the HIP both insulin sensitivity and DI declined between early puberty and early T5, regardless of body mass index (BMI). These data indicate that puberty affects both insulin sensitivity and secretion in normal weight, healthy, youth.

A secondary objective was to assess the impact of puberty on glycemia over time and explore potential factors associated with HbA1c change during puberty. Our hypotheses were that HbA1c would rise during puberty and would be inversely associated with DI.

BMI	Body mass index
DI	Disposition index
HbA1c	Hemoglobin A1c
HIP	Health Influences of Puberty
T2D	Type 2 diabetes
T5	Tanner stage 5

Methods

The HIP study was approved by the Colorado Multiple Institutional Review Board and consent and assent were obtained from all participants. Detailed methods were previously published.[3,4] Briefly, youth with normal weight (BMI 5th-85th percentile for age and sex) and obesity (BMI ≥95th percentile) recruited from general pediatrics and weight management clinics at Children's Hospital Colorado and the surrounding community were enrolled in early puberty (Tanner stages 2-3 [T2-T3]). Exclusion criteria included known history of diabetes, impaired glucose tolerance or impaired fasting glucose during an oral glucose tolerance test, dyslipidemia or hypertension requiring pharmacologic intervention, genetic syndromes, other disorders or medications known to impact glucose metabolism or weight gain, current or recent use of an insulin sensitizer, proteinuria, or weight of >300 pounds.

Tanner staging was performed by a pediatric endocrinologist every 6 months. Testicular volume was also assessed using a Prader orchidometer and assigned a Tanner stage equivalent.[4] Primary outcome visits occurred at 3 time points: baseline (T2-T3), T4, and T5, preceded by a 3-day standard macronutrient diet provided by the University of Colorado-Anschutz Clinical Translational Research Center's metabolic kitchen and 3 days of exercise restriction. Visits included frequently sampled intravenous glucose tolerance testing, fasting laboratory studies, and dual x-ray absorptiometry to measure percent fat mass. Frequently

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sampled intravenous glucose tolerance testing and laboratory methodology were previously published.[3,4] HbA1c was performed by high-performance liquid chromatography (Bio-Rad Variant TURBO, CS). Insulin sensitivity, insulin secretion, and the DI were calculated using the Bergman minimal model.[3]

The analyses reported here are secondary and exploratory. Groups were compared using t tests or the Mann-Whitney U test for continuous variables, and χ^2 or Fisher exact test for categorical variables. Linear models (unadjusted and adjusted for sex, race/ethnicity, baseline Tanner stage, and change in percent fat over time) tested group differences in change in HbA1c from baseline (T2-T3) to the end of study (T5). Mixed models assessed whether factors chosen based on their known contribution to puberty or to glycemia were associated with HbA1c at each time point. Factors included in the model were: leptin, adiponectin, highly sensitive C-reactive protein, dehydroepiandrosterone-sulfate, DI, and insulin-like growth factor-1.

Results

Eight-four youth enrolled in HIP. Baseline demographic, anthropometric, and laboratory characteristics are shown in the Table. As expected, youth with obesity had a higher BMI z-score, percent fat, and leptin, and lower insulin sensitivity and adiponectin. Fasting glucose was significantly higher in youth with obesity, but HbA1c was not statistically different (unadjusted).

The Figure shows the change in HbA1c over time and the distribution of HbA1c in youth with normal weight and obesity. In the overall cohort, HbA1c rose between baseline (T2/T3) and T4 ($\beta = 0.19 \pm 0.05$; $P < .001$) and T5 ($\beta = 0.16 \pm 0.05$; $P = .003$), but did not change significantly between T4 and T5 ($\beta = -0.03 \pm 0.04$; $P = .79$). HbA1c increased significantly in youth with normal weight at T4 ($\beta = 0.11 \pm 0.05$; $P = .03$) and T5 ($\beta = 0.14 \pm 0.05$; $P = .006$) compared with baseline, but not in youth with obesity. HbA1c was, however, higher in youth with obesity vs normal weight at T4 ($5.6 \pm 0.08\%$ vs $5.2 \pm 0.06\%$; $P < .001$) and at T5 ($5.5 \pm 0.08\%$ vs $5.2 \pm 0.06\%$; $P = .01$). Importantly, although the overall HbA1c distribution was wider in youth with obesity, the tail crossed the cut-point for diagnosis of prediabetes (5.7%) in both cohorts. Time-dependent results were unchanged after adjusting for sex and race/ethnicity and after adding percent fat to the model. However, group contrasts at each time point were no longer significant after adjusting for percent fat.

The DI was not significantly associated with HbA1c in either univariate regression ($\beta = 0.00006 \pm 0.00001$; $P = .64$) or in mixed models that included factors preselected to potentially contribute to changing HbA1c over time ($\beta = 0.00002 \pm 0.00001$; $P = .23$). Results were the same before and after adjusting for sex and race/ethnicity. In the mixed models, adjusted for sex and race/ethnicity, higher leptin ($\beta = 0.11 \pm 0.04$; $P = .01$) and lower adiponectin ($\beta = -0.06 \pm 0.03$; $P = .03$) were significantly associated with higher HbA1c. After adding percent fat to the model, the effect of adiponectin was similar ($\beta = -0.07 \pm 0.03$; $P = .01$), but leptin was no longer significant ($\beta = 0.11 \pm 0.06$; $P = .07$).

Table. Baseline study characteristics

Variables	Normal weight	Obesity	*P* value
No.	47	37	
Race/ethnicity, n (%)			.001
Hispanic	12 (25.5)	26 (70.3)	
White, non-Hispanic	23 (48.9)	6 (16.2)	
Black	8 (17.0)	3 (8.1)	
Asian	3 (6.4)	0 (0.0)	
Other	1 (2.1)	2 (5.4)	
Age (years)	12.0 (1.5)	11.3 (1.2)	.02
BMI Z-score	−0.01 (0.71)	2.89 (1.08)	<.001
Tanner stage, n (%)			.94
2	29 (62)	24 (65)	
3	18 (38)	13 (35)	
HbA1c (%)	5.14 (0.25)	5.34 (0.49)	.09
Body fat (%)	25.1 (6.4)	41.31 (6.94)	<.001
Physical activity (metabolic equivalents)*	65.66 (13.13)	66.20 (14.26)	.86
Insulin sensitivity ($\times 10^{-4}$/min^{-1}/mIU/mL)	8.49 (5.80)	3.66 (4.36)	<.001
Insulin secretion (μIU/mL)	591 (435)	1831 (1072)	<.001
DI ($\times 10^{-4}$/min^{-1})	3770 (1639)	4159 (1731)	.31
Triglycerides (mg/dL)	75.93 (38.28)	106.30 (48.12)	.003
High-density lipoprotein (mg/dL)	50.98 (8.48)	40.16 (8.27)	<.001
Low-density lipoprotein (mg/dL)	79.34 (22.94)	86.51 (22.23)	.16
Systolic blood pressure (mm Hg)	110.13 (9.68)	118.03 (12.28)	.002
Diastolic blood pressure (mm Hg)	67.02 (7.71)	66.45 (7.89)	.75
Fasting glucose (mg/dL)	83.09 (7.90)	89.86 (16.63)	.02
Aspartate aminotransferase (IU/L)	42.49 (14.69)	40.89 (18.01)	.66
Alanine aminotransferase (IU/L)	25.13 (9.63)	34.78 (21.96)	.01
Insulin-like growth factor-1 (ng/mL)	315.64 (103.38)	260.95 (91.00)	.01
Leptin (ng/mL)	6.03 (5.66)	31.30 (14.28)	<.001
Adiponectin (μg/mL)	12.11 (4.40)	9.57 (5.22)	.02
C-reactive protein (mg/L)	1.02 (2.63)	6.28 (23.41)	.13
DHEA-S (μg/dL)	90.79 (61.01)	95.76 (62.99)	.72

Data have been previously published.[3]
Values are mean (SD), unless otherwise specified.
*Estimated from the 3-day Physical Activity Recall Questionnaire.[5]

Discussion

This longitudinal analysis of a small, but deeply characterized, cohort extends our previous findings that a subset of otherwise healthy youth experience mild elevation in HbA1c in adolescence.[3] Moreover, our findings suggest that this HbA1c elevation may relate to pubertal changes in adipose tissue metabolism. These findings have important clinical implications. First, they raise a critical question about the definitions of normal and abnormal glycemia during adolescence. In particular, if HbA1c, and by extension glycemia, increases in all youth during adolescence, then the current criteria for prediabetes in adolescents based on adult norms may not be valid. Although HbA1c was, on average, higher in youth with obesity in HIP, the overall distribution overlapped between youth with normal weight and obesity. Thus, a "prediabetes range" HbA1c may not mean the same thing in youth, even those with obesity, as it does in

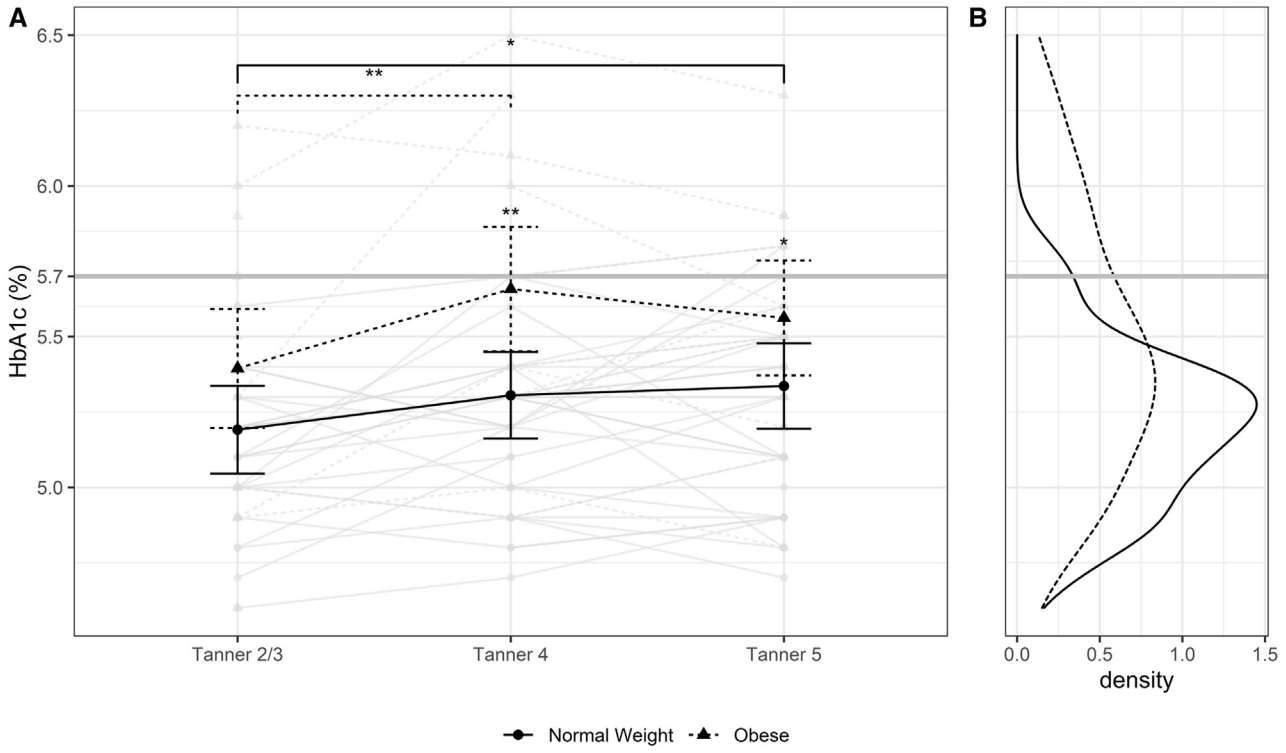

Figure. **A,** Individual and mean changes in HbA1c in youth with normal weight or obesity. Mean HbA1c is significantly higher in youth with obesity at T4 and T5. In youth with normal weight, but not obesity, there is a significant rise in HbA1c from baseline to T4 and T5, suggesting an effect of puberty itself of normal glycemia. **B,** Distribution of HbA1c in youth with normal weight and obesity. A proportion of youth in both groups experience an HbA1c above the American Diabetes Association-defined cut-point for prediabetes. *$P < .05$, **$P < .01$, ***$P < .001$.

adults. Because only a small subset of youth with obesity progress to T2D and youth-onset T2D behaves more aggressively than adult-onset disease, it is critical to define risk for progression specific to adolescents. Better defining normal glycemia and prediabetes is a critical first step in developing more reliable criteria for T2D risk in youth.

Our finding that the increase in HbA1c did not relate to DI—an estimate of β-cell function relative to insulin sensitivity—was surprising, particularly given that we previously demonstrated that the DI decreases during puberty, both in youth with normal weight and with obesity.[3] It is important to note that DI was based on an intravenous glucose tolerance test in HIP, whereas HbA1c represents glycemia in free-living conditions. Thus, postprandial glucose, which contributes strongly to HbA1c, may play a large role in average glycemia during puberty, perhaps owing to an altered incretin response or higher dietary glucose intake in youth vs adults. HbA1c did correlate with adipokines. Leptin, which showed a positive association, is thought to help regulate glucose homeostasis, although it is typically thought to decrease glucose in the healthy state.[6] Leptin increases in all youth upon entry into puberty. In girls, leptin continues to increase throughout puberty, whereas in boys, leptin subsequently decreases as puberty progresses.[7] It is certainly possible that metabolic leptin resistance occurs during puberty, but further study is needed. Adiponectin is typically positively associated with insulin sensitivity, but a direct relationship with glycemia and adiponectin is less clear. Growth hormone is also increased during puberty, which, during fasting stimulates lipolysis, elevates free fatty acids, and induces insulin resistance, which may impact different factors than assessed by an intravenous glucose tolerance test.[8] Another potential explanation for changing glycemia during puberty is altered insulin clearance, which is affected by race, is lower in obese youth than in obese adults, and correlates with decreases in β-cell function in youth.[9-12] However, little is currently known about how insulin clearance normally changes during childhood. Finally, changes in glucose mediated glucose uptake may play a role in average glycemia during puberty, another area in need of exploration.

Limitations of HIP include the relatively small sample size, the absence of continuous glucose monitoring to track free-living glycemic patterns, and the lack of repeated oral glucose tolerance testing over time. It is also possible that an intravenous glucose tolerance test may not be sensitive enough to detect subtle changes in the DI. Moreover, race and ethnicity may impact glycemia as well as measurement of HbA1c, but the racial and ethnic differences between groups confound further analyses.[13,14] Thus, more information is needed

in future studies to determine the specific characteristics of the pubertal changes in average glycemia we have demonstrated.

In summary, in a longitudinal study of youth with normal weight and obesity, we found that average glycemia increases as puberty progresses, irrespective of BMI. This finding has important implications for defining prediabetes based on HbA1c in youth. Further studies are needed to better understand mediators of glucose homeostasis during puberty and define normal glycemia during adolescence. ■

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