Is Obesity-Related Insulin Status the Cause of Blunted Growth Hormone Secretion in Turner's Syndrome?

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Growth hormone (GH) secretion is reduced in girls with Turner's syndrome (TS) at pubertal age. We have recently proposed that the impairment of GH release in TS girls might be secondary to obesity. In the present study, we assessed the influence of overweight-related insulin status on spontaneous GH secretion in a group of 15 TS girls. Eighteen age-matched short normal subjects and six short obese prepubertal children were chosen as controls. Anthropometry, spontaneous GH secretion, insulin-like growth factor-I (IGF-I) serum levels, basal fasting insulin, and glucose concentrations were determined. The percentage of ideal body weight (IBW) was used as an index of nutritional status. Baseline fasting glucose (milligrams per deciliter) to insulin (milliunits per liter) ratio (G/I) was chosen as an index of insulin resistance. GH secretion was significantly lower in TS girls than in non-obese children ($P < .005$), whereas no significant difference was seen between TS and obese subjects. IGF-I levels were not statistically different in all groups. GH secretion was confirmed to be related to the degree of overweight ($r = -.52$, $P < .05$ in TS girls and $r = -.74$, $P < .0001$ in control group). G/I was closely related to both the percentage of IBW ($r = -.59$, $P = .02$) and GH level ($r = .57$, $P = .03$) in TS patients. These results confirm that the blunted GH secretion in TS patients is dependent on nutritional status, and suggest that insulin resistance secondary to overweight might represent the pathophysiologic link between the obesity-related metabolic status and impaired GH secretion.

GROWTH HORMONE (GH) secretion has been extensively investigated in girls with Turner's syndrome (TS) in an attempt to understand the pathophysiology of the growth failure. Conflicting data exist on GH pituitary reserve explored by pharmacologic stimulation tests: some patients show normal GH responses, whereas others respond in a subnormal fashion.[1-4]

Due to these controversial results obtained by testing GH pituitary reserve, several studies have more recently focused on the assessment of spontaneous GH secretion. A progressive decline in GH secretion was initially described in TS girls older than 9 years,[5] whereas other studies have subsequently shown either a normal GH output[6,7] or a lack of the age- and puberty-related increase of GH secretion.[8] A possible explanation for these discrepancies is that other variables such as sex steroids and nutritional or metabolic status might have either independent or combined effects on GH release.

We have recently reported that TS girls have a spontaneous GH secretion somewhere between that of normal children and "classic" GH-deficient patients.[9] According to a previous report,[10] in our patients GH levels were negatively related to the severity of overweight,[9] another typical finding in TS girls,[11] which thus resembles the condition in otherwise normal obese children who show a blunted GH secretion.[12]

The aim of this study was to investigate the relationship between obesity-related insulin status and spontaneous GH release in TS girls.

SUBJECTS AND METHODS

Study Population

Fifteen prepubertal girls (mean ± SD age, 10.6 ± 2.6 years) diagnosed with TS by karyotype analysis were studied. Ten had a karyotype of 45,X, one was 46,Xi(Xq), one 45,X/46,Xi(Xq), one 45,X/46,Xi(Xp), one 45,X/46,X,r(X), and one 45,X/46,XX. None of them had received GH, oxandrolone, or ethinyl estradiol in the previous 6 months.

The retrospective control group consisted of 18 prepubertal age-matched children (four girls; mean ± SD age, 10.7 ± 2.5 years) and six short overweight but otherwise normal prepubertal children (four girls; mean ± SD age, 9.6 ± 1.3 years) with growth retardation due to familial short stature and/or constitutional growth delay.[9] According to the literature, in fact, boys and girls do not show any significant difference in GH release before puberty.[13]

Informed consent was obtained from parents of all the children.

Methods

Anthropometric measurements were performed according to standard procedures.[14] Height, height velocity, and pubertal status were evaluated according to Tanner's growth standards.[15] Bone age was estimated by the Greulich-Pyle method.[16] The percentage of ideal body weight (IBW) was chosen as an index of the degree of obesity.[17]

Spontaneous GH secretion was assessed as previously described.[18] Briefly, a catheter was inserted into an antecubital vein, and blood samples for measurement of GH concentrations were drawn at 30-minute intervals from 8 PM to 8 AM the next morning. The mathematical mean of GH levels in these 25 samples, amplitude of the maximal peak, and area under the GH curve were computed. Since the different parameters yielded overlapping results, the mean 12-hour serum GH concentration was chosen as the index of spontaneous GH secretion.[18] Pituitary GH reserve was investigated using clonidine 150 μg/m^2 body surface area administered orally. A peak GH response greater than 10 μg/L was considered normal.

The baseline fasting glucose (milligrams per deciliter) to insulin (milliunits per liter) ratio (G/I) was chosen as an index of insulin resistance, since it is a simple, reliable, and noninvasive method to investigate insulin sensitivity.[19]

Serum GH levels were measured using a commercial radioimmunoassay kit (hGH kit, liso-phase; Technogenetics, Milano, Italy). Sensitivity of the assay was 0.2 μg/L. Interassay and intraassay

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Table 1. Clinical and Endocrine Features of TS Patients and Controls (mean ± SD)

	TS Patients (n = 15)	Controls	
		Non-obese (n = 18)	Obese (n = 6)
Chronologic age, (yr)	10.6 ± 2.6	10.7 ± 2.5	9.6 ± 1.3
Bone age, (yr)	8.6 ± 2.5	8.8 ± 2.4	8.1 ± 2.2
Height (SD)	−3.1 ± 0.8	−2.4 ± 0.5*	−2.2 ± 0.2*
Height velocity	−2.6 ± 0.7	−1.0 ± 0.7†	−0.9 ± 0.8†
% IBW	121.8 ± 24	98.5 ± 9.2†	131.5 ± 15.8
Mean 12-hour GH level (μg/L)	3.45 ± 1.8	5.5 ± 1.3†	2.4 ± 1.0
Basal insulin level (mU/L)	13 ± 18.3	ND	ND
G/I (mg/dL:mU/L)	20.9 ± 27.4	ND	ND
IGF-I (μg/L)	143.4 ± 102	200.7 ± 161	175.4 ± 105

*$P < .01$, †$P < .001$: Differences between TS and control means.
Abbreviation: ND, not determined.

coefficients of variation were 4.8% to 7.2% and 2.9% to 5.7%, respectively.

Plasma insulin-like growth factor-I (IGF-I) concentrations were assessed after acid-ethanol extraction by a commercial radioimmunoassay kit (Nichols Institute, San Juan Capistrano, CA). Sensitivity of the assay was 13.5 μg/L. Interassay and intraassay coefficients of variation were 5.2% to 8.4% and 2.4% to 3.0%, respectively.

Serum insulin level was determined by a commercial radioimmunoassay (Coat-A-Count Insulin; Diagnostic Products, Los Angeles, CA). Sensitivity of the assay was 1.5 mU/L. Interassay and intraassay coefficients of variation were 4.9% to 10% and 3.5% to 12%, respectively.

Blood glucose level was determined by a hexokinase method.

Statistics

Significance of the difference between means was assessed using an unpaired two-tailed t test and one-way ANOVA. Significance was assigned for P less than .05. The relationship between parameters was evaluated by forward stepwise regression analysis. All independent variables were assessed step by step, and only those with a significant t value ($P < .05$) were included in the final regression model. All data were logarithmically transformed before regression analysis. A computer program was used for all statistical calculations SOLO 3.0; BMPD Statistical Software, Los Angeles, CA).

RESULTS

No significant difference was found in chronologic age and bone age between TS patients and non-obese controls, whereas there was a significant difference in height ($P < .005$), height velocity ($P < .0001$), and percentage IBW ($P < .001$; Table 1). All non-obese controls had weight appropriate for height (percentage IBW, 80% to 120%), whereas eight of 15 TS girls were obese, showing a percentage IBW above 120%. Overweight control subjects were significantly taller than TS girls ($P = .01$), whereas mean percentage IBW was not statistically different (Table 1).

All non-obese controls showed a normal peak GH response to a clonidine stimulation test (>10 μg/L). Four of six obese controls and 12 of 15 TS girls showed a GH peak less than 10 μg/L. Spontaneous GH levels were significantly lower in TS girls than in non-obese controls (mean ± SD, 3.5 ± 1.8 μg/L in TS girls v 5.5 ± 1.3 in controls, $P < .001$). In overweight controls, mean 12-hour GH concentration was significantly lower than in non-obese controls (2.4 ± 1.0 μg/L, $P < .01$) and was not statistically different from that in TS patients. There was no significant difference in IGF-I concentrations between TS patients and controls (mean ± SD, 143.4 ± 102 μg/L in TS girls, 200.7 ± 161 in non-obese, and 175.4 ± 105 in obese controls).

In TS patients, GH levels were inversely related to percentage IBW ($r = -.52$, $P < .05$; Fig 1). Analyzing together data from normal and overweight controls, GH levels were shown to be closely related to percentage IBW ($r = -.74$, $P < .0001$).

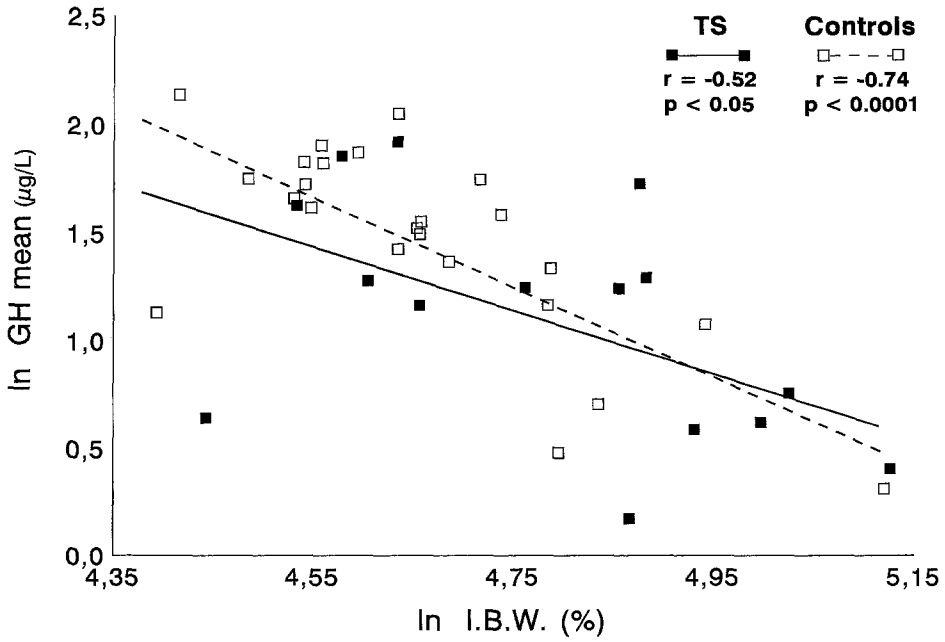

Fig 1. Inverse linear correlation between mean 12-hour nocturnal GH level (log_e GH mean) and percentage IBW (log_e IBW) in TS girls and controls.

According to Caro,[19] a G/I less than 6 was chosen as characteristic of subjects with insulin resistance. Five of 15 TS girls had a G/I less than 6. G/I was negatively related to percentage IBW ($r = -.59$, $P = .02$) and positively related to mean GH concentration ($r = .57$, $P = .03$; Fig 2). Also, basal insulin concentration significantly correlated with percentage IBW ($r = .57$, $P = .03$) and GH level ($r = -.55$, $P = .03$). The relationship between IBW, bone age, IGF-I, and G/I (independent variables) and mean GH concentration (dependent variable) was evaluated by regression analysis. Only G/I was significant ($t = 2.48$, $P < .03$) and was included in the final regression equation ($y = 0.42 + 0.28x$, $R^2 = .32$, $F = 6.16$, $P = .027$).

DISCUSSION

Reduced GH secretion is usually found in girls with TS. This finding, together with the presence of severe growth failure, has stimulated clinical trials with biosynthetic GH.[20] Preliminary results of these therapeutic trials have been encouraging, and treatment of TS has become the second worldwide-accepted indication for GH therapy. However, the rationale of GH treatment in TS is still debatable.[21]

We have recently reported that GH secretion in girls with TS is intermediate between that of normals and "classic" GH-deficient patients, being inversely related to the severity of overweight.[9] This finding led us to speculate that the reduced GH secretion might be related to the obesity. Normal obese subjects show a blunted GH secretion,[12] which normalizes after weight loss.[22] Kelijman and Frohman[23] have reported in obese adults a normalization of GH secretory status after a 3-day fast, thus indicating that the rapid change of metabolic status rather than weight loss per se might restore normal GH secretion. A strong negative correlation between GH level and degree of adiposity has been recently shown also in normal short-stature children.[24] Accordingly, our results show a close inverse relationship between spontaneous GH secretion and percentage IBW in controls and TS girls, with GH levels being subnormal in both TS patients and obese controls.

Obese subjects have been shown to have at least a dual defect leading to subnormal GH concentrations: reduced pituitary GH secretion and enhanced GH metabolic clearance.[25] Unlike otherwise normal obese subjects, TS girls have been found to have a decreased clearance of GH,[26] suggesting that the reduction of GH circulating levels is primarily due to a blunted pituitary release. We have found that G/I, a good and simple indicator of insulin resistance,[19] is closely related both to the degree of overweight and to GH level. These results indicate that obesity-related insulin status might be involved in inhibition of GH secretion.

In obese adults, GH secretion has been recently reported to be related to insulin levels and more closely to the degree of insulin resistance,[27] with multiple mechanisms being potentially involved in the insulin-dependent regulation of GH secretion. Insulin has been reported to suppress GH secretion by rat pituitary cells[28] and to regulate *in vitro* expression of the rat GH gene, exerting either a positive or negative action according to the metabolic status of the cell.[29]

Another mechanism advocated to explain the insulin-dependent inhibition of GH secretion is the induction of expression of GH receptors. TS girls, similar to obese subjects, have been shown to have high levels of GH-binding protein,[30,31] which reflects cellular GH receptor status.[32] GH-binding protein has been found to be positively related to nutritional status.[31,33] Streptozotocin-treated diabetic rats show a marked reduction of GH binding to liver receptors, which normalizes after insulin therapy,[34] and obese Zucker rats show an increase in the

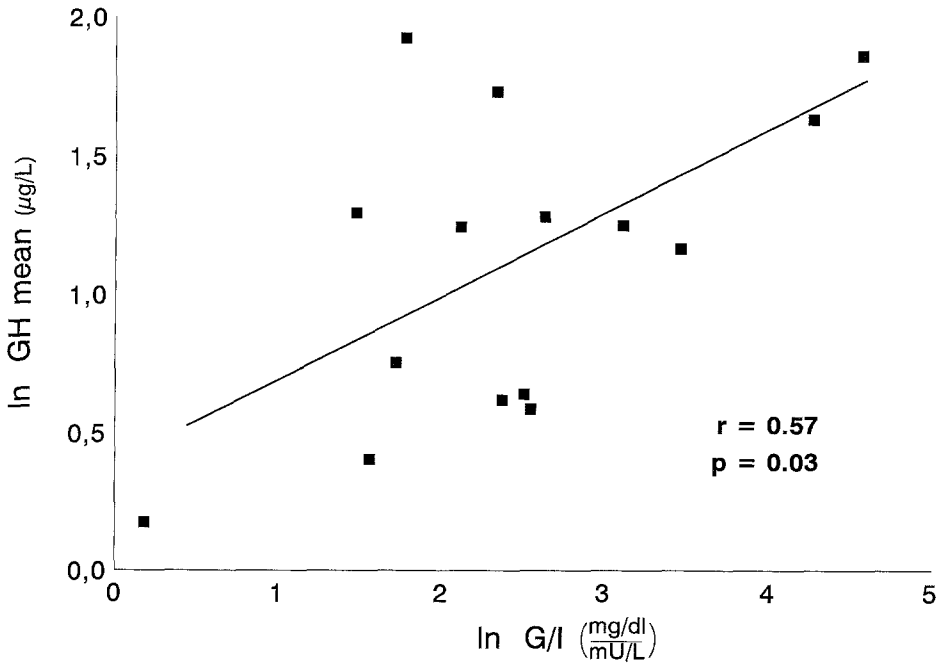

Fig 2. Positive linear correlation between G/I (log_e G/I) and mean 12-hour nocturnal GH level (log_e GH mean) in TS girls.

number of hepatic GH-binding sites shortly after the onset of hyperinsulinemia.[35] In man, a close positive relationship between serum levels of GH-binding protein and insulin dose per kilogram body weight has been recently shown in children and adolescents with insulin-dependent diabetes mellitus.[36] Taken together, these findings suggest that TS girls and obese subjects might have an insulin-induced overexpression of GH receptors. The increased number of receptors might enhance the GH negative feedback on the neuroendocrine control system, reducing the threshold of the inhibitory effect and thus eventually leading to reduced GH secretion.

The obesity-related hyperinsulinism might reduce GH output also by affecting biologic activity of IGFs. IGFs circulate bound to at least six different binding proteins (termed IGFBP-1 through IGFBP-6) that prolong IGF half-life, allocate IGFs into the various tissues, and modulate IGF bioactivity.[37] IGFBP-1 serum levels are inversely related to insulin,[38] which negatively regulates IGFBP-1 mRNA transcription.[39] Obese subjects show an insulin-dependent reduction of IGFBP-1 levels.[40,41] Since IGFBP-1 modulates IGF bioactivity, exerting an inhibitory effect,[42] it is possible to speculate that the insulin-induced reduction of IGFBP-1 levels might enhance IGF bioactivity, including the negative feedback on GH secretion. According to this hypothesis, we previously reported a close inverse relationship between IGFBP-1 level and nutritional status in TS patients.[43]

The above-proposed insulin-dependent enhancement of both GH sensitivity and IGF bioactivity in obese children might explain their tall stature and advancement of bone age, whereas it appears in contrast to the growth failure and delayed skeletal maturation of TS girls. However, the presence of skeletal dysplasia in TS might account for a peripheral resistance to GH and IGF growth-promoting actions.[44]

In conclusion, the confounding effect of obesity (and the related insulin resistance) on GH secretory status should be taken into account when interpreting the results of GH pituitary-reserve investigations in TS girls and in otherwise normal obese subjects, and when deciding whether to start them on GH treatment.

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