Effects of Maturational Stage on Insulin Sensitivity during Puberty*

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ABSTRACT

During puberty, plasma insulin levels increase, and insulin sensitivity decreases along with multiple other physical and hormonal changes. To determine 1) the time course of the decrease in insulin sensitivity in relationship to Tanner stage of genital development, and 2) how this change relates to changes in GH secretion, insulin-like growth factor-I (IGF-I), IGF-binding protein-3, and gonadal steroid secretion, we studied 58 healthy children and adolescents (34 males and 24 females; age 7–15 yr) using overnight GH sampling and frequently sampled iv glucose tolerance tests. The insulin sensitivity index (ISI) was calculated using the program MINMOD. ISI differed significantly by Tanner stage (P < 0.05, by analysis of variance) with a decrease from Tanner stage 1 to 2 (P < 0.05). IGF-I and IGF-binding protein-3 followed opposite patterns to ISI, with lower levels in Tanner stage 1 than in stages 2–5 (P < 0.05). Mean GH levels did not increase until Tanner stage 4 (P < 0.05) and then fell during Tanner stage 5. Multiple linear regression analysis revealed negative relationships among ISI, IGF-I, and body mass index. No relationship was found with GH. We conclude that the pubertal change in ISI is not necessarily associated with increased GH secretion, but is associated with increased GH peripheral effect, as indicated by the relationship between ISI and IGF-I. (J Clin Endocrinol Metab 77: 725–730, 1993)

Subjects and Methods

Subjects

The study population consisted of 58 healthy children and adolescents (34 males and 24 females) with no family history of insulin-dependent diabetes mellitus, ranging in age from 7–15 yr. The characteristics of the subjects are shown in Table 1. The protocol was approved by the Institutional Review Board of the University of Iowa. Informed consent was obtained from a parent, and informed assent from the children. Height was measured with a wall-mounted stadiometer, and weight was determined by a balance scale. Body mass index (BMI; kilograms per m²) was used as a measure of obesity. Using the normative data of Hammer et al. (18), there were 6 subjects whose BMI was greater than 95% for age (18). Overweight subjects included 1 male each in Tanner stages 1, 2, 3, and 4 and 1 female each in Tanner stages 1 and 4. Pubertal development was assessed by the criteria of Marshall and Tanner (8, 9). Children were divided into Tanner stages according to breast development in girls and genital development in boys.

Study protocol

All subjects were admitted to the Clinical Research Center at the University of Iowa for overnight monitoring of GH and a frequently sampled iv glucose concentration in glucose uptake independent of plasma insulin changes [glucose effectiveness (GE)] (17). Cutfield et al. (16) found a decrease in insulin sensitivity at puberty using this model.

TABLE 1. Characteristics of subjects by Tanner stage

<table>
<thead>
<tr>
<th>Tanner stage</th>
<th>No. of subjects</th>
<th>Age (yr)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13 M, 8 F</td>
<td>10.0 ± 1.3</td>
<td>18.8 ± 4.0</td>
</tr>
<tr>
<td>2</td>
<td>2 M, 6 F</td>
<td>10.9 ± 1.4</td>
<td>19.8 ± 3.6</td>
</tr>
<tr>
<td>3</td>
<td>4 M, 1 F</td>
<td>12.5 ± 1.3</td>
<td>21.8 ± 6.2</td>
</tr>
<tr>
<td>4</td>
<td>11 M, 5 F</td>
<td>13.5 ± 1.3</td>
<td>20.2 ± 4.6</td>
</tr>
<tr>
<td>5</td>
<td>1 M, 3 F</td>
<td>18.9 ± 0.8</td>
<td>21.6 ± 1.8</td>
</tr>
</tbody>
</table>

Values are the mean ± SD.
sampled iv glucose tolerance test (FSIVGTT) (19-21). On arrival, each child had a complete physical exam and a radiograph of the left hand and wrist. The Greulich and Pyle atlas (22) was used to determine bone age. Each child's bone age was within the mean ± 2 SD for chronological age.

All subjects were fasted from 2000 h until completion of the testing. Approximately 2 h before sampling began, an iv catheter was inserted into a forearm vein. Blood was withdrawn through this catheter every 15 min from 2200 h to 0800 h the following morning. At 0800 h, blood was obtained for IGF-I, IGFBP-3, and testosterone or estradiol measurements.

A second iv catheter was then placed in a contralateral forearm vein for the administration of dextrose (0.3 g/kg) at 0 min and tolbutamide (5 mg/kg) at 20 min. From the opposite iv catheter, blood was obtained at -10, 0, 2, 4, 6, 8, 10, 12, 14, 16, 19, 22, 27, 32, 42, 52, 62, 72, 82, and 92 min for the measurement of insulin and glucose levels.

**Laboratory methods**

Plasma glucose concentrations were measured by a YSI model 2300 glucose analyzer (Yellow Springs Instruments, Inc., Yellow Springs, OH).

Serum GH concentrations were determined in duplicate using Diagnostic Products GH RIA kits (Los Angeles, CA). The intra- and interassay coefficients of variation for GH were 6% and 9%, respectively. WHO First International Reference Preparation 66/217 was used. The GH assay has a cross-reactivity of 0.6% for PRL.

Serum insulin concentrations were measured by RIA. The intra- and interassay coefficients of variation were 5.3% and 9.4%, respectively.

Serum testosterone and estradiol concentrations were determined in duplicate using Diagnostic Product RIA kits. The lower limits of detectability were 35 nmol/L for testosterone and 36.7 pmol/L for estradiol. IGF-I was measured by RIA after acid-ethanol extraction (Endocrine Sciences, Calabasas Hills, CA), and IGFBP-3 was determined by RIA specific for the acid-stable binding subunit (Endocrine Sciences).

**Data analysis**

The insulin sensitivity index (ISI) was calculated from plasma glucose and insulin levels using the minimal model method by the computer program MINMOD. This method uses a series of iterations to solve two differential equations based on the rate of glucose disposal and insulin entry into a third space compartment. The zero minute plasma insulin and glucose concentrations were used as the 180 min values required by the MINMOD program for analysis (16, 17). In one Tanner 1 male, a low insulin response to glucose prevented the calculation of ISI. This subject was dropped from analyses involving ISI.

The mean GH concentration, GH area under the curve (GH AUC), and GH pulse frequency were calculated using the Cluster pulse detection computer algorithm that was developed and kindly furnished by Drs. Michael Johnson and Johannes Veldhuis (23).

Two-way analysis of variance (ANOVA) was used to evaluate differences in variables between sexes and Tanner stages. Stepwise multiple linear regression was used to determine which factors among BMI, IGF-I, IGFBP-3, mean GH, and plasma testosterone and estradiol levels best predicted an individual's ISI and GE.

**Results**

Plasma glucose concentrations during the FSIVGTT did not differ by sex or Tanner stage (Fig. 1a). ANOVA of repeated measures revealed that boys had significantly higher overall plasma insulin levels ($P < 0.05$) and plasma insulin responses to glucose loading over the 20 min before tolbutamide administration (sex by time interaction, $P < 0.01$; Fig. 1b) than girls. Plasma insulin levels were higher in Tanner stages 3, 4, and 5 than in Tanner stages 1 and 2, and the response to iv glucose was also higher in these groups (Tanner stage by time interaction, $P < 0.01$; Fig. 1c). The pubertal increase in insulin did not vary between the sexes (sex by Tanner stage interactions, $P = NS$).

Two-way ANOVA revealed that ISI (Fig. 2a) varied significantly with Tanner stage ($P < 0.02$), but not with sex. The change in ISI with Tanner stage did not differ with sex (sex by Tanner stage interaction, $P = 0.32$). Planned contrasts revealed that ISI in Tanner stage 1 was significantly higher than that during Tanner stages 2-5 ($P < 0.01$). ISI tended to decrease further after Tanner stage 2, but these changes did not reach statistical significance. GE did not differ by sex or Tanner stage (Fig. 2b).

IGF-I, IGFBP-3, and mean GH levels all varied signifi-
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IGF-I and IGFBP-3 were significantly greater in Tanner stages 2-5 than in Tanner stage 1 (P < 0.05). IGF-I levels also significantly differed by sex, with higher levels in males (by ANOVA, P < 0.05). Females had their highest IGF-I levels during Tanner stage 4, whereas males had the highest level during Tanner stage 5. This difference in IGF-I pattern by Tanner stage between sexes did not quite reach statistical significance (sex by Tanner stage interaction, P = 0.08). Mean GH was greater during Tanner stage 4 than during the other Tanner stages (P < 0.05) in the group as a whole. GH AUC followed a similar pattern to mean GH, but GH pulse frequency did not vary across Tanner stage. In males, testosterone levels were significantly higher in Tanner stages 2-5 than during Tanner stage 1 (P < 0.05; Table 2). Estradiol levels did not significantly vary with Tanner stage (Table 2). BMI did not vary by sex or Tanner stage, as shown in Table 1.

A significant negative relationship was found between BMI and mean GH (r = -0.31; P < 0.05), whereas a significant positive relationship was found between BMI and IGF-I (r = 0.28; P < 0.05). Both IGF-I (r = 0.54; P < 0.001) and IGFBP-3 (r = 0.29; P < 0.05) increased as the mean GH increased.

To explore possible causal relationships between ISI and the various other hormonal and physical parameters, stepwise multiple linear regression was performed using ISI as the independent variable and BMI, IGF-I, IGFBP-3, and mean GH levels as dependent variables. In all subjects, the best fit for the data was found to include BMI and IGF-I (r² = 0.42; P < 0.001). ISI decreased as both BMI (P < 0.001; Fig. 4a) and IGF-I (P < 0.01; Fig. 4b) increased. Only BMI was found to be a significant predictor of GE (r² = 0.18; P < 0.01). GE decreased as BMI increased. The relationships among ISI, GE, and BMI did not significantly change when those subjects with BMI greater than the 95% for age were not included in the analysis.

Similar multiple linear regression analysis was performed
in each sex, with the addition of either plasma estradiol or testosterone levels as dependent variables. In girls, BMI and mean GH, but not IGF-I, IGFBP-3, or estradiol, were found to be significant predictors of ISI (r² = 0.61; P < 0.01). ISI decreased as both BMI (P < 0.001) and mean GH (P = 0.013) increased. Estradiol levels were positively correlated with IGF-I (r = 0.69; P < 0.01), but not with mean GH. Again, only BMI was found to significantly predict GE (r² = 0.30; P < 0.01).

In boys, BMI and testosterone, but not mean GH, IGF-I or IGFBP-3, were found to be significant predictors of ISI (r² = 0.36; P < 0.001). Again, ISI decreased as BMI increased (P < 0.01). ISI was negatively related to plasma testosterone as well (P = 0.025). Plasma testosterone levels were positively correlated with IGF-I levels (r = 0.40; P < 0.05) and mean GH levels (r = 0.62; P < 0.01). GE, again, was related only to BMI (r² = 0.05; P < 0.05).

Discussion

Previous studies of insulin sensitivity in adolescence have not divided puberty into stages based on sexual maturation and, thus, have not been able to determine how insulin sensitivity changes during the course of puberty. We found that the pubertal decrease in insulin sensitivity occurs early in puberty. ISI decreased significantly between Tanner stage 1 and Tanner stage 2. ISI tended to decrease further in Tanner stages 3, 4, and 5. ISI did not differ by sex. The changes in insulin sensitivity followed a pattern opposite that of changes in IGF-I, IGFBP-3, and testosterone, which all increased significantly from Tanner stage 1 to Tanner stage 2. Mean GH levels followed a slightly different pattern, in that mean GH levels were not statistically elevated until Tanner stage 4.

The abbreviated minimal model method has previously been shown to be a valid technique for the measurement of insulin sensitivity in children (16). A limitation of this technique is the inability to measure insulin sensitivity when insulin release was low, which may be a problem in prepubertal children. In spite of this limitation, we were able to quantify insulin sensitivity in all but one subject.

BMI was found to be a negative predictor of ISI in all subjects and in individual subsets of subjects assigned by sex. The negative relationship between insulin sensitivity and BMI is consistent with previous studies that have demonstrated insulin resistance in obese adults and children (24, 25). Furthermore, because we found a negative relationship between GE and BMI, this indicates that obesity impairs not only insulin-mediated glucose uptake but noninsulin-mediated glucose uptake as well. The negative relationship between BMI and ISI was present even when markedly overweight subjects were excluded. Because insulin resistance has been implicated in the pathogenesis of hypertension and a variety of cardiovascular disorders in adults (26), this increased insulin resistance in obese children and adolescents may be a poor prognostic sign. The negative relationship between BMI and ISI does not appear to be responsible for the pubertal decrease in ISI, because BMI did not vary by Tanner stage.

Multiple linear regression found IGF-I, but not mean GH, to be a significant predictor of ISI in the group as a whole, although IGF-I was strongly correlated with mean GH. Bloch et al. (6) and Caprio et al. (7) have previously demonstrated a negative relationship between insulin sensitivity and IGF-I using insulin and glucose infusion clamp techniques. Neither of these two studies directly assessed GH secretion. On the other hand, Amiel et al. (5), in a combination of healthy and diabetic children, found no relationship between insulin sensitivity and IGF-I, but did find a significant relationship between insulin sensitivity and mean GH. Arslanian et al. (27) found increased GH secretion and decreased insulin sensitivity in adolescent females compared to males with insulin-dependent diabetes mellitus. We found no sex differences in ISI in our population of healthy children and adolescents.

It is unlikely that IGF-I itself is the cause of the insulin resistance seen at puberty because IGF-I cross-reacts with and stimulates the insulin receptor and has been shown to have a hypoglycemic effect when given to humans (28) and animals (29). More likely, IGF-I is marker of the peripheral and hepatic effects of GH (30, 31). The fact that no relationship was seen between mean GH and ISI may be due to the fact that BMI and mean GH were negatively correlated, whereas BMI and IGF-I were positively correlated, thus indicating that body habitus may play a role in altering the peripheral effect of GH. Cordido et al. (32) found that obese and lean adults have similar IGF-I levels in spite of having markedly lower basal and peak GH levels in response to
binding protein, as Martha et al. (33) found that the GH-binding protein increases as BMI increases.

The relationship between ISI and IGF-I was not found when the data from each sex were analyzed separately. In males, BMI and plasma testosterone levels were negative predictors of ISI, whereas in females, BMI and mean GH negatively predicted ISI. Previous studies in animals and adult humans on the effects of sex steroids have also shown similar variability (34-38). In children, Bloch et al. (6) found a negative relationship between sex steroid and GH secretion. We found a positive relationship between both the effects of sex steroids in males and females may be due to the relationship between sex steroid and GH secretion. We found a positive relationship between both testosterone and estradiol and IGF-I, but only testosterone significantly correlated with mean GH. This finding suggests that the testosterone effect may be mediated through increasing GH levels.

One limitation of our study is the small number of subjects in the higher Tanner stages, which may influence our ability to detect small differences between Tanner stages or differences between sexes. A second limitation may the overnight variability in the mean GH pattern (39), although overnight measurement of plasma GH levels has been found to be more reliable than provocative stimulation (40). The patterns of mean GH and IGF-I in our study are very similar to those reported by others. Several studies (11, 15) have demonstrated an increase in GH secretion in mid- to late puberty, with a fall after Tanner stage 4. Interestingly, in our study, this drop in mean GH was not associated with a fall in IGF-I or IGFBP-3. Rosenfeld et al. (41) and Luna et al. (42) reported similar findings, with a rise in IGF-I during early Tanner stage 5, but a fall in adults. Our subjects were adolescents and, thus, in early Tanner stage 5.

In conclusion, we found a significant fall in insulin sensitivity early in puberty, with a trend toward further decreases as Tanner stage progressed. The time course of the change in ISI was opposite that of changes in IGF-I, IGFBP-3, and gonadal steroids. BMI was the best predictor of ISI in all subjects and in both girls and boys when analyzed separately. This relationship to BMI does not appear to explain the pubertal differences in ISI, because BMI did not vary with changes in Tanner stage. In the group as a whole, IGF-I, but not mean GH, also, negatively predicted ISI. Thus, the pubertal decrease in insulin sensitivity is not necessarily caused by increased GH secretion, but is associated with increased peripheral GH effect. The mechanism of this increased GH effect may differ between girls and boys, because decreased ISI was associated with increased sex steroid secretion in boys, but not in girls, and increased mean GH in girls, but not in boys. Further studies will be needed to explore the cellular mechanism responsible for this dissociation between GH effect and secretion and to assess the role of the increased insulin resistance and accompanying increase in insulin secretion on pubertal growth.

References
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