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Pubertal alterations in growth and body composition: IX. Altered spontaneous secretion and metabolic clearance of growth hormone in overweight youth

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Abstract

Deconvolution analysis was used to determine 12-hour spontaneous nocturnal growth hormone (GH) secretion and GH half-life in lean (body mass index, <85th percentile; n = 39) and overweight (body mass index, ≥85th percentile; n = 18) youth. The integrated GH concentration, GH burst mass, and half-life were lower ($P < .05$) in overweight than in lean youth. For each unit increase in percentage of body fat, integrated serum GH concentrations, secretory burst mass, and half-life declined by 83.6 μg/L per minute ($r = -0.39$, $P < .01$), 0.22 μg/L ($r = -0.28$, $P < .05$), and 0.2 minute ($r = -0.38$, $P < .01$), respectively. The effect of overweight on GH secretion was independent of pubertal status. Hierarchical regression models tested the hypothesis that altered GH secretion in youth is more related to total adiposity than abdominal visceral fat. When age, sex, fat-free mass, testosterone, and estradiol were held constant, the sequential addition of abdominal visceral fat did not increase R^2 for any GH secretion variable. Sequential addition of percentage of body fat increased R^2 ($P < .05$) for integrated GH concentration, total secretory rate, secretory burst mass, and pulsatile production rate. We conclude that serum GH concentrations are reduced in overweight youth primarily because of reduced GH burst mass with no change in the number of secretory events and secondarily to reduced GH half-life. Based on the model that GH-releasing hormone predominantly increases GH pulse amplitude whereas somatostatin primarily controls GH pulse frequency, these results suggest that overweight in youth diminishes GH-releasing hormone stimulation resulting in truncated GH bursts but does not alter the number of somatostatin withdrawal intervals so that GH burst frequency is conserved.

1. Introduction

There are large individual differences in circulating growth hormone (GH) concentrations, and a primary factor influencing GH concentrations is adiposity. In adults, mean 24-hour serum GH concentrations decrease with increases in central and total adiposity [1-4]. The potential result of diminished GH concentrations is reduced GH-induced lipolysis and increased fat accrual at the same energy intake, leading to even greater reductions in GH concentrations. Circulating GH concentrations are a function of the rate of GH secretion and its metabolic clearance. The neuroregulation of GH secretion determines the number and amplitude of GH secretory events through the interplay of GH-releasing hormone (GHRH), somatostatin, insulin, and insulin-like growth factor (IGF)–I, and metabolic signals such as free fatty acids, glucose, and amino acids [5,6].

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The alterations in neuroregulation of GH secretion that occur with obesity are not yet fully understood. Deconvolution analysis allows for quantification of the number, mass, duration, and amplitude of GH secretory events and offers indirect information regarding GH neuroregulation [7,8]. Deconvolution analysis has demonstrated that alterations in GH secretion of obese adults include a reduced mass of GH secreted per burst possibly because of reduced GHRH stimulation of the somatotropes and, to a smaller extent, decreased endogenous GH half-life [5,6,9-13]. The influence of adiposity on the neuroregulation of GH secretion in adults has been independently confirmed from the approximate entropy (ApEn) metric, which demonstrates greater irregularity of GH secretion in the obese, suggesting greater feedback activity within the axis [5-7,14]. In contrast, there are very few data on GH neuroregulation of overweight youth, and data from adults may not be directly applicable to youth because the neuroregulation of GH secretion likely differs in youth and adults [15,16].

Inverse relationships have been reported between adiposity and GH concentrations in youth [17,18], but neither the neuroregulation of spontaneous GH secretion nor its half-life have been compared in lean and overweight youth. The influence of abdominal visceral fat (AVF) on GH secretion of youth has not been widely studied, so it is unclear if the consistent inverse relationship between AVF and GH secretion found in adults [1-4] is also present in youth.

Pubertal maturation may influence the relationship between adiposity and GH secretion. Pubertal increases in total daily GH secretion are accompanied by altered neuroregulation of GH secretion [19], so the relationships between adiposity and GH secretion may be quite different in prepubertal and pubertal youth. Contrasting the neuroregulation of GH secretion in lean and overweight prepubertal and pubertal youth is important for understanding the mechanisms of lower serum GH concentrations in overweight youth.

Therefore, the purpose was to determine differences in the neuroregulation of spontaneous nocturnal GH secretion and GH half-life of lean and overweight prepubertal and late-adolescent youth, and to determine the relationships among total and regional adiposity and GH secretion. This was accomplished by combining serial 10-minute blood sampling, an ultrasensitive chemiluminescence GH assay, deconvolution analysis, the ApEn metric, an accurate 4-compartment model of body composition, and determination of AVF by magnetic resonance imaging. We hypothesized that (*a*) overweight youth would have lower mean GH concentrations resulting from a reduced mass of GH secreted per burst and decreased half-life of endogenous GH, and (*b*) greater irregularity of GH secretion as indicated by increased ApEn; and that (*c*) overweight pubertal youth would have a greater disruption of normal GH secretion than overweight prepubertal youth and (*d*) pulsatile GH secretion and the regularity of GH secretion would be inversely related to total adiposity but not related to AVF.

2. Methods

2.1. Subjects

Prepubertal (n = 19) and pubertal (n = 20) lean (body mass index [BMI], <85th percentile for sex and age [20]) youth, and prepubertal (n = 8) and pubertal (n = 10) youth at risk for overweight or overweight (BMI, ≥85th percentile for sex and age) were evaluated. Subjects were participating in a longitudinal study of the endocrine control of growth, maturation, and body composition. This article consists of a cross-sectional analysis of the data. Study entry criteria required a height and height velocity within 2 SDs of the mean for chronological age and sex. Height was measured as recommended [21], and bone age was determined by the Fels method [22] by an experienced assessor (JNR). Stage of secondary sex characteristics was assessed by a trained pediatric endocrinologist by the method of Tanner [23]. Prepubertal boys and girls were stage I for genital and breast development, respectively. Pubertal boys and girls were stage IV or V for genital and breast development, respectively. Written informed consent was obtained from a parent and written assent was obtained from each child before entrance into the study. The sex distribution of each subject group is given for descriptive purposes. The specific effects of sex and maturation on GH secretion of these youth have been described [19], but the important effect of adiposity on GH secretion and metabolic half-life was not considered. We present the effects of adiposity on GH secretion in prepubertal and pubertal youth.

2.2. Clinical protocol

Subjects were admitted to the University of Virginia General Clinical Research Center at 8:00 AM. Starting with breakfast, the subjects consumed meals and snack that are constant for energy, fat (30% of calories), protein (15% of calories), and carbohydrate (55% of calories) at standard times. All subjects had a normal medical history, physical examination, and screening blood tests of hepatic, renal, hematologic, metabolic, and electrolytic function. No subjects had acute illness or chronic disease. A catheter was inserted into a forearm vein at 4:00 PM and kept patent with a heparin lock. Serial blood sampling (every 10 minutes) was initiated at 6:00 PM and continued until 6:00 AM. Activity was limited to walking and rest, and the subjects remained in bed with the lights out after 10:00 PM. A single morning blood sample was withdrawn at 6:00 AM for screening endocrine measurements, including testosterone, estradiol, and IGF-I.

2.3. Assays

Serum GH concentrations were determined in each sample in duplicate using an automated Nichols Luma Tag hGH chemiluminescence assay (Nichols Institute, San Juan Capistrano, Calif) modified for ultrasensitivity. All 73 serum samples for each subject were assayed together. Use of the assay in our laboratory has been previously described [7,24].

The sensitivity of the assay is 0.005 μg/L. The intra-assay coefficients of variation (CVs) are 4.9% at 0.2 μg/L, 6.7% at 2 μg/L, and 6.4% at 4.9 μg/L, whereas the interassay CVs were 7.2% at both 1.7 μg/L and 4.2 μg/L. Insulin, testosterone, and estradiol were measured by radioimmunoassay kits (Diagnostic Products Corporation, Los Angeles, Calif). The sensitivity of the insulin assay was 1.3 μIU/mL, with an intra-assay CV of 8.3% to 6.4% within the range of 4.8 to 54.6 μIU/mL and interassay CV of 12.2% to 4.7% within the range of 4.9 to 52.9 μIU/mL. The sensitivity of the testosterone assay was 10.0 ng/dL, with an intra-assay CV of 5% to 6% within the range of 100 to 800 ng/dL and interassay CV of 9.2% to 12.9% within the range of 70 to 840 ng/dL. The sensitivity of the estradiol assay was 10.0 pg/mL, with an intra-assay CV of 4% to 7% within the range of 50 to 1100 pg/mL. The interassay CV ranged from 4.2% to 8.1% within the range of 50 to 1025 pg/mL. Estradiol concentrations were not considered in relation to ovarian cycles. Insulin-like growth factor–I concentrations were measured by radioimmunoassay (Nichols Institute) after acid ethanol extraction and had an intra-assay CV of 2.4% and 3.0% at 0.53 ng/mL and 0.92 ng/mL and an interassay CV of 5.2% and 8.4% at 0.54 ng/mL and 0.82 ng/mL. The sensitivity was 0.06 ng/mL.

2.4. Deconvolution analysis

Basal and pulsatile GH secretion was determined by a multiparameter deconvolution technique [7,8]. The following specific measures were estimated: secretory burst frequency (number of secretory episodes per 12 hours), amplitude (maximal rate of GH secretion attained within a secretory episode), mass (integral of the calculated secretory pulse), basal GH secretion rate (GH secretion per distribution volume per time), and the endogenous GH half-life (minutes). Total pulsatile GH secretion is calculated as the product of the secretory burst frequency and the mean mass of GH released per pulse. Basal GH secretion rate is the product of the mean basal secretion rate and total duration of sampling. The percentage of pulsatile GH secretion is the ratio of daily pulsatile GH release to the total of pulsatile plus basal GH secretion. Deconvolution analysis has been validated for determining the total secretion rate and half-life of a variety of hormones including GH [11,25], cortisol [26], and luteinizing hormone [27].

2.5. Approximate entropy

Approximate entropy was applied as a scale- and model-independent metric to quantify the orderliness or regularity of GH release patterns [28]. Approximate entropy measures the logarithmic likelihood that runs of patterns of data length m that are similar remain similar with a tolerance r on the next incremental comparisons. Normalized ApEn parameters of $m = 1$ (series length) and $r = 20\%$ (threshold) of the intraseries SD were applied as previously described [28]. Approximate entropy assigns a single nonnegative number to a time series. Greater absolute ApEn values given the same series lengths and ApEn parameter values as those used here denote greater disorderliness of subpatterns in the time series. Approximate entropy is an indirect measure of feedback activity within a neuroendocrine axis.

2.6. Overnight (12-hour) rhythmicity

Cosine regression with a 720-minute periodicity was used to quantify the inherent overnight rhythms in deconvolution-calculated GH secretory burst mass and intersecretory pulse intervals in each of the 4 study groups [29]. Only rhythms with significantly nonzero ($P < .05$) amplitudes were considered further.

2.7. Height, weight, and BMI

Subjects weighed on a calibrated scale (Metro Equipment, Sunnyvale, Calif) accurate to 0.1 kg. Height was assessed using a calibrated Harpenden stadiometer (Holtain, Crymch, Wales) to the nearest 1.0 mm. The height and weight data were used to determine BMI, BMI percentile [20], and BMI z score. Body mass index z score was calculated as (BMI − 50th percentile BMI for sex and chronological age)/SD of BMI at that age.

2.8. Underwater weighing

Body composition was measured using a 4-compartment model as previously described [30]. Briefly, residual volume was measured using an oxygen dilution technique [31] on land with the subject seated in the same position as that used during the hydrostatic weighing and repeated until 2 trials were within ±50 mL. Hydrostatic weighing was then completed to an accuracy of ±50 grams. Urine samples were collected the morning after the overnight blood draws at the University of Virginia General Clinical Research Center and before the subjects ate breakfast. The subjects consumed an oral dose of 99.9% enrichment ^2H_2O (0.05 g/kg; Isotec, Miamisburg, Ohio). Urine samples were collected immediately before dosing and 4 and 5 hours after dosing, and were analyzed by isotope ratio mass spectroscopy (Europa Hydra 20/20 Gas Isotope Ratio Mass Spectrometer; Metabolic Solutions, Inc, Concord, New Hampshire). Measurement of bone mineral content was made by dual-energy x-ray absorptiometry using a Hologic QDR 2000 bone densitometer (Hologic, Waltham, Mass). The subjects were placed in a supine position, and a series of transverse scans were made with a pencil beam from head to toe of the subject at 1-cm intervals. All scans were analyzed with Hologic software version 5.64 by a single trained technologist. A 4-compartment model [32] was used to adjust the body density for total body water and mineral content and to estimate the percentage of body fat.

2.9. Magnetic resonance imaging

Fat and lean tissue areas at the level of the L4-L5 intervertebral space were measured with magnetic resonance imaging using a Siemens Vision 1.5T scanner (Siemens,

Table 1
Subject characteristics by adiposity and pubertal maturation group

	Prepubertal		Pubertal	
	Lean (n = 19)	Overweight (n = 8)	Lean (n = 20)	Overweight (n = 10)
Age (y)*	10.5 ± 0.3	10.0 ± 0.4	14.6 ± 0.3	14.1 ± 0.4
Height (cm)*	141.3 ± 1.9	137.5 ± 2.9	169.6 ± 1.8	165.1 ± 2.6
Weight (kg)*,**	32.7 ± 1.8	38.4 ± 2.8	57.2 ± 1.8	68.1 ± 2.5
BMI percentile**	37.1 ± 4.4	86.8 ± 6.7	50.4 ± 4.3	89.7 ± 6.0
BMI *z* score**	−0.39 ± 0.14	1.14 ± 0.21	−0.05 ± 0.14	1.32 ± 0.19
Percentage of body fat (%)**	18.1 ± 1.2	28.0 ± 1.9	19.4 ± 1.2	28.9 ± 1.7
Fat mass (kg)*,**	6.0 ± 0.8	11.1 ± 1.3	10.9 ± 0.8	19.9 ± 1.1
Fat-free mass (kg)*	26.7 ± 1.5	27.4 ± 2.4	46.2 ± 1.5	48.2 ± 2.1
AVF (cm^2)*,**	34.5 ± 4.3	52.0 ± 6.6	54.6 ± 4.2	63.5 ± 5.9
IGF-I (ng/mL)*	197.3 ± 29.8	195.8 ± 45.9	475.1 ± 29.1	546.1 ± 41.1
Estradiol (pg/mL)*	12.2 ± 10.8	19.5 ± 16.5	66.2 ± 10.5	53.1 ± 14.8
Testosterone (ng/dL)*	12.4 ± 5.3	40.1 ± 7.7	291.9 ± 48.8	176.1 ± 68.9
Insulin (mIU/mL)*,**	6.1 ± 0.7	7.8 ± 1.1	8.2 ± 0.7	10.4 ± 0.9

Values are presented as mean ± SE.
* Significant ($P \leq .05$) maturation effect.
** Significant ($P \leq .05$) adiposity effect.

Munich, Germany). Adipose tissue area was assessed using a T1-weighted spin echo sequence and MedX Software (Sensor Systems, Sterling, Va).

2.10. Statistics

Two-way analysis of variance was used to test the main and interaction effects of pubertal maturation (pre-, late) and adiposity (lean, overweight) on body composition, body fat distribution, serum hormone concentrations, and GH secretion and half-life parameters. Linear regression was used to examine the strength of the relationship between percentage of body fat, BMI percentile, or serum hormone concentrations and GH secretion and half-life parameters. Hierarchical regression was used to determine whether BMI percentile and percentage of body fat were related to GH secretion parameters and ApEn after initial adjustment for a block of variables pertaining to body size and maturation (chronological age, sex, fat-free mass), for a block of variables pertaining to serum testosterone and estradiol concentrations, and then for AVF. Thus, a series of 4 blocks was used in the hierarchical regression: block 1 (chronological age, sex, fat-free mass), block 2 (testosterone and estradiol concentrations), block 3 (AVF), and block 4 (percentage of body fat). The predictors in each successive block were added to those in previous blocks. The increase in R^2 between consecutive blocks corresponds to the proportion of variance of the dependent variable that is shared by the newly added variable(s). The order of the blocks determines the variables that are being controlled. The effects of variables entered in earlier steps are partialed from relationships in later steps [33]. By adding percentage of body fat as the final block, we are asking if it is related to GH secretion and clearance parameters and ApEn independently of (after accounting for the variance in) chronological age, sex, fat-free mass, serum testosterone and estradiol concentrations, and AVF.

Table 2
Growth hormone secretion by adiposity and pubertal maturation groups

	Prepubertal		Pubertal	
	Lean	Overweight	Lean	Overweight
Mean GH concentration (μg/L)*,**	2.6 ± 0.4	1.6 ± 0.6	5.7 ± 0.3	3.6 ± 0.5
Integrated GH (μg/L per min)*,**	1878 ± 284	1157 ± 437	3921 ± 277	2611 ± 391
Total secretory rate (μg/L per 12 h)*,**	77.5 ± 10.5	48.5 ± 16.2	158.3 ± 10.5	103.5 ± 14.5
Secretory burst mass (μg/L)*,**	7.5 ± 0.9	4.9 ± 1.4	14.7 ± 0.8	8.6 ± 1.2
Burst amplitude (μg/L per min)*,**	0.30 ± 0.03	0.22 ± 0.05	0.42 ± 0.03	0.30 ± 0.04
Pulsatile production rate (μg/L per 12 h)*,**	73.3 ± 10.1	47.0 ± 15.6	158.2 ± 9.8	99.5 ± 13.9
Burst half-duration (min)*	22.8 ± 1.5	21.0 ± 2.3	31.9 ± 1.4	28.4 ± 2.0
GH burst frequency (per 12 h)	10.0 ± 0.6	10.6 ± 1.0	11.0 ± 0.6	12.0 ± 0.9
Interburst interval (min)	72.1 ± 4.0	68.2 ± 6.1	61.2 ± 3.8	60.8 ± 5.5
Pulsatile secretion (%)	95.4 ± 0.9	96.6 ± 1.3	96.3 ± 0.9	96.0 ± 1.2
Basal secretory rate (μg/L per min × 10^{-3})*	2.9 ± 0.6	2.0 ± 0.9	5.4 ± 0.6	5.3 ± 0.8
GH half-life (min)**	17.7 ± 0.7	16.7 ± 1.1	18.7 ± 0.7	16.2 ± 0.9
ApEn (1,20%)	0.62 ± 0.04	0.66 ± 0.05	0.70 ± 0.04	0.75 ± 0.05

Values are presented as mean ± SE.
* Significant ($P \leq .05$) maturation effect.
** Significant ($P \leq .05$) adiposity effect.

3. Results

The pubertal subjects had a greater ($P < .01$) chronological age, height, body weight, fat mass, fat-free mass, AVF, and greater fasting serum IGF-I, estradiol, testosterone, and insulin concentrations as compared with the prepubertal group (Table 1). The overweight subjects had a greater ($P < .01$) BMI percentile, BMI z score, percentage of body fat, fat mass, AVF, and fasting serum insulin concentrations than the lean subjects.

The 12-hour nocturnal GH secretion characteristics are shown in Table 2. Measures of total GH secretion, including mean and integrated serum GH concentrations and total GH secretory rate, were greater ($P < .01$) in late pubertal subjects than in prepubertal subjects and lower ($P < .001$) in overweight subjects than in lean subjects. For each unit increase in percentage of body fat, the mean GH concentration, integrated serum GH concentration, and total GH secretory rate declined by 0.10 μg/L ($r = -0.31$, $P < .05$), 83.6 μg/L per minute ($r = -0.39$, $P < .01$; see Fig. 1), and 2.6 μg/L per 12 hours ($r = -0.30$, $P < .05$), respectively. Maturation and adiposity effects were also observed for pulsatile aspects of GH secretion. The mass of GH secreted per secretory burst of late pubertal youth was double that of prepubertal youth ($P < .001$) and lower in overweight than in lean subjects ($P < .001$). For each unit increase in percentage of body fat, the mass of GH secreted per secretory burst declined by 0.22 μg/L ($r = -0.28$, $P < .05$; see Fig. 1). The greater GH burst mass in the pubertal subjects was accompanied by a greater burst amplitude ($P = .01$), greater ($P < .001$) GH pulsatile production rate, and greater ($P < .001$) GH secretory burst half-duration. Growth hormone burst amplitude ($P < .01$) and pulsatile production rate ($P < .01$) were lower in the overweight subjects. For each unit increase in percentage of body fat, the GH burst amplitude and pulsatile production rate declined by 0.005 μg/L per minute ($r = -0.22$, $P = .11$) and 2.2 μg/L per 12 hours ($r = -0.25$, $P = .06$), respectively. Growth hormone secretory burst half-duration ($P = .16$) was not dependent on adiposity. Growth hormone burst frequency was not dependent on either pubertal maturation ($P = .13$) or obesity ($P = .30$). The interburst interval was similar ($P = .07$) in prepubertal than late pubertal youth and not dependent on adiposity ($P = .67$). The percentage of pulsatile GH secretion was not dependent on maturation ($P = .90$) or adiposity ($P = .64$). Basal GH secretion was also dependent on maturation because the late pubertal youth had greater ($P < .001$) basal GH secretory rate than the prepubertal youth but was invariant of adiposity status ($P = .52$). Growth hormone half-life was shorter ($P = .05$) in overweight than in lean youth and decreased by 0.2 minute for every unit increase in percentage of body fat ($r = -0.38$, $P < .01$), but was maturation-independent ($P = .76$). Mean GH ApEn was similar ($P = .07$) in pubertal youth and invariant across adiposity groups ($P = .71$). The influence of adiposity on altering GH secretion was consistent across both maturation groups because there was no significant maturation by adiposity interactions.

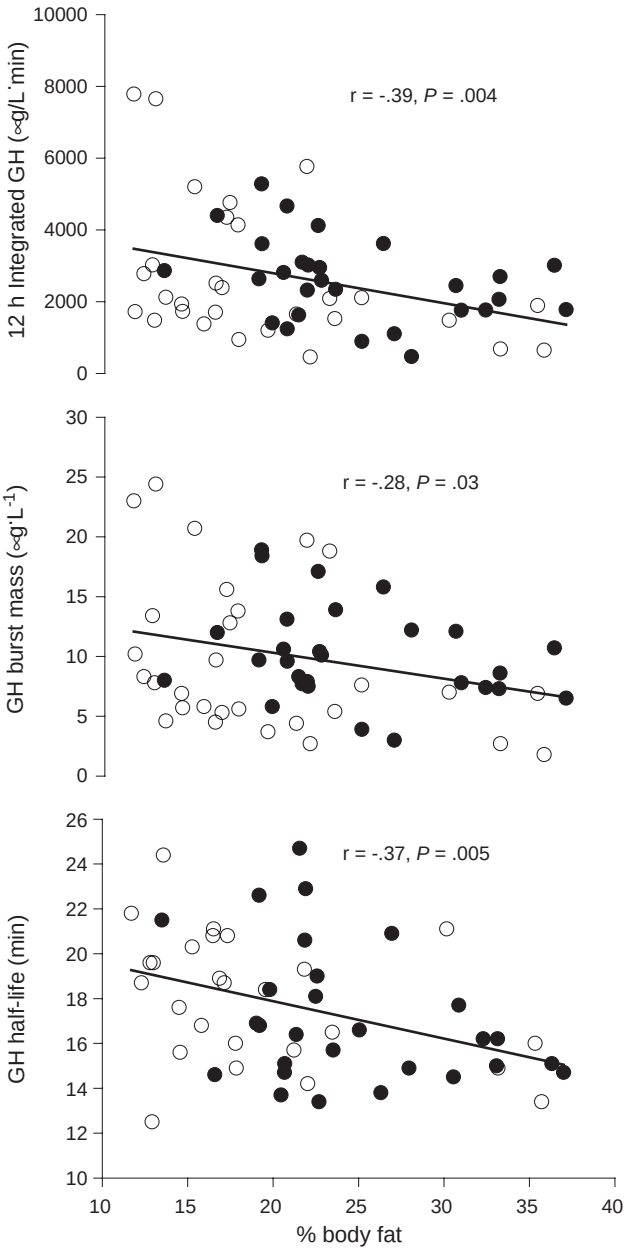

Fig. 1. Inverse relationships between percentage of body fat and the 12-hour integrated spontaneous nocturnal GH concentration (upper panel), mass of GH secreted per secretory burst (middle panel), and GH half-life (lower panel). Filled circles indicate boys; open circles, girls.

Maturational and adiposity influences on the magnitude and timing of the overnight rhythm in mass of GH secreted per burst are shown in Table 3. The mesor (mean) and amplitude of the rhythm of GH secretory burst mass were greater ($P < .001$) in pubertal than in prepubertal subjects and greater ($P < .05$) in lean than in overweight youth. The acrophase or timing of this rhythm was invariant to pubertal status ($P = .90$) and adiposity ($P = .54$). Percentage of body fat was inversely related to the mesor ($r = -0.31$, $P = .02$) and amplitude ($r = -0.28$, $P = .04$) of the rhythm

Table 3
Overnight rhythms in deconvoluted-calculated GH secretory burst mass

	Prepubertal		Pubertal	
	Lean	Overweight	Lean	Overweight
Mesor*,**	2.6 ± 0.4	1.6 ± 0.5	5.8 ± 0.4	3.7 ± 0.5
Amplitude*,**	2.2 ± 0.3	1.4 ± 0.5	4.0 ± 0.3	3.1 ± 0.5
Acrophase	309 ± 13	330 ± 23	322 ± 12	321 ± 18

Values are presented as mean ± SE.
* Significant ($P \leq .05$) maturation effect.
** Significant ($P \leq .05$) adiposity effect.

in GH secretory burst mass but was not related ($P > .20$) to the acrophase.

As shown in Table 4, there were no significant relationships between AVF area (or AVF as a percentage of the total abdominal cross-sectional area [data not shown]) and GH secretory characteristics. Additional univariate correlations between age, fasting serum insulin concentrations, testosterone concentrations, estradiol concentrations, and GH secretion parameters are also shown in Table 4.

Table 5 shows the hierarchical regression results, mainly, the R^2 after entry of each block of independent variables. Block 1 consisted of physical characteristic variables, including chronological age, sex, and fat-free mass. These variables were added first so that the predictive value of the remaining independent variables could be determined while holding constant initial differences in body size, age and sex, which influence GH secretion. For the remaining blocks, order of entry was according to our hypotheses that pulsatile GH secretion is inversely related to percentage of body fat independent of gonadal steroids (block 2) and AVF (block 3). The addition of serum testosterone and estradiol concentrations (block 2) to the physical characteristics produced a significant ($P < .05$) increment in R^2 (sR_i^2) for mean GH concentration ($sR_i^2 = 0.09$), integrated GH concentration ($sR_i^2 = 0.14$), secretory burst mass ($sR_i^2 = 0.08$), percentage pulsatile GH secretion ($sR_i^2 = 0.12$), GH half-life ($sR_i^2 = 0.12$), and ApEn ($sR_i^2 = 0.26$). The addition of AVF did not significantly increase in sR_i^2 for any variable. After accounting for physical characteristics, gonadal steroids, and AVF, percentage of body fat reliably ($P < .05$) increased sR_i^2 for mean GH concentration ($sR_i^2 = 0.08$), integrated GH concentration ($sR_i^2 = 0.06$), total secretory rate ($sR_i^2 = 0.10$), secretory burst mass ($sR_i^2 = 0.06$), pulsatile production rate ($sR_i^2 = 0.05$), percentage of GH secretion that was pulsatile ($sR_i^2 = 0.15$), and GH half-life ($sR_i^2 = 0.07$). Fasting serum insulin concentration was not included in the hierarchical regression models because it was not correlated to any GH pulse parameters when using univariate analyses.

Table 4
Univariate correlation results

Model	AVF	Age	Insulin	Testosterone	Estradiol
Mean GH concentration (μg/L)	0.16	0.56*	0.15	0.50*	0.19
Integrated GH (μg/L per min)	0.07	0.51*	0.05	0.53*	0.22
Total secretory rate (μg/L per 12 h)	0.10	0.52*	0.03	0.34*	0.25
Secretory burst mass (μg/L)	0.09	0.51*	0.08	0.40*	0.25
Burst amplitude (μg/L per min)	0.02	0.23	0.11	0.25	0.05
Pulsatile production rate (μg/L per 12 h)	0.06	0.52*	0.05	0.32*	0.21
Burst half-duration (min)	0.20	0.56*	0.03	0.25	0.46*
GH burst frequency (per 12 h)	0.05	0.17	−0.05	−0.02	−0.02
Interburst interval (min)	−0.12	−0.22	0.11	−0.01	−0.10
Pulsatile secretion (%)	0.22	0.07	0.22	0.27*	−0.25
Basal secretory rate (μg/L per min × 10^{-3})	−0.24	0.06	0.11	−0.10	0.14
GH half-life (min)	−0.06	0.12	−0.06	0.37*	−0.16
ApEn (1,20%)	0.13	0.16	0.10	−0.29*	0.49*

* $P < .05$.

Table 5
Hierarchical regression results

Model	Block 1	Block 2	Block 3	Block 4
Mean GH concentration (μg/L)	0.33	0.42*	0.43	0.51*
Integrated GH (μg/L per min)	0.27	0.41*	0.44	0.50*
Total secretory rate (μg/L per 12 h)	0.27	0.30	0.32	0.42*
Secretory burst mass (μg/L)	0.27	0.35*	0.36	0.42*
Burst amplitude (μg/L per min)	0.08	0.10	0.12	0.16
Pulsatile production rate (μg/L per 12 h)	0.28	0.30	0.32	0.37*
Burst half-duration (min)	0.39	0.44	0.45	0.45
GH burst frequency (per 12 h)	0.03	0.10	0.10	0.10
Interburst interval (min)	0.05	0.09	0.09	0.09
Pulsatile secretion (%)	0.07	0.19*	0.22	0.37*
Basal secretory rate (μg/L per min × 10^{-3})	0.10	0.12	0.16	0.17
GH half-life (min)	0.05	0.17*	0.18	0.25*
ApEn (1,20%)	0.11	0.37*	0.37	0.37

Values are R^2. Block 1 consists of chronological age, sex, free fat-free mass; block 2, serum testosterone and estradiol concentrations added to variables in block 1; block 3, AVF added to variables in blocks 1 and 2; block 4, percentage of body fat added to variables in blocks 1 through 3.
* Significant ($P < .05$) incremental increase in R^2 between successive blocks.

4. Discussion

This is the first investigation to use frequent blood sampling, an ultrasensitive GH assay, deconvolution analysis, the ApEn metric, and very accurate measures of body composition and body fat distribution to determine the influence of total and regional adiposity on GH neuroregulation in overweight but otherwise healthy youth compared with that in lean youth of similar age and height. These analyses established that mean GH concentrations are lower in overweight youth because of lower pulsatile GH secretion and secondarily to a greater rate of GH disappearance. Basal GH secretion is unaffected by obesity. In disagreement with our original hypothesis, pubertal maturation did not influence the effect of adiposity on pulsatile GH secretion. Thus, the neuroregulatory mechanisms for

reduced pulsatile GH secretion appear to be similar in prepubertal and pubertal overweight youth, and consist of reduced secretory burst mass and amplitude, but burst half duration and frequency are similar to lean youth. Overweight youth also have a reduced mesor and amplitude of the overnight rhythm of GH secretion, but inconsistent with our hypothesis, the regularity of GH secretion is conserved as measured by the ApEn metric. Furthermore, hierarchical regression analysis demonstrated that total body adiposity modulates GH secretory burst characteristics and GH half-life independent of the effects of age, sex, fat-free mass, serum testosterone and estradiol concentrations, and AVF, but that AVF is not related GH secretion.

Inverse relationships have been reported between mean or integrated serum GH concentrations and total adiposity in youth [18,34-36] but not consistently [37]. The current investigation contributes to the understanding of how adiposity influences circulating GH concentrations of youth by focusing on alterations in GH secretory dynamics, which is necessary for understanding the neuroregulatory mechanisms of reduced GH secretion. In agreement with our hypothesis, we found that for each unit increase in percentage of body fat, secretory burst mass declined by 0.22 μg/L and GH half-life declined by 0.2 minute, but that burst half duration and frequency were not affected by adiposity.

Two hypothalamic hormones, GHRH and somatostatin, control the timing and the mass of GH secreted from the pituitary. The current neuroregulatory model [5,6] proposes that GH is secreted as a burst when a GHRH pulse coincides with a nadir in the sinusoidal pattern of somatostatin tone. Evidence strongly suggests that GHRH predominantly increases GH pulse amplitude whereas somatostatin primarily controls GH pulse frequency. Growth hormone–releasing hormone infusions in healthy adults augment GH pulse amplitude, whereas GH pulse number is unchanged [38]. When GHRH action is diminished in humans through competitive antagonists to the GHRH receptor, pulsatile GH secretion is reduced by 75%, but there is no effect on GH pulse frequency or interpulse GH concentration [39]. Evidence that somatostatin primarily modulates GH pulse timing in humans includes a rebound-like pulse of GH that occurs during short-term somatostatin withdrawal [40] and a reduction in the frequency and area of GH peaks but conserved GH pulsatility during intravenous somatostatin infusion [41]. The separate functions of GHRH and somatostatin for controlling GH pulse amplitude and frequency are uniquely demonstrated in young adults with a recessive point mutation that results in truncated and inactive GHRH receptors [42]. Compared with healthy adults, these individuals have extremely low serum GH concentrations because of very small GH burst amplitudes, but the GH secretory burst frequency is 2- to 3-fold greater than age- and sex-matched control subjects [42].

In the present study, obesity in youth primarily reduced GH burst mass and amplitude but did not alter GH burst frequency. Although, to the best of our knowledge, no other studies have directly compared the pulsatile aspects of endogenous GH secretion in overweight and in lean youth, similar to the present study, several have reported that measures of adiposity are more inversely related to GH pulse amplitude than GH pulse frequency [18,34,35]. A relevant mechanism could involve diminished hypothalamic GHRH responsiveness as well as possible augmentation of somatostatin release per burst with no evident change in signal timing [5]. Indeed, GHRH-stimulated GH secretion is blunted in overweight children [43,44] and adults [45,46]. The reduction in GHRH stimulation in overweight youth appears to be more of a function of reduced pituitary responsiveness to GHRH than reduced GHRH pulse magnitude because infusions of both GHRH and arginine or pyridostigmine (somatostatin inhibitors) increase but do not fully restore GH secretion to that of normal weight individuals [43,44,47-50]. Moreover, GHRH sensitivity is increased with weight loss as measured by augmented GHRH-stimulated GH secretion [51-53]. Weight loss in children increases 24-hour spontaneous pulsatile GH secretion 1.9-fold but increases GH burst frequency by only 6% [54], which again suggests that adiposity has a predominant neuroendocrine influence on GHRH responsiveness rather than somatostatin tone.

In adults, hyposomatotropism coincident with altered GH neurosecretory activity [2,14] and reduced half-life [11,12] occur mainly in those with large stores of AVF. In contrast, total body fat, but not AVF, was related to alterations in GH neuroregulation in the present study of youth, and this agrees with our hypothesis (Table 5). During adulthood, there is an inevitable decrease in sex steroid secretion and increase in AVF resulting in reduced GH secretion, which may initiate a vicious cycle of further gains in AVF and reductions in GH secretion. In contrast, pubertal increases in sex steroid secretion are a robust stimulus for GH secretion [19,55] and are protective against gains in AVF [56,57], which may account for the weak relationship in adolescents, but not in the prepubertal youth who have yet to experience a reawakening of the hypothalamic-pituitary-gonadal axis. So, although sex steroid concentrations may explain some of the differences between the role of AVF in modulating GH secretion in youth and adults, the similar relationships among AVF and GH secretion in both prepubertal and pubertal groups suggest additional age-related differences. In adults, accumulation of AVF is associated with hyperinsulinemia [58], which, in turn, reduces GH secretion by directly inhibiting the somatotropes [59] and indirectly by reducing circulating IGF–binding protein–1 resulting in increased free IGF-I and IGF-I negative feedback on GH [60,61]. In children and adolescents, insulin secretion and action are modulated more by total adiposity than AVF [62,63], suggesting that insulin-mediated effects on GH secretion in youth would occur more as a result of increases in total adiposity rather than AVF.

Another possibility is that a critical amount of AVF is necessary to produce metabolic alterations such as

hyperinsulinemia with proposed AVF thresholds of 130 cm^2 in adults [64] and 58 cm^2 in youth [65]. The overweight adolescent group in the present study had a mean AVF greater than 58 cm^2 (Table 1), and when correlation analyses were limited to subjects within this group, basal GH secretion ($r = -0.68$, $P = .02$) and GH secretory burst mass ($r = -0.56$, $P = .07$) were indeed inversely related to AVF, but fasting serum insulin concentrations were not ($r = 0.40$, $P = .22$). Perhaps inverse relationships between AVF and GH secretion would have been observed for the whole group if additional youth with greater amounts of AVF had been studied, thereby increasing the range of AVF data and the likelihood of an inverse correlation with GH secretion. The lack of significant relationships among fasting serum insulin concentrations and GH secretion for the whole group and within just the overweight pubertal group suggests other metabolic signals such as free fatty acids may contribute to obesity hyposomatotropism in youth [66,67].

Previously, we [68] proposed that serum leptin concentrations may act as an inhibitory signal to the hypothalamus to reduce GH secretion. However, more recent experimental evidence strongly suggests that leptin plays little, if any, role in GH secretion. Stimulated GH secretion parallels total adiposity but not leptin concentrations in lean control subjects, extremely obese patients with homozygous mutations of the leptin gene who produce little leptin and those with heterozygous mutations who have intermediate levels of adiposity and leptin [69]. For this reason and the difficulty in separating the independent contributions of leptin and adiposity to GH secretion in correlational studies due to high colinearity among adipose and leptin variables [70], leptin was not included in the hierarchical regression models.

In agreement with our hypothesis, a secondary mechanism for reduced mean GH concentrations in overweight youth was a 14% shorter endogenous GH half-life. This is a novel finding because only one other study has investigated the influence of adiposity on GH half-life of youth, and although lean and overweight youth were not directly compared, no relation between BMI SDS and GH half-life was found [35]. Increased GH metabolic clearance has been reported in obese adults, and although the mechanisms are unclear, it has been suggested that adipose tissue clears GH and increases the GH distribution volume [10,14,71].

The hierarchical regression analyses demonstrated the robust stimulatory effect of sex steroids on total and pulsatile GH secretion during puberty [55] as the block of testosterone and estradiol significantly increased R^2 after prior adjustment for age and sex. The sex steroid block also increased R^2 of ApEn, which agrees with previous research that sex steroids, especially estrogen, increase the disorder of GH secretory patterns perhaps by altering GH and/or IGF-I auto–negative feedback [19]. Unexpectedly, addition of sex steroids to the model increased R^2 for GH half-life and was almost entirely due to the direct relationship between testosterone concentrations and GH half-life. We are unsure why testosterone would be independently related to GH half-life as previous research has shown that neither androgen administration [72] nor androgen receptor blockade [73] influences GH half-life. In agreement with our results, others have shown that large perturbations in estradiol concentrations have little effect on GH half-life [74,75]. As hypothesized and discussed previously, AVF did not reliably increase the prediction of any GH secretion parameters. However, total adiposity was inversely related to total and pulsatile GH secretion and to GH half-life independent of the effects of age, sex, fat-free mass, serum testosterone and estradiol concentrations, and AVF.

In summary, serum GH concentrations are reduced in overweight youth primarily because of reductions in pulsatile but not basal GH secretion, and secondarily because of a reduced endogenous GH half-life, perhaps due to accelerated GH metabolic clearance. Based on the neuroregulatory model that GHRH amplifies GH pulses whereas somatostatin alters GH pulse frequency, the results suggest that overweight in youth diminishes GHRH stimulation, resulting in truncated GH bursts, but does not alter the number of intervals of somatostatin withdrawal so that GH burst frequency is conserved.

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