Longitudinal Study on Pubertal Insulin Resistance

Michael I. Goran[1] and Barbara A. Gower[2]

Previous cross-sectional studies show that puberty is associated with a reduction in insulin sensitivity (S_I), but no longitudinal studies have examined this change in detail. This study is a longitudinal study in 60 children (33 male and 27 female subjects; 32 Caucasian and 28 African-American) examined at Tanner stage I (age 9.2 ± 1.4 years) and after 2.0 ± 0.6 years of follow-up, by which time 29 children remained at Tanner stage I and 31 had progressed to Tanner stage III or IV. Tanner stage was assessed by physical examination. S_I, the acute insulin response (AIR), and the disposition index (DI) were determined by the tolbutamide-modified intravenous glucose tolerance test and minimal modeling, body fat mass was assessed by dual-energy X-ray absorptiometry, visceral fat was determined by computed tomography, and fasting blood was analyzed for hormone levels. In children progressing to Tanner stage III, S_I fell significantly by 32% (4.4 ± 3.0 to 3.0 ± 1.7 × $10^{-4}min^{-1}/[\mu IU/ml]$), AIR increased by 30%, DI fell by 27%, and there was a significant increase in fasting glucose (93.5 ± 5.0 to 97.0 ± 4.1 mg/dl) and insulin (14.3 ± 8.1 to 18.6 ± 11.0 μIU/ml). In children remaining at Tanner stage I, there was a slight increase in S_I (6.4 ± 3.1 to 7.4 ± 3.5 × $10^{-4}min^{-1}/[\mu IU/ml]$) with no significant change in AIR or fasting glucose and insulin. The pubertal fall in S_I was more consistent in African-Americans; remained significant after controlling for age, sex, and change in fat mass, visceral fat, and fat-free mass; and was similar in children at low, medium, and high body fat. Change in S_I was not significantly related to change in fasting hormone levels, but change in AIR was significantly related to change in androstendione ($r = 0.39$; $P = 0.04$). Pubertal transition from Tanner stage I to Tanner stage III was associated with a 32% reduction in S_I, and increases in fasting glucose, insulin, and AIR. These changes were similar across sex, ethnicity, and obesity. The significant fall in DI suggests conservation in β-cell function or an inadequate β-cell response to the fall in S_I. The fall in S_I was not associated with changes in body fat, visceral fat, IGF-I, androgens, or estradiol. *Diabetes* 50:2444–2450, 2001

From the [1]Departments of Preventive Medicine and Physiology & Biophysics, Keck School of Medicine, University of Southern California, Los Angeles, California; and the [2]Department of Nutrition Sciences, School of Health-Related Professions, University of Alabama at Birmingham, Birmingham, Alabama.

Address correspondence and reprint requests to Dr. Michael I. Goran, 1540 Alcazar St., Room 208-D, Institute for Prevention Research, Department of Preventive Medicine, University of Southern California, Los Angeles, CA 90033. E-mail: goran@usc.edu.

Received for publication 12 January 2001 and accepted in revised form 27 July 2001.

AIR, acute insulin response; CT, computed tomography; CV, coefficient of variation; DEXA, dual-energy X-ray absorptiometry; DI, disposition index; FSH, follicle-stimulating hormone; FSIGTT, frequently sampled intravenous glucose tolerance test; GCRC, General Clinical Research Center; RIA, radioimmunoassay; S_I, insulin sensitivity; UAB, University of Alabama at Birmingham.

Pubertal insulin resistance has been well documented in several cross-sectional studies (1–7). The fall in insulin sensitivity (S_I) during puberty is associated with a compensatory increase in insulin secretion (7). The regulatory purpose of these changes in insulin action and secretion is not clear but is thought to be selective for glucose but not protein metabolism (2) and to provide a mechanism for increasing the anabolic effects of insulin and growth hormone during a period of rapid somatic growth (1,8). The original observation of pubertal insulin resistance was reported in 1987 when Amiel et al. (5) showed that insulin-stimulated glucose metabolism was ~30% lower in a sample of children at Tanner stages II–IV compared with children at Tanner stage I or adults. Previous cross-sectional reports consistently show that pubertal development is associated with a ~25–30% reduction in S_I, with the peak reduction occurring at Tanner stage III, followed by a recovery by Tanner stage V (3). Despite the existence of numerous cross-sectional studies, there are no published longitudinal studies that have examined changes within subjects over time, or how these changes might be influenced by factors such as sex, ethnicity, and body fat, and no study has attempted to separate the effects of puberty on S_I from the effects of aging per se.

Although the phenomenon of pubertal insulin resistance is well documented, the mechanism has not been clearly determined. None of the previous studies that have examined pubertal insulin resistance have incorporated strong measures of body fat or circulating hormone levels. Thus, the mechanism and cause of the rapid decline in S_I during puberty is entirely unknown. It is generally thought that the transient fall in S_I in puberty is not related to changes in body fat, since body fat increases continuously before and during puberty, whereas the fall in S_I is transient, occurring in mid-puberty and recovering to prepubertal levels by the end of puberty (3). However, some studies have hypothesized that the effect may be due to changes in body fat (6,9) because of the more accelerated increase in body fat that occurs during, compared with before, puberty. It is also frequently stated that the fall in S_I is not likely to be related to changes in sex-steroid hormones, because hormone levels rise rapidly and remain elevated, whereas S_I falls and then recovers (10). Alternatively, it has been hypothesized that the fall in S_I may be driven by transient changes in growth hormone levels in puberty (10–12). This is thought to be a more likely scenario because the transient changes in growth hormone levels are similar to those observed for S_I. However, all of these hypotheses are entirely speculative and unsubstantiated because there are no previous studies that have examined

the within-subject associations between changes in S_I and changes in body fat, body fat distribution, and hormone levels during the dynamic phase of puberty.

Therefore, there were three major objectives of this study. First, we examined pubertal changes (Tanner stage I to Tanner stage III or IV) in S_I and insulin secretion using a longitudinal study design across sex and ethnic groups (Caucasians compared with African-Americans). Second, to separate the effects of change in Tanner stage from the effects of growth and aging per se, we compared changes in S_I, body fat, and hormone levels in a group of children who progressed from Tanner stage I at baseline to Tanner stage III or IV at follow-up, with changes in a group of children who remained at Tanner stage I over a similar follow-up period. This design also allowed us to control for any potential effects of repeated exposure to the various tests. Third, by comparing the pattern of changes of various variables across time within subjects, we examined whether the pubertal reduction in S_I was associated with changes in body fat, visceral fat, sex-steroid hormones, or IGF-I.

RESEARCH DESIGN AND METHODS

Subjects. Study subjects were part of an ongoing longitudinal study on body composition, energy expenditure, and risk factors for type 2 diabetes and cardiovascular disease in children and adolescents. Findings regarding body fat distribution, aerobic capacity, energy expenditure, insulin action, and dietary factors in this cohort have been previously reported (13–16). Children were recruited by newspaper and radio advertisements and by word of mouth. No child was taking medications known to affect body composition (e.g., ritalin or growth hormone), diagnosed with syndromes or diseases known to affect body composition or fat distribution (e.g., Cushing sydrome, Down syndrome, or type 1 diabetes), or diagnosed with any major illness since birth. Ethnicity was determined by self-report. This study was approved by the Institutional Review Board at the University of Alabama at Birmingham (UAB), and parents provided informed consent before testing commenced.

Protocol. Children were admitted to the General Clinical Research Center (GCRC) in the late afternoon for an overnight visit. Upon the children's arrival, anthropometric measurements were obtained. A computed tomography (CT) scan was conducted in the Department of Radiology at UAB at ~1700 h. The children were served dinner and an evening snack, with all food consumed before 2000 h. All children were fed a fixed meal consisting of 55% carbohydrate, 15% protein, and 30% fat. Consumption of only water and noncaloric noncaffeinated beverages was permitted between 2000 h and testing the following morning. Two weeks after testing at the GCRC, children returned to the Department of Nutrition Sciences at UAB for body composition analysis by dual-energy X-ray absorptiometry (DEXA).

Tanner staging. Tanner stage was determined by a pediatrician and was based on breast stage and pubic hair development in girls (17) and genitalia development in boys (18).

Tolbutamide-modified frequently sampled intravenous glucose tolerance test. S_I, the acute insulin response (AIR), and the disposition index (DI) (product of S_I and AIR) were determined using a frequently sampled intravenous glucose tolerance test (FSIGTT) in the early morning after an overnight fast, as previously reported (15). Briefly, fasting blood samples were drawn, glucose was administered intravenously at time zero (25% dextrose; 11.4 g/m^2), tolbutamide (125 mg/m^2) was injected intravenously at 20 min postglucose, and 18 blood samples were collected over 3 h. Sera were analyzed for glucose (Ektachem DT II System; Johnson & Johnson Clinical Diagnostics) and insulin (radioimmunoassay [RIA]; Diagnostic Products, Los Angeles, CA), and values were entered into the MINMOD computer program (version 3.0, Richard N. Bergman) for determination of S_I, AIR, and DI, which is the product of S_I and AIR and serves as an index of β-cell function (19).

Total body fat and abdominal fat. Whole-body composition (fat mass and fat-free mass) was measured by DEXA using a Lunar DPX-L densitometer (LUNAR Radiation, Madison, WI) as previously described (20). Subcutaneous abdominal fat and visceral fat were measured by CT scanning with a HiLight/Advantage Scanner (General Electric, Milwaukee, WI), as previously described (13).

Hormone assays. Three samples of blood were collected over 40 min after an overnight fast, and sera were separated, pooled, stored at −85°C, and assayed for various hormones. Estradiol, testosterone, androstendione, and follicle-stimulating hormone (FSH) were measured as indexes of reproductive maturation. IGF-I was measured as an index of growth hormone action. Leptin was measured because it is closely related to body fat levels in children (21) and has been hypothesized to play a role in pubertal development (22). Cortisol was measured because this hormone is negatively associated with S_I (23). The details for each of the hormone assays are as follows:

- Estradiol (Diagnostic Products; double-antibody RIA): intra-assay coefficient of variation (CV) 3.6%; interassay CV 5.2%; and assay sensitivity 15.42 pmol/l.
- Total testosterone (Diagnostic Products; solid-phase RIA): intra-assay CV 2.7%; interassay CV 8.6%; and assay sensitivity 0.41 nmol/l.
- Androstendione (Diagnostic Systems Laboratories, Webster, TX; solid-phase RIA): intra-assay CV 11%; interassay CV 6.1%; and assay sensitivity 0.03 ng/ml.
- FSH (Diagnostic Products; immunoradiometric assay): intra-assay CV 4.47%; interassay CV 4.60%; and assay sensitivity ~0.06 mIU/ml.
- Total IGF-I (Diagnostic Systems Laboratories; immunoradiometric assay): intra-assay CV 3.7%; interassay CV 7.3%; and assay sensitivity ~2.06 ng/ml.
- Leptin (Linco Research, St. Charles, MO; double-antibody RIA): intra-assay CV 3.1%; interassay CV 6%; and assay sensitivity 0.4 ng/ml.
- Cortisol (Diagnostic Products; solid-phase RIA): intra-assay CV 5.2%; interassay CV 6.8%; and assay sensitivity 0.39 μg/dl.

Data analysis. Variables that were not normally distributed were log transformed before analysis. These variables included fasting insulin, S_I, AIR, and hormone levels. For ease of interpretation, data are presented in the measured untransformed scale. Simple, unadjusted within-subject changes were assessed by paired t test. Repeated-measures analysis of variance was used to examine the influence of time on changes on each of the dependent variables (e.g., S_I) after controlling for the between-subject effects of sex, ethnicity, and puberty transition group (i.e., remaining at Tanner stage I or progressing to Tanner stage III) as well as the covariates of age and body fat at baseline. Interaction effects were examined to determine whether the pattern of time-related changes was similar across puberty transition groups, sex, and ethnicity. Pearson's correlation coefficients were used to examine the association between changes in the main outcome variables (e.g., change in S_I) and the hypothesized factors (e.g., change in body fat mass and change in hormone level). All analyses were conducted using SPSS version 9.0, and data are presented as the means ± SD.

RESULTS

Data from 60 children were used in this analysis. The group was evenly split between the sexes (33 male and 27 female subjects) and ethnic groups (32 Caucasians and 28 African-Americans). All children were at Tanner stage I at their original visit and had a mean age of 9.2 ± 1.3 years. After a mean follow-up period of 2.0 ± 0.6 years, 31 of these children had progressed to Tanner stage III or IV, whereas 29 remained at Tanner stage I. The children who progressed to Tanner stage III or IV were older, with greater body fat than those remaining at Tanner stage I (Table 1).

The children who progressed from Tanner stage I to Tanner stage III or IV were aged 10.0 ± 1.1 years at baseline and 12.3 ± 1.1 at follow-up (Table 1). As expected, both fat mass and fat-free mass were significantly higher by Tanner stage III, with fat-free mass increasing by 37% and fat mass increasing by 44%. Fasting glucose and fasting insulin were significantly higher at Tanner stage III or IV compared with Tanner stage I (Table 1). S_I fell significantly by 32%, AIR increased significantly by 30%, DI fell significantly by 30% (Table 1), and glucose effectiveness remained constant (data not shown).

To separate the effects of changing Tanner stage from age-related changes, we compared the changes noted above with a group of children who remained at Tanner stage I over a similar follow-up period (Table 1). These children were younger, with a mean age of 8.4 ± 1.1 years at baseline and 10.0 ± 1.1 years at follow-up. As expected,

TABLE 1
Within-subject changes in children increasing to Tanner stage III or IV versus those remaining at Tanner stage I

	Children remaining at Tanner stage I (n = 29)		Children progressing from Tanner stage I to III or IV (n = 31)		Within-subject effects	Between-subject effects
	Initial visit	Follow-up visit	Initial visit	Follow-up visit		
Age (years)						
Caucasian	8.6 ± 1.2	10.2 ± 1.3*	10.2 ± 0.9	12.4 ± 1.2*	—	None
African-American	8.0 ± 0.8	9.5 ± 0.6*	9.8 ± 1.2	12.2 ± 1.1*		
Fat-free mass (kg)						
Caucasian	22.7 ± 5.0	26.1 ± 5.5*	26.7 ± 5.9	35.4 ± 8.0*	Time	Sex
African-American	21.9 ± 3.6	24.9 ± 3.7*	27.9 ± 3.9	39.1 ± 6.3*		
Fat mass (kg)						
Caucasian	8.2 ± 6.5	11.0 ± 7.9*	15.0 ± 9.8	21.5 ± 14.1*	Time	None
African-American	5.8 ± 7.6	7.6 ± 3.4*	12.9 ± 8.0	18.7 ± 12.9*		
Fasting glucose (mg/dl)						
Caucasian	92.7 ± 4.8	97.1 ± 4.2*	94.8 ± 6.4	95.6 ± 5.4	None	Sex
African-American	94.0 ± 5.2	96.8 ± 4.2	92.2 ± 5.6	96.2 ± 4.5*		
Fasting insulin (μIU/ml)						
Caucasian	10.3 ± 3.9	11.4 ± 5.1	14.7 ± 8.0	18.8 ± 8.7*	None	None
African-American	11.2 ± 5.0	10.9 ± 2.2	13.9 ± 8.3	18.5 ± 12.6*		
S_I [$\times 10^{-4}$ min^{-1}/(μIU/ml)]						
Caucasian	7.4 ± 3.2	7.9 ± 3.9	5.0 ± 3.7	3.4 ± 1.6	Time*group	Race
African-American	4.4 ± 1.4	6.4 ± 2.4*	4.0 ± 2.4	2.6 ± 1.7*	Time*group	
AIR (μIU/ml)						
Caucasian	652 ± 365	650 ± 540	823 ± 435	781 ± 449	None	Race
African-American	1,715 ± 1,782	1,239 ± 1,024	2,071 ± 1,714	2,899 ± 3,718		
DI ($\times 10^{-4}$ min^{-1})						
Caucasian	4,123 ± 1,508	3,972 ± 1,856	3,025 ± 2,224	2,224 ± 973	Time*group	Race
African-American	6,174 ± 4,394	7,284 ± 4,717	6,905 ± 4,304	5,009 ± 2,937		

Data are means ± SD *Significantly different at follow-up compared with initial visit by paired *t* test. Within-subject effects were from repeated-measures analysis of variance (ANOVA) after adjusting for age, sex, ethnic group, and initial fat mass. Between-subject effects of race or sex were from repeated-measures ANOVA model. Time effects are the effects of time; significant time*group interaction indicates that change over time was significantly different in those children remaining at Tanner stage I versus those progressing to Tanner stage III or IV.

both fat and fat-free mass also increased, but at a lower rate (14% increase in fat-free mass and 34% increase in fat mass). Fasting glucose and fasting insulin remained unchanged at follow-up, S_I increased slightly but significantly (by 15%), and AIR, DI, and glucose effectiveness did not change significantly over time.

To fully maximize the power of this study and examine the data collectively, we conducted a repeated-measures analysis of variance to examine the within-subject effects and compare the change over time in those children progressing to Tanner stage III or IV, versus those children remaining at Tanner stage I. As shown in Table 1, there was a significant time*group interaction effect for fasting insulin, S_I, and DI, indicating that the pattern of change in these variables was significantly different in the two groups of children. There were no significant interaction effects between time and sex or ethnicity in these models, indicating that the degree of change was similar across sex and ethnicity. In addition, these effects remained significant after controlling for age, fat-free mass, and body fat content. The group mean values for S_I across time in the sex and ethnic groups are shown in Fig. 1. Since the two groups of children varied in age, we examined changes in subgroups matched for age at baseline and at follow-up. Similar findings were observed in these age-matched subgroups. In fact, in this analysis the fall in S_I in those progressing to Tanner stage III or IV was slightly higher (39% fall), with a 53% increase in AIR, and no significant changes in either variable in the children remaining at Tanner stage I (data not shown).

To examine the effect of body fat on pubertal changes in S_I, we examined pubertal changes in children who fell in the lower, middle, and upper tertiles for body fat percentage at baseline among those who progressed to Tanner stage III (Table 2). The three tertiles were similar for age (9.8 ± 1.4, 10.0 ± 1.1, and 10.1 ± 0.8 years, respectively), but, varied greatly for percentage of body fat (17.8 ± 6.7, 28.7 ± 3.3, and 41.3 ± 5.0% fat at baseline, respectively). In repeated-measures analysis of variance, there was a significant within-subject effect of time ($P = 0.001$) on S_I that was consistent across the fat groups (*P* value for time*fat-group interaction = 0.38), as well as a significant between-subject effect of fat ($P < 0.001$). The reduction in S_I over time was 33% in the low-fat group, 30% in the medium-fat group, and 36% in the high-fat group. Results were similar when examining pubertal changes across fat groups for AIR, DI, and fasting insulin and glucose, as summarized in Table 2.

Changes in various hormone concentrations over time in those children progressing from Tanner stage I to Tanner stage III or IV versus those remaining at Tanner stage I are shown in Table 3. In those children remaining at Tanner stage I, there were no significant changes in estradiol, testosterone, cortisol, or leptin, but there were significant increases in FSH (70% increase) and a small but significant increase in IGF-I (13% increase). In those children progressing to Tanner stage III or IV, there were no significant changes in cortisol or leptin, but there were large increases in estradiol and testosterone (both undetectable at baseline), a doubling of androstendione, a

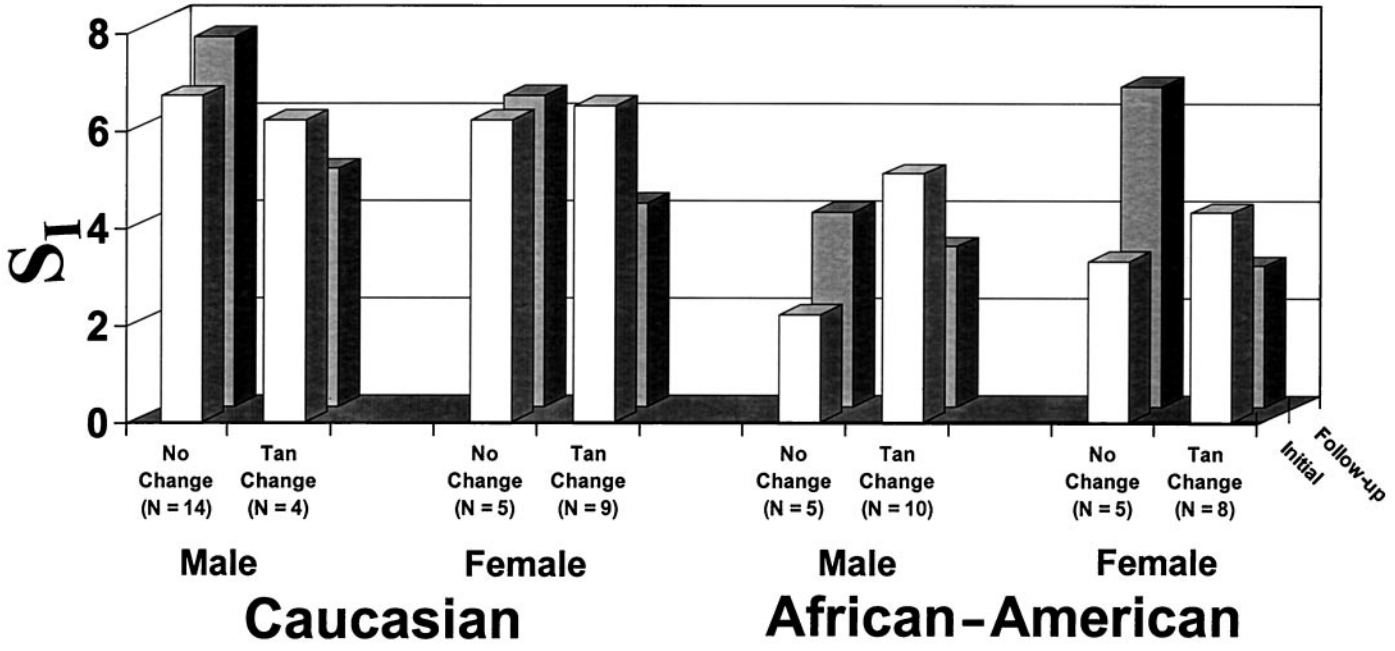

FIG. 1. Mean values for S_I across sexes and ethnic groups in children remaining at Tanner stage I and children progressing to Tanner stage III at follow-up. S_I measured at baseline (open bars) and then after 2.0 ± 0.6 years of follow-up (filled bars) in children who remained at Tanner stage I (groups indicated by "No Change"), compared with children progressing to Tanner stage III (groups indicated by "Tan Change").

threefold increase in FSH, and a 1.7-fold increase in IGF-I. Among those children who progressed from Tanner stage I to Tanner stage III or IV, change in S_I was not significantly associated with change in fat mass, change in subcutaneous or abdominal adipose tissue, or any of the hormone levels, in the group as a whole or when analyzed in sex and ethnic subgroups. Change in the AIR was not associated with change in any other variable except the change in androstendione ($r = 0.39$; $P = 0.04$; $n = 29$). This relationship was evident but not significant when examined separately in boys ($r = 0.44$; $P = 0.11$; $n = 14$) or girls ($r = 0.43$; $P = 0.11$; $n = 15$).

DISCUSSION

The phenomenon of pubertal insulin resistance has been well described in numerous studies (1–7), but there have been no longitudinal studies that have examined this change in detail. It is therefore unclear whether pubertal insulin resistance is related to compensatory changes in insulin secretion and is associated with pubertal changes in body fat and/or hormone levels. We used a longitudinal study design to compare within-subject changes over time, and our main findings were that pubertal transition from Tanner stage I to Tanner stage III or IV was associated with a 32% reduction in S_I and a disproportionately low increase in the AIR. These changes were consistent across sex, ethnicity, and obesity and were not apparent in growing children who did not experience a change in Tanner stage. The disproportionately low increase in the AIR observed in response to the decline in S_I suggests a conservation in β-cell function or an inadequate β-cell response. Finally, the fall in S_I was not associated with changes in body fat, IGF-I, or sex steroid hormone levels.

The present findings regarding pubertal changes in S_I generally agree with those of previous cross-sectional observations. The largest cross-sectional study was reported in data from 357 children (10–14 years of age) in whom insulin action was assessed by the euglycemic clamp technique (3). In this study, S_I began to fall by Tanner stage II, reached a nadir at Tanner stage III, and then recovered to near prepubertal levels by Tanner stage V (3).

We did not have substantial numbers of children within each of the four sex by ethnic subgroups to thoroughly examine the influence of sex and ethnicity on the magnitude of changes in S_I or AIR. In the repeated-measures analysis of variance, we did not detect any significant interactions between sex and time or ethnicity and time, other than the interaction of time*group*ethnicity (Table

TABLE 2
Pubertal changes in children at low, medium, and high percentage fat

	Low percentage fat		Medium percentage fat		High percentage fat		Significant effects
	Tanner I	Tanner III	Tanner I	Tanner III	Tanner I	Tanner III	
Fasting glucose (mg/dl)	92.8 ± 4.5	97.3 ± 3.8	93.6 ± 6.1	96.9 ± 5.1	93.9 ± 4.6	96.7 ± 3.8	Time
Fasting insulin (μIU/ml)	10.1 ± 5.4	13.5 ± 2.6	11.3 ± 2.2	16.8 ± 5.6	20.7 ± 9.6	25.0 ± 15.8	Time; fat
S_I [$\times 10^{-4}$ min^{-1}/(μIU/ml)]	6.8 ± 0.8	4.5 ± 0.4	4.0 ± 0.8	2.8 ± 0.4	2.6 ± 0.8	1.7 ± 0.4	Time; fat
AIR (μIU/ml)	1,351 ± 750	1,608 ± 1,158	1,250 ± 621	1,412 ± 793	1,997 ± 2,279	2,922 ± 4,891	None
DI ($\times 10^{-4}$ min^{-1})	7,816 ± 4,985	6,159 ± 3,153	4,254 ± 1,467	3,394 ± 1,574	3,902 ± 3,216	2,141 ± 1,314	Time; fat

Data are means ± SD. Time*fat interaction was not significant for any variable.

TABLE 3
Changes in circulating hormone concentrations and correlation of these changes with changes in insulin sensitivity

	Subjects who remained at Tanner stage I		Subjects who became Tanner stage III or IV	
	Initial visit	Follow-up	Initial visit	Follow-up
Estradiol (pg/ml)	4.2 ± 0.0	5.0 ± 3.5	4.2 ± 0.0	13.2 ± 14.6*
Testosterone (ng/dl)	11.8 ± 0.0	14.3 ± 12.0	13.4 ± 5.6	112.4 ± 147.9*
Androstendione (ng/ml)	0.49 ± 0.22	0.61 ± 0.4	0.65 ± 0.25	1.12 ± 0.55*
Cortisol (ug/dl)	10.7 ± 3.3	10.6 ± 4.5	10.8 ± 3.1	10.0 ± 3.2
FSH (mIU/ml)	0.79 ± 0.65	1.34 ± 1.37*	1.32 ± 0.86	3.40 ± 1.87*
IGF-I (ng/ml)	216.3 ± 84.1	243.1 ± 97.3*	273.0 ± 97.3	472.6 ± 172.6*
Leptin (ng/ml)	11.9 ± 9.1	13.2 ± 10.1	15.2 ± 14.3	17.1 ± 18.3

Data are means ± SD. *$P < 0.05$ for change over time by paired t test (note that the SD for estradiol and testosterone appears as zero in some cases because all values were equal to the minimal detectable level).

1). This finding indicates that the fall in S_I may have been stronger in blacks than in whites. This was also evident in the simple paired t test analysis, in which the fall in S_I in children progressing to Tanner stage III was significant in African-Americans (4.0 ± 2.4 to 2.6 ± 1.7; $P = 0.001$; $n = 18$) but not in Caucasians (5.0 ± 3.7 to 3.4 ± 1.6; $P = 0.16$; $n = 13$). However, the lack of effect in Caucasians may be due to the smaller sample ($n = 13$) and greater variability in S_I.

The pubertal fall in S_I was accompanied by an increase in the AIR. However, based on the hyperbolic relationship between S_I and AIR (24), the increase in AIR (30% overall) was lower than expected to match the overall 32% reduction in S_I. This is reflected in the overall 30% fall in DI. According to the hyperbolic relationship, AIR would have to be increased by 50% to fully compensate for the fall in S_I. Such an increase would maintain a constant DI and reflect a healthy response at the level of the β-cell. The accompanying rise in AIR is consistent with previous findings (7), but the finding of a lower-than-expected increase is a new observation. This finding potentially indicates either that there is a conservation of β-cell activity or that the β-cell is failing.

Substantial indirect evidence suggests that growth hormone may play a key role in pubertal insulin resistance. This hypothesis is attractive because growth hormone and IGF-I are transiently higher in mid-puberty, mirroring the changes in S_I, and S_I is correlated with growth hormone and IGF-I (5). In addition, growth hormone–deficient children have increased S_I (25), and growth hormone has strong effects on β-cells (26). Also, the increase in growth hormone during puberty may contribute to insulin resistance via its effect on increasing lipolysis and free fatty acid concentration.

However, not all studies have found supporting evidence for the growth hormone theory. One study that performed detailed measures of growth hormone (9) found no significant difference across pubertal groups in overnight growth hormone secretion, peripheral growth hormone responsiveness (as indicated by growth hormone–binding protein), or growth hormone action (as indicated by circulating IGF-I). However, it may not be appropriate to base conclusions regarding the influence of growth hormone on pubertal S_I on these results, because no pubertal change in S_I was observed in this study. In our study, although there was a large increase in circulating IGF-I during puberty, this was not correlated with the fall in S_I. Since we only had a fasting plasma sample, we were unable to thoroughly evaluate the influence of growth hormone beyond an examination of IGF-I as a surrogate marker of growth hormone action. Clearly, there is a strong need to examine more carefully and thoroughly the relationship between changes in growth hormone action and insulin action and secretion during puberty.

Although changes in sex hormones may contribute to pubertal insulin resistance, this theory has not been previously examined in detail. In general, changes in sex steroids are not thought to drive changes in pubertal insulin resistance because sex steroids increase in early puberty but remain high, whereas S_I returns to normal levels by the end of puberty. Travers et al. (6) compared S_I in 50 Tanner stage II boys and girls with 47 Tanner stage III boys and girls. The effect of Tanner stage on S_I was significant only in girls, and neither testosterone nor estradiol was correlated with S_I. However, no other hormones were examined. In addition, this study is limited because it compared only Tanner stage II with Tanner stage III, and significant changes may have already occurred by Tanner stage II. In another study, 4 months of testosterone administration to adolescents with delayed puberty led to an increase in fat-free mass (through reductions in protein breakdown and protein oxidation), an increase in circulating testosterone (23 ± 4 to 422 ± 45 ng/dl), IGF-I (210 ± 28 to 505 ± 40 ng/ml), and mean nocturnal growth hormone (2.5 ± 0.5 to 6.0 ± 0.8 ng/ml), but had no effect on S_I (11). The lack of effect of testosterone administration on S_I in adolescents is consistent with other studies in adults (27).

The longitudinal data presented in this study are consistent with previous studies in that we did not find any correlation between the change in S_I and changes in circulating hormone levels. The only significant relationship we observed was between the change in AIR and the change in androstendione (the relationship did not reach statistical significance when analyzed separately in boys and girls, although the trend remained the same in both sexes). Androstendione is the biosynthetic precursor of testosterone and estradiol, and it is the predominant adrenal androgen in mid-puberty in both boys and girls. This hormone has been hypothesized to contribute to bone and muscle development during growth (28), but it may not be effective in adults since exogenous administration to young men had no anabolic effects on muscle protein metabolism (29). Circulating levels of androstendione gradually increase during pubertal development, with higher

levels in girls than boys; the increase in androstendione in the first half of puberty is thought to derive predominantly from the adrenal glands, with a shift to production from the gonads by the end of puberty (30). Further studies in larger samples and with more detailed hormone measures are necessary to more fully examine the role of sex hormones on insulin resistance and insulin secretion during puberty. On the basis of the current data and power analysis, we estimated that sample sizes of 46 children per subgroup would be needed to detect a significant correlation of 0.4 (similar to what we observed for the correlation between change in S_I and change in androstendione) with a power of 0.8. In addition, more extended longitudinal observations in the current cohort should also be useful to reveal which factors are associated with the recovery of insulin resistance by the end of puberty.

Our results have several implications for the prevention of type 2 diabetes during puberty. Since insulin resistance is likely to play an integral role in healthy somatic growth, we do not think that it is prudent to attempt to prevent the pubertal decline in S_I. When a more detailed mechanism is identified, it may be important to identify interventions that ensure that S_I recovers during later puberty and that β-cell function remains adequate to support this period of insulin resistance. More importantly, dietary and physical activity interventions should be explored for decreasing body fat, increasing S_I, and sustaining β-cell function before and during pubertal development, especially in those subjects with very low levels of S_I. In the present study, early pubertal development was evident in fatter, age-matched children (Table 2). Early puberty is also a known risk factor for breast cancer (31) and could be hypothesized to contribute to risk for type 2 diabetes if the pancreas is unable to compensate for the pubertal decline in S_I. Thus, early puberty and a longer period of maturation may be additional risk factors for the development of type 2 diabetes during puberty, and further studies are needed to examine these issues.

In summary, the pubertal transition from Tanner stage I to Tanner stage III or IV was associated with a 32% reduction in S_I and a lower-than-expected increase in AIR. These changes were consistent across sex, ethnicity, and obesity subgroups and were not observed in growing children remaining at Tanner stage I. The lower-than-expected rise in AIR and the significant fall in DI suggest a conservation in β-cell function or an inadequate β-cell response to the fall in S_I. The fall in S_I was not associated with changes in body fat, visceral fat, IGF-I, testosterone, or estradiol, but the increase in AIR was positively correlated with the pubertal increase in androstendione concentration.

ACKNOWLEDGMENTS

This study was supported by the National Institute of Child Health and Development (to M.I.G.: grants R29 HD 32668 and R01 HD/HL 33064), the National Institute of Aging (to B.A.G.: grant K01AG00740), and by GCRC Grant M01-RR-00032.

We are extremely grateful to Tena Hilario, who coordinated this project; the staff of the GCRC; and the children and their families who participated in this study.

REFERENCES

1. Caprio S, Tamborlane WV: Effect of puberty on insulin action and secretion. *Semin Reprod Endocrinol* 12:90–96, 1994
2. Caprio S, Cline G, Boulware S, Permnente C, Shulman GI, Sherwin RS, Tamborlane WV: Effects of puberty and diabetes on metabolism of insulin-sensitive fuels. *Am J Physiol* 266:E885–E891, 1994
3. Moran A, Jacobs DR Jr, Steinberger J, Hong C-P, Prineas R, Luepker RV, Sinaiko AR: Insulin resistance during puberty: results from clamp studies in 357 children. *Diabetes* 48:2039–2044, 1999
4. Bloch CA, Clemons PSMA: Puberty decreases insulin sensitivity. *J Pediatr* 110:481–487, 1987
5. Amiel SA, Sherwin RS, Simonson DC, Lauritano AA, Tamborlane WV: Impaired insulin action in puberty: a contributing factor to poor glycemic control in adolescents with diabetes. *N Engl J Med* 315:215–219, 1986
6. Travers SH, Jeffers BW, Bloch CA, Hill JO, Eckel RH: Gender and Tanner stage differences in body composition and insulin sensitivity in early pubertal children. *J Clin Endocrinol Metab* 80:172–178, 1995
7. Caprio S, Plewe G, Diamond MP, Simonson DC, Boulward SD, Sherwin RS, Tamborlane WV: Increased insulin secretion in puberty: a compensatory response to reductions in insulin sensitivity. *J Pediatrics* 114:963–967, 1989
8. Caprio S, Jones J, Tamborlane W: Developmental changes in insulin action and secretion in childhood health and disease. *Adv Endocrinol Metab* 5:171–201, 1994
9. Hoffman RP, Vicini P, Sivitz WI, Cobelli C: Pubertal adolescent male-female differences in insulin sensitivity and glucose effectiveness determined by the one compartment minimal model. *Pediatr Res* 48:384–388, 2000
10. American Diabetes Association: Type 2 diabetes in children and adolescents. *Pediatrics* 105:671–680, 2000
11. Arslanian S, Suprasongsin C: Testosterone treatment in adolescents with delayed puberty: changes in body composition, protein, fat, and glucose metabolism. *J Clin Endocrinol Metab* 82:3213–3220, 1997
12. Arslanian SA, Kalhan SC: Correlations between fatty acid and glucose metabolism: potential explanation of insulin resistance of puberty. *Diabetes* 43:908–914, 1995
13. Goran MI, Nagy TR, Treuth MT, Trowbridge C, Dezenberg C, McGloin A, Gower BA: Visceral fat in Caucasian and African-American pre-pubertal children. *Am J Clin Nutr* 65:1703–1709, 1997
14. Lindquist C, Gower BA, Goran MI: Role of dietary factors in ethnic differences in early risk for cardiovascular disease and type 2 diabetes. *Am J Clin Nutr* 71:725–732, 2000
15. Gower BA, Nagy TR, Goran MI: Visceral fat, insulin sensitivity, and lipids in prepubertal children. *Diabetes* 48:1515–1521, 1999
16. Ku C-Y, Gower BA, Hunter GR, Goran MI: Racial differences in insulin secretion and sensitivity in prepubertal children: role of physical fitness and physical activity. *Obes Res* 8:506–515, 2000
17. Marshall WA, Tanner JM: Variations in the pattern of pubertal changes in girls. *Arch Dis Child* 44:291–303, 1969
18. Marshall WA, Tanner JM: Variations in the pattern of pubertal changes in boys. *Arch Dis Child* 45:13–23, 1970
19. Bergman RN, Phillips LS, Cobelli C: Physiologic evaluation of factors controlling glucose tolerance in man. *J Clin Invest* 68:1456–1467, 1981
20. Goran MI, Driscoll P, Johnson R, Nagy TR, Hunter GR: Cross-calibration of body composition techniques against dual-energy X-ray absorptiometry in young children. *Am J Clin Nutr* 63:299–305, 1996
21. Nagy TR, Gower BA, Trowbridge CA, Dezenberg C, Shewchuk RM, Goran MI: Effects of gender, ethnicity, body composition, and fat distribution on serum leptin concentrations in children. *JCEM* 82:2148, 1997
22. Ahmed ML, Morrell D, Ong K, Drayer N, Perry L, Preece MA, Dunger D: Longitudinal study of leptin concentrations during puberty: sex differences and relationship to change in body composition. *J Clin Endocrinol Metab* 84:899–905, 1999
23. Bjorntorp P: Neuroendocrine perturbations as a cause of insulin resistance. *Diabetes Metab Rev* 15:427–441, 1999
24. Bergman RN: Toward physiological understanding of glucose tolerance. *Diabetes* 38:1512–1527, 1989
25. Merimee TJ, Burgess JA, Rabinowitz D: Influence of growth hormone on insulin secretion. *Diabetes* 16:478–482, 1967
26. Nielsen JH: Effects of growth hormone, prolactin, and placental lactogen on insulin content and release, and deoxyribonucleic acid synthesis in cultured pancreatic islets. *Endocrinology* 110:606, 1982
27. Friedl KE, Jones RE, Hannan CJ Jr, Plymate SR: The administration of pharmacological doses of testosterone or 19-nortestosterone to normal

men is not associated with increased insulin secretion or impaired glucose tolerance. *J Clin Endocrinol Metab* 68:975, 1989
28. Sheffield-Moore M: Androgens and the control of skeletal muscle protein synthesis. *Ann Med* 32:181–186, 2000
29. Rasmuseen BB, Volpi E, Gore DC, Wolfe RR: Androstenedione does not stimulate muscle protein anabolism in young healthy men. *J Clin Endocrinol Metab* 85:55–59, 2000
30. Williams RH: *Textbook of Endocrinology*. Philadelphia, WB Saunders, 1981
31. MacMahon B, Trichopoulos D, Brown J, Andersen AP, Cole P, DeWaard F, Kauraniemi T, Polychronopoulou A, Ravnihar B, Stormby N, Westlund K: Age at menarche, urine estrogens and breast cancer risk. *Int J Cancer* 30:427–431, 1982