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Alterations in Growth Hormone Secretory Dynamics in Adolescent Girls with Anorexia Nervosa and Effects on Bone Metabolism

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Anorexia nervosa (AN) is a disorder that is increasing in frequency in adolescents, and the age of onset is often in the prepubertal years, potentially affecting the development of peak bone mass and linear growth. The GH-IGF-I axis plays an important role in bone formation, and alterations in GH secretory patterns have been described in adult women with AN. However, GH secretory dynamics in adolescents with AN have not been described, and the effects of alterations in GH secretory patterns and GH concentration on bone metabolism in AN are not known. We examined patterns of GH secretion by deconvolutional analysis, and GH concentration by Cluster analysis, in adolescent girls with AN (n = 22) and controls (n = 20) of comparable bone age and pubertal stage. We also examined the roles of cortisol, leptin, and estradiol in the regulation of GH secretion and concentration, and the relationship of GH secretory patterns and concentration to bone metabolism. Basal GH secretion and secretory pulse number in adolescent girls with AN were increased compared with control values (*P* = 0.03 and 0.007, respectively), and increased disorderliness of GH secretion (approximate entropy) was found in AN (*P* = 0.004). Mean and nadir GH concentrations and total area under the concentration curve were increased (*P* = 0.03, 0.002, and 0.03, respectively), and IGF-I levels were decreased (*P* = 0.0002) in girls with AN compared with healthy adolescent girls. IGF-I levels correlated negatively with nadir GH concentrations (r = −0.35; *P* = 0.02). Serum cortisol levels were higher in girls with AN than in controls (*P* < 0.0001) and correlated inversely with IGF-I (r = −0.58; *P* = 0.0001) and weakly with GH concentration (area under the concentration curve; r = −0.43; *P* = 0.05). A strong inverse relationship between markers of nutritional status (body mass index, fat mass, and leptin) and basal and pulsatile GH secretion, and mean and nadir GH concentrations was observed. GH concentration predicted levels of all markers of bone formation and a marker of bone resorption (N-telopeptide) in healthy controls, but not in AN. We demonstrate increases in basal GH secretion, number of secretory bursts, and GH concentration in adolescents with AN compared with controls, accompanied by low IGF-I levels. These data are consistent with the hypothesis that an acquired GH resistance occurs in this undernourished group. We also demonstrate that GH secretion and concentration are nutritionally regulated, and that the effects of nutrition exceed the effects of cortisol on GH concentration. Acquired GH resistance may play a role in the osteopenia and decreased peak bone mass frequently associated with AN. (*J Clin Endocrinol Metab* 88: 5615–5623, 2003)

ANOREXIA NERVOSA (AN) is a disorder that has increased in prevalence at least 5-fold over the last few decades, and 0.2–4% of all adolescent girls in the United States now suffer from this disorder (1, 2), making it the third most common chronic illness in adolescent girls (1). Because the disorder goes undiagnosed as much as 50% of the time, the true prevalence is probably even higher. Bone mineral accrual is affected in more than half of all girls suffering from this eating disorder (3). Osteopenia and osteoporosis are established complications of AN, and onset of disease during adolescence is associated with more severe bone mass reduction (3–9). Importantly, poor bone mineral accrual during a time of establishment of peak bone mass results in deficits in bone mass that may be permanent despite disease recovery (10, 11).

Multiple abnormalities in the hypothalamic-pituitary axis have been described in AN, including abnormal thyroid function (12–14), hypercortisolemia (12, 13, 15–17), and hypothalamic hypogonadism. Abnormalities of the GH-IGF-I axis (13, 15, 18–21) have also been reported. In adult women with AN, low IGF-I levels with high values of GH have been demonstrated and have been attributed to a nutritionally acquired GH resistance (15, 20, 21). In adolescents, data are less conclusive. Although studies have consistently reported low IGF-I levels in adolescents with this eating disorder, GH levels have been reported as low, high, or normal (18, 19). In addition, GH secretory patterns in adolescents with AN have not been studied. The one study that examined GH concentration by Cluster analysis (not secretory patterns) reported on fully mature (Tanner 5) adolescent girls (19). Because GH production is maximum around midpuberty, it is also im-

Abbreviations: AN, Anorexia nervosa; ApEn, approximate entropy; AUC, area under the concentration curve; BA, bone age; BMAD, bone mineral apparent density; BMD, bone mineral density; BMI, body mass index; BSAP, bone-specific alkaline phosphatase; DPD, deoxypyridinoline; DXA, dual energy x-ray absorptiometry; GCRC, General Clinical Research Center; NTX, N-telopeptide; OC, osteocalcin; PICP, C-terminal propeptide of type I procollagen; UFC, urinary free cortisol.

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portant to examine alterations in GH secretion and concentration in a pubertal adolescent population. We have used deconvolutional analysis to better understand changes in GH secretion in girls with AN and have studied adolescent girls in Tanner stages 2–5 of puberty as more representative of a pubertal adolescent population. Although the cause of poor bone mineral accrual in AN is not well understood, decreased lean body mass, body mass index, and low IGF-I levels resulting from nutritional deficiency, hypoestrogenemia, and hypercortisolemia have been implicated. Lean body mass (8, 9, 11, 22, 23), body mass index (3, 22–25), and IGF-I levels (8, 26) have been demonstrated to impact on bone density and bone formation markers in studies of adults and adolescents. GH stimulates IGF-I secretion from the liver and also locally at the level of bone. The effects of the GH-IGF-I axis on bone stem from both the direct stimulatory effects of GH on the proliferation and differentiation of osteoblast precursors and its indirect effects, through IGF-I, on the differentiation of these precursors (27, 28). GH may also have a direct inhibitory effect on osteoclasts. The effects of changes in GH secretory patterns and GH concentration on bone metabolism in AN have not been determined.

In this study we examined the pathophysiology of GH secretion and acquired GH resistance in adolescent girls with AN and also investigated the effects of GH secretory patterns and concentration on bone density and bone formation markers in this disorder.

Subjects and Methods

Subject selection

Twenty-two adolescent girls with AN diagnosed by DSM-IVR criteria and 20 healthy controls were enrolled in the study after a screening visit. All subjects were Caucasian and ranged in age from 12.2–18.8 yr. Healthy controls had body mass indexes (BMIs) ranging from 16.8–28.0 kg/m^2, between the 14th and 94th percentiles for girls for age (29). The time since diagnosis ranged from 1–36 months for girls with AN. Four subjects with AN had not attained menarche, and 18 had secondary amenorrhea. Three controls were premenarchal. None of the healthy adolescent girls had a present or past history of eating disorders. Subjects with AN were recruited through referrals from primary care providers, nutritionists, psychiatrists, and therapists and also from inpatient and day-treatment eating disorder programs in Massachusetts, New Hampshire, and Maine. Healthy controls were recruited through mass mailings to primary care providers in the greater Boston area, advertisements in community newspapers, and within hospitals in the Partners Health Care Network. All subjects with AN were receiving out-patient multidisciplinary treatment at study initiation. The study was approved by the institutional review board of the Partners Health Care system. Informed assent and consent were obtained from all subjects and their parents.

Experimental protocol

Eligibility for participation in the study was determined at a screening visit in the General Clinical Research Center (GCRC) of Massachusetts General Hospital, which included a history, physical examination, and screening laboratory tests. Blood was drawn for measurement of TSH, FSH, LH, PRL, hematocrit, potassium, and glucose. Values within the normal range for TSH, FSH, LH, and PRL, a hematocrit greater than 30%, potassium greater than 3 mmol/liter, and glucose greater than 50 mg/dl were necessary for study eligibility.

Eligible subjects were subsequently admitted overnight to the GCRC. A 24-h urine collection for urinary free cortisol, calcium, and creatinine was begun on the day of admission and completed at the GCRC. Bone density testing was performed, and a bone age was determined. Subjects had dinner before 1930 h, after which a 20-gauge iv catheter was inserted into a vein in the forearm or antecubital fossa for blood sampling. Frequent sampling for GH and cortisol determinations was carried out every 30 min from 2000 h on the night of admission until 0800 h the following morning. After frequent sampling, a fasting blood sample was obtained for IGF-I, estradiol, leptin, and bone formation markers [bone-specific alkaline phosphatase (BSAP), osteocalcin (OC), and C-terminal propeptide of type I procollagen (PICP)]. A 2-h second morning urine sample was collected for markers of bone resorption [N-telopeptide (NTX) and deoxypyridinoline (DPD)].

Anthropometric measurements

A single stadiometer was used to measure the heights of the subjects (the average of triplicate measurements was used), and subjects were weighed on an electronic scale while wearing a hospital gown. BMI was calculated (weight in kilograms divided by height in meters squared), and BMI percentiles were determined using published charts (29). Parental heights were determined by report, and midparental height was calculated by subtracting 13 cm from the sum of the parents' heights in centimeters and dividing the result by 2. Bone age (BA) was determined from an x-ray of the left wrist and hand using the methods of Greulich and Pyle (30). Tanner stage for pubic hair and breast development was assessed in all patients. However, the results of Tanner breast staging are not reported because Tanner breast stage is often difficult to determine accurately in this population, as breast tissue is often so atrophied that it may appear as an early or midpubertal breast. Many adolescents with AN go through puberty at a normal age and therefore have had full pubertal maturation before the onset of AN. In such cases, although breast tissue appears immature, skeletal age may be mature due to the past exposure to high levels of sex steroids and normal nutrition. For these reasons, BA and Tanner staging for pubic hair were determined to be better estimates of pubertal stage.

Pubertal staging and BA determination for all subjects was performed by one study investigator, a pediatric endocrinologist. Girls in Tanner stages 2 and 3 for pubic hair were determined to be in early puberty, and girls in Tanner stages 4 and 5 were determined to be in late puberty. A BA of 15 yr was used as the cut-off for classifying girls as pubertally immature (BA, $<$15 yr) or mature (BA, $\geq$15 yr). Five healthy controls and four girls with AN were immature based on BA and in early puberty based on Tanner stage.

Biochemical assessment

The hospital laboratory measured ionized calcium, phosphorus, glucose, and urinary creatinine using previously described methods (31).

GH levels were measured using an immunoradiometric assay (Nichols Institute Diagnostics, Inc., San Juan Capistrano, CA) with a detection limit of 0.05 ng/ml and an intraassay coefficient of variation of 2.4–9.4%. More sensitive assays for GH estimation now exist with lower limits of detection. However, GH secretion is higher in adolescents than in adults; thus, there is a smaller likelihood of missing small pulses in a younger population. In our subjects only 2 healthy controls had GH values below the limit of detection by the assay used: 1 adolescent had 3 of 25 values below the detection limit, and the other had 2 of 25 values below the detection limit. It is thus unlikely that significant pulses were missed. An immunoradiometric assay (Nichols Institute Diagnostics, Inc.) was used to measure serum IGF-I (detection limit, 30 μg/liter; coefficient of variation, 3.1–4.6%).

We measured urinary free cortisol by the GammaCoat ^{125}I RIA (Diasorin, Inc., Stillwater, MN; detection limit, 1 μg/dl; coefficient of variation, 7.0%) using the extraction method. The concentration of free cortisol in the 24-h urine sample was multiplied by the total volume over 24 h to obtain the value for urinary free cortisol in micrograms per day. Urinary cortisol values were corrected for creatinine and for surface area and creatinine. This standardization was performed 1) because of questions regarding the adequacy of a 24-h urine collection in our subjects, and 2) because of a recent report demonstrating the need to correct urinary free cortisol for creatinine and for surface area in healthy adolescents (32). Serum cortisol values were measured with an RIA (Diagnostic Products Corp., Los Angeles, CA; limit of sensitivity, 1.0 μg/dl; coefficient of variation, 2.5–4.1%). This assay measures not only cortisol, but also other steroid metabolites. However, this limitation of the assay is of significance primarily in infancy; in older children RIA remains the standard assay for cortisol. We measured estradiol levels by ultrasen-

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sitive RIA (Diagnostic Systems Laboratories, Inc., Webster, TX; detection limit, 8.1 pmol/liter; coefficient of variation, 6.5–8.9%). RIA was used to measure serum leptin (Linco Diagnostics, Inc., St. Louis, MO; sensitivity, 0.5 μg/liter; coefficient of variation, 3.4–8.3%).

PICP was measured by RIA (Diasorin, Inc.) with a limit of detection of 25 ng/ml and a coefficient of variation of 1.3–3.8%. For serum OC we used an immunoradiometric assay (Nichols Institute Diagnostics, Inc.; sensitivity, 0.5 ng/ml; coefficient of variation, 3.2–5.2%). BSAP was measured using an ELISA (Quidel, Inc., Mountain View, CA; sensitivity, 0.7 U/liter; coefficient of variation, 3.9–5.8%). We measured urinary DPD and NTX in a 2-h, second morning urine sample normalized for creatinine excretion. DPD and NTX were measured by ELISAs [Quidel, Inc. (minimum detection limit, 1.1 nmol/liter; coefficient of variation, 4.3–8.4%) and Ostex International, Inc. (Seattle, WA; detection limit, 20 nmol bone collagen equivalent; intraassay coefficient of variation, 5–19%)]. Serum samples were stored at −80 C until analysis. All samples were run in duplicate.

Analysis of GH secretion and concentration

Cluster analysis. We first used the computerized mathematical algorithm, Cluster, to determine mean nocturnal GH concentration, nadir GH concentration, and total area under the curve (AUC) (33). Cluster analysis does not provide information about individual secretory events, but allows determination of mean GH concentration, nadir of the GH concentration, and total AUC. Cluster also allows assessment of peaks of GH concentration (as opposed to secretion). A 1 × 2 cluster configuration (one sample in the test nadir and two in the test peak) was used. In this algorithm, a peak GH concentration is defined as a significant increase in a cluster of GH values, followed by a significant decrease in a second cluster of GH values. Areas of GH concentration were calculated using the trapezoidal rule. In contrast to GH secretion, GH concentration is a function not only of GH secretion at any point of time, but also of GH secreted at earlier time points that has not been metabolized or cleared from the circulation.

Deconvolutional analysis. We then performed deconvolutional analysis to characterize GH secretory patterns in girls with AN and in healthy adolescent girls. GH secretion is pulsatile, and the GH concentration at any time point represents the effects of both GH secretion into blood and its clearance from blood. The GH concentration is thus related to the occurrence of secretory pulses, the mass of each secretory pulse, pulse duration, and GH half-life. We used the multiple parameter deconvolutional analysis described by Veldhuis *et al.* (34, 35) to uncover individual secretory bursts. This analysis considers the secretion and elimination parameters to be simultaneous unknowns and then solves the convolutional integral. This method allows estimation of basal GH secretion rate, total basal GH secretion (basal GH secretion rate × duration of sampling), GH half-life, frequency of secretion bursts, area under secretion bursts (burst mass), secretory burst amplitude, and total pulsatile production (mean burst mass × number of secretory bursts). Total GH secretion was calculated by adding basal and pulsatile GH secretion. The ratio of pulsatile to total GH secretion was determined.

Approximate entropy (ApEn). The orderliness of GH secretion was determined using ApEn. This score increases in magnitude as the secretory pattern becomes more disorderly. ApEn is not affected by small changes in background noise or by occasional artifacts in the data. It makes no assumptions of periodicity or waveform and detects differences in underlying episodic behavior not reflected in other measures of secretion analysis (15).

Bone density and body composition

Bone density was measured at the lumbar spine (L1–L4; in anteroposterior projection) and hip by dual energy x-ray absorptiometry (DXA; QDR-4500, Hologic, Inc., Waltham, MA). The SD for lumbar bone density measurement is 0.01 g/cm^2 and does not vary with bone density. The z-scores were calculated from the applet of Bachrach, Hastie, and Narasimhan (http://www-stat-class.stanford.edu/pediatric-bones/). Although this applet is based on measurements using a Hologic QDR 1000 bone densitometer, studies have demonstrated very minimal differences in bone density measurements at the lumbar spine using a Hologic QDR 4500 machine *vs.* a Hologic QDR 1000 machine (r^2 = 0.985 and 0.990 with mean bone mineral density (BMD) differences of 0.68% and 0.003 g/cm^2). An estimate of volumetric bone density, bone mineral apparent density (BMAD), was calculated using the formula described by Katzman *et al.* (36).

Body composition, including validated measures of fat mass and lean body mass, was determined by whole body DXA (Hologic QDR 4500) (37, 38). Lean body mass refers to soft tissue only and does not include bone. The precision error (SD) of DXA has been reported to be 425 g for whole body fat and fat-free mass (37), with a correlation of 0.99 with a four-compartment model, body composition method for measuring fat-free mass, and 0.93–0.97 with multislice computed tomography for measuring regional fat-free mass (38).

Statistical methods

All data are presented as the mean ± SD. The data were analyzed using the JMP program (version 4, SAS Institute, Inc., Cary, NC). A *t* test was used to calculate differences between means. We employed univariate and multiple regression analyses to determine predictors of GH secretion, levels of bone turnover markers, and bone density.

Results

Clinical and anthropometric data

Clinical and anthropometric data are summarized in Table 1. Chronological age, BA, and Tanner stage were not different in adolescent girls with AN compared with control subjects. The mean duration since diagnosis of AN was 7.0 ± 8.2 months (range, 1–36 months), and the mean duration of amenorrhea was 4.2 ± 3.5 months (range, 3–12 months). As expected, girls with AN had significantly lower weights, BMIs, and percent ideal body weight compared with controls. In this study population, girls with AN were taller than healthy controls. Fat mass was much lower in girls with AN than in controls, whereas lean body mass was comparable in the two groups.

Bone density and markers of bone turnover

Bone density data and markers of bone turnover are summarized in Table 2. Lumbar spine BMD z-scores, lumbar spine BMAD, and lumbar spine BMAD z-scores were all significantly lower in girls with AN. Femoral neck and hip BMD z-scores were significantly lower in AN than in controls. Levels of markers of bone formation and bone resorption were all lower in AN than in healthy controls, but did not reach statistical significance. Levels of ionized calcium and phosphorus did not differ in the two groups, but 24-h urinary calcium/creatinine excretion was significantly higher in girls with AN than in controls.

TABLE 1. Baseline demographic data

	Controls (n = 20)	AN (n = 22)	*P*
Chronological age (yr)	15.5 ± 1.7	16.3 ± 1.6	NS
BA (yr)	15.9 ± 1.9	15.8 ± 1.6	NS
Tanner stage (pubic hair)	4.5 ± 0.8	4.2 ± 0.7	NS
Weight (kg)	57.0 ± 10.8	46.4 ± 5.7	0.0002
BMI (kg/m^2)	21.9 ± 2.9	16.7 ± 1.2	<0.0001
Height (cm)	161.0 ± 7.3	166.6 ± 5.7	0.008
Midparental height (cm)	162.4 ± 4.9	164.8 ± 5.1	NS
Surface area (m^2)	1.59 ± 0.17	1.46 ± 0.11	0.006
Fat mass (kg)	17.6 ± 5.7	8.9 ± 2.8	<0.0001
% Fat mass (%)	29.5 ± 5.5	18.5 ± 4.1	<0.0001
Lean body mass (kg)	39.2 ± 6.4	37.1 ± 4.1	NS

NS, Not significant.

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TABLE 2. Bone density and bone turnover markers

	Controls (n = 20)	AN (n = 22)	P
Lumbar spine BMD z-score	−0.44 ± 0.72	−0.99 ± 0.90	0.04
Lumbar BMAD (g/cm^3)	0.130 ± 0.014	0.120 ± 0.011	0.02
Lumbar BMAD z-score	−1.5 ± 0.8	−2.2 ± 0.8	0.01
Hip BMD z-score	0.31 ± 0.84	−0.35 ± 1.11	0.04
Femoral neck BMD z-score	0.20 ± 0.95	−0.46 ± 1.05	0.04
PICP (ng/ml)	225 ± 127	174 ± 95	NS
OC (ng/ml)	44.6 ± 29.1	35.1 ± 21.3	NS
BSAP (U/liter)	40.0 ± 32.2	29.4 ± 20.0	NS
NTX (nmol BCE/mmol creatinine)	155 ± 142	104 ± 69	NS
DPD (nmol/mmol creatinine)	13.8 ± 14.0	9.0 ± 3.8	NS
Ionized calcium (mmol/liter)	1.23 ± 0.03	1.22 ± 0.05	NS
Phosphorus (mg/dl)	3.80 ± 0.50	3.84 ± 0.48	NS
24-h urinary calcium/creatinine	0.11 ± 0.05	0.15 ± 0.07	0.02

NS, Not significant; BCE, bone collagen equivalent.

TABLE 3. Hormonal data

	Controls (n = 20)	AN (n = 22)	P
Mean serum cortisol (μg/dl)	6.0 ± 1.1	8.8 ± 2.2	<0.0001
Peak serum cortisol (μg/dl)	17.8 ± 2.3	21.3 ± 2.5	<0.0001
UFC (μg/d)	30.9 ± 12.0	32.0 ± 16.8	NS
24-h creatinine (mg)	986 ± 313	707 ± 284	0.004
UFC/creatinine ($\times 10^{-3}$)	0.033 ± 0.013	0.051 ± 0.029	0.01
UFC/creatinine·SA ($\times 10^{-3}$)	0.021 ± 0.008	0.035 ± 0.003	0.008
Estradiol (pmol/liter)	75.4 ± 28.0	58.0 ± 22.8	0.03
Leptin (μg/liter)	13.1 ± 4.8	3.4 ± 2.7	<0.0001

SA, Surface area; NS, not significant.

Hormonal data

Cortisol, estradiol, and leptin. Mean nocturnal serum cortisol secretion was markedly higher in girls with AN than in controls (Table 3), as was peak cortisol secretion. Absolute values of urinary free cortisol (UFC) were not different in the two groups. No subject had an UFC value that was above the normal range. However, when UFC was standardized for creatinine and for surface area and creatinine, levels were significantly higher in girls with AN than in controls. This standardization was performed after a recent report suggesting that controlling UFC values for creatinine and surface area is necessary in adolescents (32). Mean serum cortisol levels correlated with urinary free cortisol/creatinine ratio (r = 0.50; $P = 0.0006$) and with urinary free cortisol/creatinine.surface area (r = 0.53; $P = 0.0006$). Serum cortisol levels did not correlate with urinary free cortisol or with the ratio of urinary free cortisol/surface area. Lean body mass was not different in the two groups; thus, differences in creatinine excretion could not be accounted for by differences in muscle mass.

Levels of estradiol were significantly lower in AN than in controls (58.0 ± 22.8 *vs.* 75.4 ± 28.0 pmol/liter; $P = 0.03$) even though all controls were studied in the early follicular phase of their menstrual cycles, when estradiol levels are lowest. Leptin levels were markedly decreased in girls with AN compared with healthy adolescent girls (3.4 ± 2.7 *vs.* 13.1 ± 4.8 ng/ml; $P < 0.0001$).

GH concentration (Cluster analysis) and IGF-I. Mean IGF-I levels were significantly lower in girls with AN than in controls (Table 4), with levels in AN being reduced by almost 30%. Mean GH values over 12 h of nocturnal sampling were significantly higher in AN than in healthy subjects (Table 4). Adolescent girls with AN thus had lower IGF-I levels despite higher GH concentrations. Girls with AN had higher nadir GH concentrations (2.3 ± 1.4 *vs.* 1.1 ± 0.9 ng/ml; $P = 0.002$) and a greater total AUC than healthy controls (3710 ± 1881 *vs.* 2661 ± 1099 ng/ml; $P = 0.03$). The width of GH concentration peaks and the area under these concentration peaks were greater in AN than in controls. Cluster data for two subjects with AN and two controls are shown in Fig. 1A.

GH secretory dynamics (deconvolutional analysis). The basal GH secretion rate was significantly higher in adolescent girls with AN than in controls (0.020 ± 0.017 *vs.* 0.011 ± 0.006 ng/ml·min; $P = 0.03$), and girls with AN also had a larger number of secretory bursts over the 12-h sampling period (11.8 ± 1.4 *vs.* 10.4 ± 1.7; $P = 0.007$; Table 5). Subsequent to increased nocturnal burst frequency in AN, this group had a shorter interpulse interval (60.7 ± 5.8 *vs.* 68.0 ± 10.6 min; $P = 0.008$). Mean amplitude and area of secretory bursts did not differ in the two groups, but the nocturnal pulsatile production of GH trended higher in AN than in controls (222 ± 136 *vs.* 166 ± 66 ng/ml; $P = 0.09$). The ratio of pulsatile to total GH secretion did not differ in girls with AN and controls. The half-life of GH was higher in AN than in controls, but did not reach statistical significance. Deconvolutional analysis of GH concentration and corresponding secretory bursts in two girls with AN and two controls are illustrated in Fig. 1b. Subjects with AN had a greater disorderliness of GH secretion, as measured by ApEn, compared with healthy adolescent girls (1.45 ± 0.46 *vs.* 1.06 ± 0.33; $P = 0.004$).

Girls in early puberty or with immature BA had higher GH

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TABLE 4. GH concentration and IGF-I (Cluster analysis)

	Controls (n = 20)	AN (n = 22)	*P*
IGF-I (ng/ml)	340 ± 77	244 ± 72	0.0002
12-h mean GH (ng/ml)	3.6 ± 1.5	5.1 ± 2.5	0.03
Nadir of GH (ng/ml)	1.1 ± 0.9	2.3 ± 1.4	0.002
Mean no. of GH peaks	3.1 ± 0.9	2.3 ± 0.8	0.01
Mean interval between GH peaks (min)	172 ± 50	162 ± 52	NS
Mean amplitude of GH peaks (ng/ml)	8.8 ± 3.7	10.9 ± 5.0	NS
Mean width of GH peaks (min)	113 ± 37	163 ± 94	0.03
Mean area under GH peak (ng/ml)	511 ± 290	768 ± 533	0.06
Total AUC (ng/ml)	2661 ± 1099	3710 ± 1881	0.03

NS, Not significant.

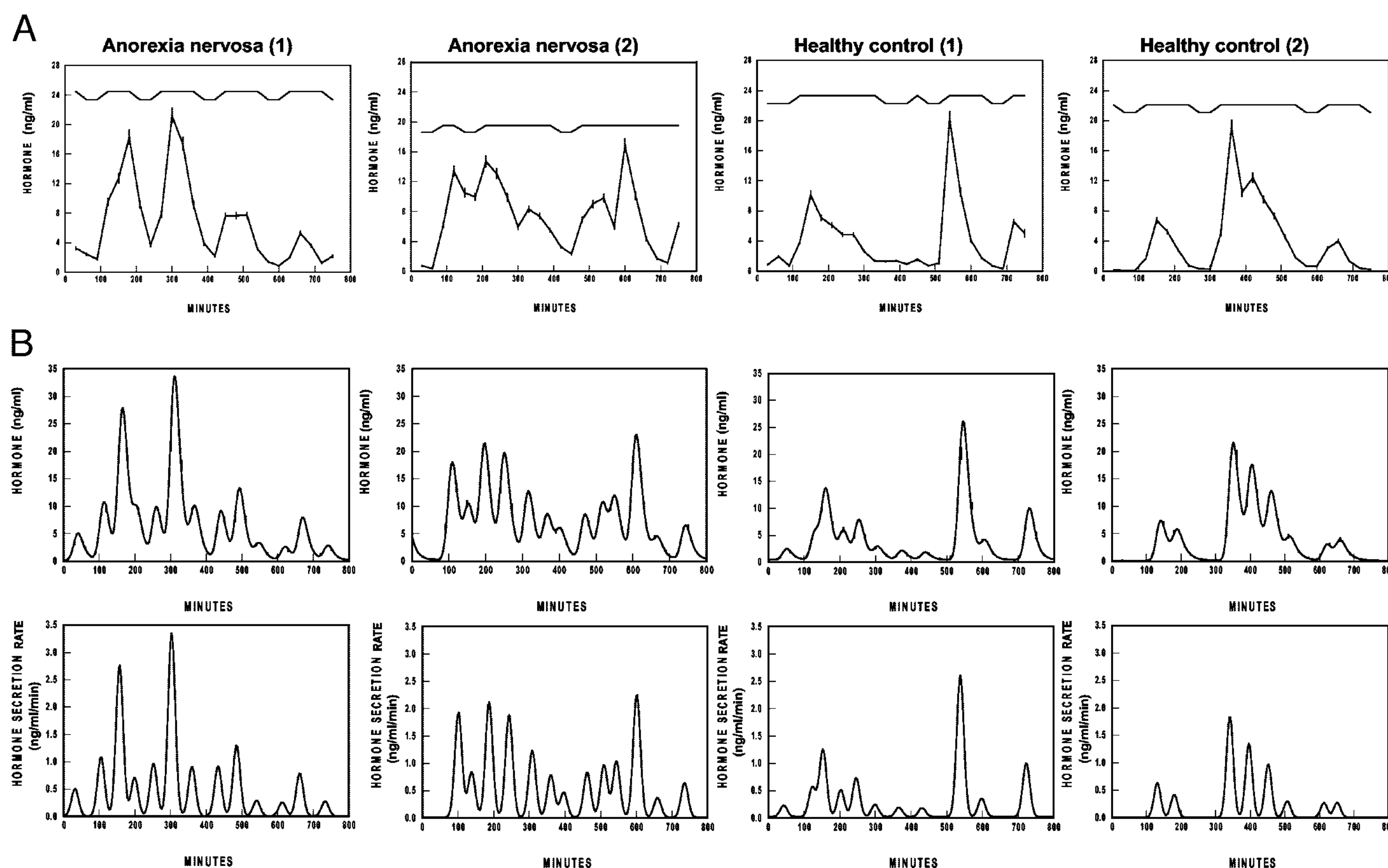

FIG. 1. Cluster and deconvolutional analyses in AN patients and controls. A, Cluster analysis in two girls with AN (*two left panels*) and two healthy adolescent girls (*two right panels*). The mean and nadir GH concentrations and the total AUC were greater in girls with AN than in controls. B, Deconvolutional analysis in the two girls with AN and the two healthy controls analyzed by Cluster in A. The *upper panels* show GH concentrations over the sampling period; the *lower panels* show the individual secretory bursts. Girls with AN had higher basal GH secretion and a greater number of secretory episodes than healthy adolescents of comparable chronological and bone ages.

concentration and secretion than those in late puberty or with mature BA in both groups; immature AN had higher GH values than immature controls, and mature AN had higher GH values than mature controls. However, these differences did not reach statistical significance.

Relationship of GH to cortisol secretion. A negative correlation was noted between IGF-I levels and mean nocturnal cortisol secretion (r = −0.58; *P* = 0.0001) when both groups were considered; thus, girls with the lowest IGF-I values had the highest mean cortisol secretion. In girls with AN, weak negative correlations were observed between mean cortisol values and basal GH secretion (r = −0.40; *P* = 0.07), mean GH concentration (r = −0.41; *P* = 0.06), and total AUC (r = −0.43; *P* = 0.05). However, despite the significantly higher mean cortisol secretion in girls with AN and an inverse relationship between cortisol and GH secretion, these subjects had higher levels of mean and nadir GH concentration, greater basal GH secretion, and a higher pulse frequency than controls.

Relationship of GH to nutritional parameters. In both groups taken together, basal and pulsatile GH secretion, mean and nadir GH concentrations, and total AUC correlated nega-

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TABLE 5. GH secretory dynamics and ApEn (deconvolutional analysis)

	Controls (n = 20)	AN (n = 22)	*P*
IGF-I (ng/ml)	343 ± 77	244 ± 72	<0.0001
Basal GH secretion rate (ng/ml·min)	0.011 ± 0.006	0.020 ± 0.017	0.03
Total nocturnal basal GH secretion (ng/ml·12 h)	8.2 ± 4.3	14.7 ± 12.1	0.03
No. of nocturnal secretory bursts/12 h	10.4 ± 1.7	11.8 ± 1.4	0.007
Mean interval between secretory bursts (min)	68.0 ± 10.6	60.7 ± 5.8	0.008
Mean amplitude of secretory bursts (ng/ml)	0.65 ± 0.22	0.79 ± 0.48	NS
Amplitude of largest secretion burst (ng/ml)	1.74 ± 0.65	2.15 ± 1.52	NS
Area under secretion bursts (ng/ml)	16.0 ± 6.3	18.8 ± 2.4	NS
Total nocturnal pulsatile GH secretion (ng/ml·12 h)	166 ± 66	222 ± 136	0.09
Total nocturnal GH secretion (ng/ml·12 h)	174 ± 68	237 ± 139	0.07
Pulsatile/total secretion	0.95 ± 0.03	0.93 ± 0.07	NS
Half life (min)	11.4 ± 1.8	12.8 ± 4.0	NS
ApEn	1.06 ± 0.33	1.45 ± 0.46	0.004

NS, Not significant.

TABLE 6. Relationship between GH secretion and concentration, IGF-I, and markers of nutritional status (all subjects)

	Basal GH secretion		Pulsatile GH secretion		Mean GH		Nadir of GH	
	r	*P*	r	*P*	r	*P*	r	*P*
BMI (kg/m^2)	−0.34	0.03	−0.33	0.03	−0.46	0.002	−0.45	0.003
% Fat mass	−0.34	0.03	−0.31	0.05	−0.41	0.008	−0.51	0.0007
Leptin (μg/liter)	−0.36	0.02	−0.32	0.04	−0.45	0.003	−0.54	0.0003

tively with measures of nutritional status, including body mass index, percent fat mass, and leptin (Table 6), whereas IGF-I values correlated positively with these parameters (r = 0.38; *P* = 0.01, r = 0.38; *P* = 0.01, and r = 0.43; *P* = 0.005, respectively). On multiple regression analysis including BMI, fat mass, and leptin, serum leptin was the single most important predictor of the nadir GH concentration (r^2= 30%) and number of GH pulses (r^2= 17%), whereas BMI was the most important predictor of mean GH concentration (r^2= 22%) and total AUC (r^2= 21%). Fat mass predicted basal GH secretion (r^2= 17%), pulsatile GH secretion (r^2= 13%), and total GH secretion (r^2= 14%). IGF-I values correlated inversely with the nadir GH concentration (r = −0.35; *P* = 0.02), and a trend toward an inverse correlation was observed with basal GH secretion (r = −0.29; *P* = 0.06). No relationship was observed between GH values and the height of the subjects.

Relationship of GH to bone density. An inverse relationship was observed between the nadir GH concentration and lumbar BMD z-scores (r = −0.38; *P* = 0.01) and lumbar BMAD z-scores (r = −0.41; *P* = 0.008) in the whole group. A weaker inverse correlation was observed with hip BMD z-scores (r = −0.26; *P* = 0.09). Basal GH secretion correlated negatively with femoral neck BMD z-scores (r = −0.33; *P* = 0.03) and weakly with hip BMD z-scores (r = −0.27; *P* = 0.08). On stepwise regression analysis, including GH secretion and concentration and BMI, the contribution of GH secretion and concentration to BMD parameters was no longer significant. BMI accounted for 25% of the variability of lumbar BMD z-scores, 20% of the variability in lumbar BMAD z-scores, 14% in femoral neck BMD z-scores, and 13% in hip BMD z-scores.

Relationship of GH to markers of bone turnover. In healthy controls, strong positive correlations were noted between all markers of bone formation (PICP, OC, and BSAP) and total AUC, mean GH concentration, and nadir GH concentration (Table 7). Similar positive correlations were observed between NTX (a marker of bone resorption) and these measures of GH concentration, but not between DPD (the other marker of bone resorption) and GH. These correlations were lost in girls with AN. Serum IGF-I and leptin levels did not predict levels of markers of bone turnover in healthy controls or in girls with AN. In all subjects taken together, mean serum cortisol values correlated inversely with PICP (r = −0.41; *P* = 0.008), OC (r = −0.31; *P* = 0.04), NTX (r = −0.37; *P* = 0.01), and DPD (r = −0.30; *P* = 0.05).

Discussion

We demonstrate increased GH concentrations in adolescent girls with AN in the presence of decreased IGF-I levels despite elevated cortisol levels. Because undernutrition directly inhibits IGF-1 production from the liver, GH levels may increase because of negative feedback. Therefore, GH action, as measured by IGF-I, is impaired despite elevated GH levels. We also demonstrate significantly greater basal GH secretion and increased pulse frequency seen in girls with AN compared with healthy adolescents. Nutritional status predicts GH secretion and concentration, with an inverse relationship demonstrable with all measures of nutritional status. GH concentration predicts levels of markers of bone turnover in healthy adolescents, but not in girls with AN. Our data suggest a state of acquired GH resistance in adolescent girls with AN, which may contribute to the lower BMD seen in this disorder.

Adults with AN have high GH values in the setting of low IGF-I levels, suggestive of GH resistance (15, 20, 21, 39). However, GH data from adults cannot be extrapolated to adolescents. One study performed Cluster analysis on data

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TABLE 7. Relationship between GH concentration and markers of bone turnover

	Total AUC		Mean GH		Nadir of GH	
	r	*P*	r	*P*	r	*P*
PICP						
Controls	0.57	0.009	0.58	0.007	0.64	0.002
AN	0.38	0.08	0.38	0.09	0.10	0.67
BSAP						
Controls	0.57	0.01	0.56	0.01	0.62	0.005
AN	0.23	0.23	0.26	0.24	−0.17	0.45
OC						
Controls	0.56	0.01	0.53	0.02	0.69	0.0007
AN	0.21	0.35	0.20	0.38	−0.14	0.52
NTX						
Controls	0.65	0.002	0.64	0.002	0.46	0.04
AN	0.25	0.27	0.24	0.29	−0.10	0.67
DPD						
Controls	0.35	0.15	0.34	0.17	0.22	0.38
AN	0.12	0.60	0.12	0.59	−0.10	0.69

from fully pubertal adolescents with AN and controls and reported two groups of Tanner stage 5 adolescents with AN, one over secreted and the other under secreted GH despite low IGF-I levels in both groups (19). IGF-I values did not predict the GH concentration in this study. Deconvolutional analysis demonstrating GH secretory patterns has not been examined in adolescents with AN.

In our study the GH concentration (mean, nadir, and total AUC) was significantly higher in adolescent girls with AN than in healthy controls on Cluster analysis. The greater width of and area under peaks of GH concentration in girls with AN appear to be subsequent to the increased frequency of secretory bursts in this group (as demonstrated on deconvolutional analysis). The increase in basal and pulsatile GH secretion on deconvolutional analysis in AN in our study was of lesser magnitude than that noted in the adult study by Stoving *et al.* (15). In addition, we noted no differences in secretory burst mass in the two groups, whereas the adult study reported higher burst mass in women with AN than in controls. This may be related to the shorter duration of disease and the less severe disease in adolescents. Adolescents are already in a state of maximal GH stimulation (40, 41), and a less marked difference from controls compared with an adult population may suggest a maximal GH threshold even with negative feedback from low IGF-I levels. Our results are in direct contrast to those observed in obese individuals, who have decreased basal and pulsatile GH secretion (42). However, decreased pulsatile GH secretion in obesity is a function of smaller burst mass, whereas pulse frequency does not change.

The sampling frequency for GH was every 30 min to minimize the amount of blood drawn from our young subjects. Because the half-life of GH is shorter than 30 min, data from this study, especially from Cluster analysis, should not be used as a database. However, data obtained from deconvolutional analysis in our healthy subjects are very similar to data obtained by Veldhuis *et al.* (43) in healthy adolescents in whom sampling was performed more frequently.

Girls with AN had higher nocturnal serum cortisol levels similar to those in adult women with AN (15). Previous studies in adolescent girls with AN have reported no differences in urinary free cortisol values compared with healthy adolescents (8, 26). In our study we similarly noted no differences in urinary free cortisol values in AN and controls despite higher serum cortisol values in AN. However, urinary free cortisol standardized for creatinine and surface area was significantly higher in AN than in healthy adolescents. There is evidence that hypercortisolism can affect the somatotropic axis at various levels (44, 45) and can cause a decrease in burst mass (46, 47). We found no differences in burst mass in the two groups. Weak inverse relationships were noted between mean serum cortisol and GH secretion and concentration in girls with AN, suggesting that high concentrations of cortisol may affect GH levels. However, because GH secretion and concentration were higher in girls with AN than in controls despite elevated cortisol levels in AN, it appears that the effects of undernutrition on GH secretion and concentration override the effects of hypercortisolism.

Inverse correlations observed in our study between markers of nutritional status (such as BMI, fat mass, and leptin) and measures of GH secretion and concentration provide further evidence that the alteration in hypothalamic control of GH secretion in AN is nutritionally regulated. Unlike other studies (15, 18–21), we found a negative correlation between IGF-I values and basal GH secretion as well as nadir GH concentration, suggesting that in adolescents with AN, negative feedback subsequent to low IGF-I values may play a role in increased GH secretion.

Interestingly, we observed a negative correlation between leptin values and basal and pulsatile GH secretion and with GH concentration. One hypothesis is that decreased fat mass in adolescents with AN is subsequent to the lipolytic effect of increased GH secretion and concentration. This is supported by reports of low serum leptin levels and decreased fat mass in GH-deficient adults receiving recombinant human GH therapy (48). However, adults with Laron's syndrome (a genetic condition of GH resistance associated with a defect in the GH receptor or in postreceptor mechanisms) have a decrease in lean body mass and an increase in fat mass (49). Given the inverse relationship reported between leptin values and ghrelin in recent studies (50, 51), the correlations between GH secretory patterns and leptin in this study might, in fact, reflect the effects of ghrelin on basal and pulsatile GH secretion. Ghrelin values, however, were not measured in this group of patients.

Serum estradiol levels have been demonstrated to affect GH secretory burst frequency (15, 52) and half-life (53). Estradiol values were lower in girls with AN than in controls in our study, but did not predict any parameter of GH secretion. About 20–25% of subjects in each group were pubertally immature. However, we do not believe that the effects of puberty affected our results because 1) the numbers of immature and mature subjects were not different in the two groups; and 2) similar trends in GH values were seen in these subgroups (with immature AN having higher GH values than immature controls, and mature AN having higher GH values than mature controls). Moreover, results across a range of pubertal stages appear similar to those in adults with AN.

Because GH and IGF-I play an important role in bone

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metabolism and linear growth, it is important to determine whether alterations in GH secretory patterns affect bone metabolism and stature. Bachrach *et al.* (49) reported an increase in markers of bone resorption in adults with Laron's syndrome with no difference in levels of markers of bone formation compared with healthy adults. We have demonstrated a decrease in all markers of bone turnover in girls with AN in previous studies (11), although in this study these decreases in bone turnover markers did not reach statistical significance. The shorter duration of AN and possible improvement in weight before enrollment in these subjects (11) may account for this difference, as bone turnover markers change rapidly with nutritional status (54).

GH plays an important role in stimulating the proliferation of osteoblast precursors, and then both directly and through IGF-I activates the differentiation of these precursors as well as the differentiated functions of mature osteoblasts (27). In healthy controls, GH concentration (but not IGF-I) predicted the levels of all markers of bone formation and one marker of bone resorption, supporting these data. These correlations were lost in girls with AN, suggesting that increased secretion and concentration of GH are unable to directly stimulate bone turnover in undernutrition, which is further evidence of impaired GH action in AN.

IGF-I levels did not predict levels of markers of bone turnover in healthy controls. However, serum IGF-I levels may not reflect local IGF-I production by osteoblasts in response to GH, and the inadequate response of bone turnover markers to the high GH levels in AN may be related to decreased local, rather than systemic, IGF-I production. In contrast, recombinant human IGF-I increases levels of markers of bone formation in adult patients with Laron's syndrome, suggesting a role for systemic IGF-I levels in bone metabolism (55). Another possibility is that our results reflect resistance to direct effects of GH on osteoblasts and osteoclasts in the early phases of undernutrition. The fact that GH concentration, but not the nature of GH secretion, correlated positively with markers of bone turnover in healthy adolescents suggests that the effects of GH on bone are mediated by net GH in serum more than by individual secretory events. We emphasize, however, that these are data derived from correlational analyses and do not imply causality.

We thus demonstrate increased basal GH secretion and increased frequency of GH secretory pulses in adolescent girls with AN. We also demonstrate that increased GH in AN is not associated with increased bone turnover, suggesting that GH resistance plays a role in the low bone turnover state and the low BMD associated with AN.

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