

# Effects of Growth Hormone Replacement on Parathyroid Hormone Sensitivity and Bone Mineral Metabolism

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Adult GH deficiency (AGHD) is associated with reduced bone mineral density, and decreased end-organ sensitivity to the effects of PTH has been suggested as a possible underlying mechanism. We investigated the effects of GH replacement (GHR) on PTH circulating activity and its association with phosphocalcium metabolism and bone turnover in 16 (8 men and 8 women) AGHD patients. Half-hourly blood and 3 hourly urine sampling was performed on each patient over a 24-h period before GHR and then after 1, 3, 6, and 12 months of GHR. GH was commenced at a dose of 0.5 IU/d and was titrated to achieve and maintain an IGF-I SD score within 2 SD of the age-related reference range.

The target IGF-I SD score was achieved within 3 months and was maintained at 12 months after GHR in all patients. Our results demonstrated a significant decrease in serum PTH at all visits after GHR compared with baseline values ( $P < 0.001$ ), with a concomitant increase in nephrogenous cAMP excretion at 1 ( $P < 0.001$ ) and 3 ( $P < 0.05$ ) months and increases in serum calcium ( $P < 0.001$ ), serum phosphate ( $P < 0.001$ ), 1,25-dihydroxyvitamin D<sub>3</sub> ( $P < 0.001$ ), type I collagen C-telopeptide (a bone resorption marker;  $P < 0.001$ ), and procollagen type I amino-terminal propeptide (a bone formation marker;  $P <$

0.001). Simultaneously, we observed a significant decrease in urinary calcium excretion ( $P < 0.001$ ) and an increase in maximum tubular phosphate reabsorption ( $P < 0.001$ ). Together these results suggest increased end-organ responsiveness to the effects of circulating PTH resulting in increased bone turnover and reduced calcium excretion. Significant circadian rhythms were observed for serum PTH, phosphate, type I collagen C-telopeptide, and procollagen type I amino-terminal propeptide before and after GHR. However, sustained PTH secretion was observed between 1400–2200 h, with a reduced nocturnal rise in untreated AGHD patients, whereas PTH secretion decreased significantly between 1400–2200 h ( $P < 0.001$ ), with a significant increase in nocturnal PTH secretion ( $P < 0.001$ ) after 12 months of GHR.

Our results demonstrate that GH may have a regulatory role in bone mineral metabolism, and our data provide a possible underlying mechanism for the development of osteoporosis in AGHD patients. The changes observed after GHR may further explain the beneficial effects of GHR on bone mineral density that have consistently been reported. (*J Clin Endocrinol Metab* 88: 2860–2868, 2003)

**A**DULT GH DEFICIENCY (AGHD) is associated with an increased prevalence of osteoporosis and reduced bone mass at different skeletal sites compared with healthy control subjects (1). The causes of osteoporosis are complex and multifactorial. Studies have suggested that the GH/IGF-I axis is one of the major determinants of adult bone mass (2). An increasing proportion of men and women with advancing age and no clinical evidence of pituitary pathology show a decline in GH secretion and serum IGF-I concentration (3). As AGHD and normal aging are both associated with a decrease in bone mass, it is possible that reduced GH secretion and IGF-I concentration in AGHD may account at least in part for this effect.

Although GH plays an important role in bone metabolism, the underlying mechanisms remain unclear. Among other hormones, PTH plays an important role in bone metabolism and has both catabolic and anabolic effects on bone (4, 5). These effects are mediated via PTH receptors in bone and

indirectly through regulation of the vitamin D/calcium axis via receptors in the kidney (6). These receptors in the kidneys activate mitochondrial vitamin D 1 $\alpha$ -hydroxylase, leading to increased serum 1,25-dihydroxyvitamin D<sub>3</sub> [1,25(OH)<sub>2</sub>D<sub>3</sub>], which, in turn, is a potent inducer of intestinal calcium absorption and bone resorption (7), whereas PTH exerts an antiapoptotic effect on osteoblasts via cAMP-mediated signals (5). We have previously demonstrated decreased bone and renal target cell sensitivity to the effects of PTH in untreated AGHD patients with significantly lower nephrogenous cAMP (NcAMP), bone turnover, and higher calcium excretion in the presence of significantly higher PTH concentrations compared with age- and gender-matched healthy controls (8).

PTH is secreted in a circadian pattern in healthy individuals (8, 9), and although the role of PTH circadian rhythm is not established, there is accumulating evidence of the importance of the nocturnal rise and subsequent fall in PTH secretion over 24 h in health and disease (8–12). The PTH rhythm is lost in patients with primary hyperparathyroidism and is restored after parathyroid surgery (13). Abnormalities in circadian rhythms of bone resorption and renal calcium conservation in women with postmenopausal osteoporosis are associated with blunting of the nocturnal rise in PTH secretion (12), suggesting that the dynamics of PTH secretion

Abbreviations: AGHD, Adult GH deficiency; BMD, bone mineral density; CTX, type I collagen C-telopeptide; CV, coefficient of variance; 1,25(OH)<sub>2</sub>D<sub>3</sub>, 1,25-dihydroxyvitamin D<sub>3</sub>; GFR, glomerular filtration rate; GHR, GH replacement; MESOR, midline estimate statistic of rhythm; NcAMP, nephrogenous cAMP; PcAMP, plasma cAMP; PINP, procollagen type I amino-terminal propeptide; SDS, SD score; TmPO<sub>4</sub>/GFR, tubular phosphate reabsorption.

may have an important role in calcium metabolism and bone remodeling. Animal and human studies have shown the importance of intermittent PTH injections in increasing trabecular bone mass, whereas continuous PTH infusions favor bone resorption (14–16).

There are as yet no reports of GH regulating PTH secretion, but a possible role has previously been suggested (17). Although GH replacement (GHR) is consistently reported to increase bone turnover and phosphocalcium balance (18–20), these effects were suggested to be independent of PTH, as these studies failed to observe any consistent changes in PTH (21–24). As PTH is secreted in a circadian pattern in healthy individuals (8, 9), the variability in PTH results may reflect the single time point methodology used in the previous studies (21–24).

The effects of GHR on PTH secretory pattern and its association with changes in phosphocalcium metabolism and bone turnover in AGHD patients have not been fully explained. We investigated the effects of GHR on PTH secretory pattern in AGHD patients and its association with changes in bone turnover and phosphocalcium homeostasis in a 12-month prospective study with an aim to determine the possible underlying mechanisms responsible for changes in bone metabolism.

## Patients and Methods

### Patients

Sixteen patients (eight men and eight women) with confirmed AGHD were recruited for the study. The local ethics committee approved the study, and all patients provided written informed consent before recruitment. All patients had undergone pituitary surgery, and the original diagnoses are presented in Table 1. Severe AGHD was defined as a peak GH response of less than 9 mU/liter (3  $\mu$ g/liter) to insulin-induced hypoglycemia (blood glucose, <2.2 mmol/liter) (25). Eight patients had a peak GH response of less than 0.5 mU/liter (1.6  $\mu$ g/liter) to provocative tests, with the peak GH response in seven patients between 0.5–5.0 mU/liter (0.16–1.6  $\mu$ g/liter). All patients required additional pituitary hormone replacement and were receiving optimal doses at recruitment (Table 1), which were stable for more than 3 yr before recruitment. None of these patients previously received GH therapy. The mean age  $\pm$  SD at recruitment was 49.5  $\pm$  10.7 yr, and the mean time  $\pm$  SD from diagnosis of AGHD to recruitment into the study was 10.7  $\pm$  6.3 yr. All patients were trained in the use of an automated pen device (Humatrope-Pen II, Eli Lilly & Co., Basingstoke, UK) for sc

self-injection of GH before recruitment. After baseline measurements, GH (Humatrope, Eli Lilly & Co.) was commenced at a daily dose of 0.5 IU/d (0.17 mg/d), self-injected at 2200 h every night. The GH dose was titrated at 2 wk after commencement, by increments of 0.25 IU/d (0.085 mg/d), according to the IGF-I concentration with an aim to maintain IGF-I within the 2 SD score (SDS) of the age-related reference range.

### Methods

Subjects were hospitalized at 1300 h for a 25-h period before and after 1, 3, 6, and 12 months of GHR. Blood samples were obtained half-hourly from 1400–1400 h via indwelling venous cannulae inserted at the time of admission. Each time 5-ml blood was sampled, and the samples were immediately centrifuged and separated. Routine analysis was performed on each sample, and then an aliquot was frozen at –20 C before further analysis.

Urine samples were collected at 3-h intervals between 1400–2300 and 0800–1400 h, and aliquots of the samples were stored at –20 C before further analysis. During their stay all patients were ambulant during 1400–2300 h and 0800–1400 h and were recumbent between 2300–0800 h. Standard hospital meals were served at 1800, 0800, and 1200 h and were consumed within 30 min.

### Biochemistry

**Plasma.** Serum adjusted calcium, phosphate, creatinine, and albumin were measured on all samples by the standard autoanalyzer method (Hitachi 747, Roche, Lewes, UK). Serum calcium was adjusted for albumin (26). Serum adjusted calcium has been shown to strongly correlate with ionized calcium and has been found to be precise in subjects with calcium and albumin within the reference range (26–28). Serum PTH-(1–84) was measured on all samples using a commercial assay (Nichols Institute Diagnostics, San Juan Capistrano, CA) with a detection limit of 0.5 pmol/liter and inter- and intra-assay coefficients of variance (CVs) of less than 7% across the working range.

Plasma cAMP (PcAMP) was measured by RIA (BIOTRAC cAMP, Amersham Pharmacia Biotech, Little Chalfont, UK). The intraassay CV was less than 8%, and the interassay CV was less than 10% across the working range, with a detection limit of 5 nmol/liter. NcAMP, which reflects the circulating activity of PTH (29), was determined according to the formula:  $NcAMP = [S_{Cr} \times (U_{cAMP}/U_{Cr})] - P_{cAMP}$ , where NcAMP is expressed as nanomoles per liter of glomerular filtration rate (GFR),  $S_{Cr}$  is serum creatinine in micromoles per liter,  $U_{cAMP}$  is urinary cAMP in micromoles per liter,  $U_{Cr}$  is urinary creatinine in millimoles per liter, and  $P_{cAMP}$  is plasma cAMP in nanomoles per liter. Serum IGF-I was measured by RIA as previously described (30), and the IGF-I SDS was then calculated from these values (30).

Serum 1,25-(OH)<sub>2</sub>D<sub>3</sub> was extracted by acetonitrile, purified through a C<sub>18</sub>-OH reverse phase column, and measured by RIA (kit from Nichols Institute Diagnostics) with tritiated recovery on each sample. The in-

**TABLE 1.** Original diagnoses and additional replacement hormones of AGHD patients

Patients	Gender	Diagnosis	Pituitary replacement hormones
1	M	Nonfunctioning pituitary adenoma	Hydrocortisone, T <sub>4</sub> , desmopressin
2	M	Craniopharyngioma	Hydrocortisone, T <sub>4</sub>
3	M	Prolactinoma	Hydrocortisone, T <sub>4</sub> , testosterone
4	M	Cystic adenoma	Hydrocortisone, T <sub>4</sub>
5	M	Craniopharyngioma	Hydrocortisone, T <sub>4</sub> , testosterone
6	M	Nonfunctioning pituitary adenoma	T <sub>4</sub> , testosterone
7	M	Nonfunctioning pituitary adenoma	T <sub>4</sub>
8	M	Nonfunctioning pituitary adenoma	Hydrocortisone, T <sub>4</sub>
9	F	Nonfunctioning pituitary adenoma	Hydrocortisone, T <sub>4</sub>
10	F	Prolactinoma	T <sub>4</sub>
11	F	Epidermal cyst	Hydrocortisone, T <sub>4</sub>
12	F	Nonfunctioning pituitary adenoma	Hydrocortisone, T <sub>4</sub> , cycloprognova
13	F	Craniopharyngioma	Hydrocortisone, T <sub>4</sub>
14	F	Prolactinoma	T <sub>4</sub> , premique
15	F	Nonfunctioning pituitary adenoma	Hydrocortisone, T <sub>4</sub> , premique
16	F	Prolactinoma	Hydrocortisone, T <sub>4</sub>

M, Male; F, female.

traassay CV was less than 9%, and the interassay CV was less than 12% across the working range, with a detection limit of 15 nmol/liter. Serum 25-hydroxyvitamin-D<sub>3</sub> was measured using an RIA kit (DiaSorin, Inc., Stillwater, MN) after acetonitrile extraction. The intraassay CV was less than 8%, and the interassay CV was less than 11% across the working range, with a detection limit of 4 μmol/liter.

Serum concentrations of type I collagen C-telopeptides (CTX), which is a bone resorption marker, were measured on all samples using an electrochemiluminescence assay (ELECSYS, Roche). The intraassay CV was less than 4%, and the interassay CV was less than 5% across the working range, with a detection limit of 0.01 ng/ml. Serum concentrations of procollagen type I amino-terminal propeptide (PINP), which is a bone formation marker, were measured on all samples using an electrochemiluminescence assay (ELECSYS, Roche). The intraassay CV was less than 2%, and the interassay CV was less than 2.5% across the working range, with a detection limit of 4 μg/liter. All serum samples obtained from a given individual over a 24-h period were assayed within a single batch to obviate interassay variability, and the CVs of the assays were independently determined in our Clinical Chemistry department.

### Urine

Urinary creatinine, calcium, and phosphate were analyzed on all samples according to standard laboratory methods (Roche). The renal threshold for maximum tubular phosphate reabsorption rate (TmPO<sub>4</sub>/GFR; millimoles per liter of GFR) was determined from serum and urine measurements and was derived from the nomogram by Walton and Bijvoet (31). Urinary cAMP (UcAMP) was measured using an in-house RIA method that has previously been described (32). The intraassay CV was less than 8%, and the interassay CV was less than 10% across the working range, with a detection limit of 0.2 μmol/liter. Urinary type I collagen cross-linked N-telopeptide (NTx), which is bone resorption marker, was measured using chemiluminescence immunoassay (VITROS ECI, Johnson & Johnson, Amersham, UK) and expressed per millimole of excreted creatinine (NTx/UCr). The intraassay CV was less than 4%, and the interassay CV was less than 5%, with a detection limit of 10 nmol bone collagen equivalent. All urine samples obtained from a given individual over a 24-h period were assayed within a single batch to obviate interassay variability, and the CVs of the assays were independently determined in our Clinical Chemistry department.

### Statistical analysis

Individual and population-mean cosinor analysis, to determine the circadian rhythm parameters of each variable, was performed using CHRONOLAB 3.0 (Universidade de Vigo, Vigo, Spain), a software package for analyzing biological time series by least squares estimation (30, 33). Population-mean cosinor analysis is based on the means of parameter estimates obtained from individuals in the study sample. The software thus provides the following circadian parameters: 1) midline estimate statistic of rhythm (MESOR), defined as the rhythm-adjusted mean or the average value of the rhythmic function fitted to the data; 2) amplitude, defined as half the extent of rhythmic change in a cycle approximated by the fitted cosine curve (difference between the maximum and MESOR of the fitted curve); and 3) acrophase, defined as the lag between a defined reference time (1400 h of the first day in our study when the fitted period is 24 h) and time of peak value of the crest time in the cosine curve fitted to the data.

The general linear model ANOVA for repeated measures was used to analyze the data, and a *t* test for paired data with Bonferroni's correction, to allow for multiple comparisons, was then applied to determine the significance of the differences between visits. Correlations were sought using Pearson's linear correlation coefficient. Values are expressed as the mean ± SEM unless otherwise stated. *P* < 0.05 was considered significant.

## Results

### GH dose and IGF-I levels

The GH dose increased significantly from 0.5 IU/d (0.17 mg/d) to 0.73 IU/d (0.24 mg/d) at 1 month (*P* < 0.01), 0.77 IU/d (0.26 mg/d) at 3 months (*P* < 0.01), and 0.83 IU/d (0.28

mg/d) at 12 months (*P* < 0.01). There were no significant differences in the GH dose between 1 and 12 months. Target IGF-I was achieved within 3 months of commencing GHR and remained within the target range at 12 months in all patients. Serum IGF-I increased from 7.7 ± 1.1 μmol/liter (58.8 ± 8.4 μg/liter) to 24.1 ± 3.3 μmol/liter (184.0 ± 25.2 μg/liter) within 1 month (*P* < 0.001), 26.1 ± 2.4 μmol/liter (199.2 ± 18.3 μg/liter) at 3 months (*P* < 0.001), and 30.2 ± 2.5 μmol/liter (230.5 ± 19.1 μg/liter) at 12 months (*P* < 0.001). Similarly, the IGF-I SDS increased from -4.47 ± 0.86 at baseline to -0.85 ± 0.82 at 1 month (*P* < 0.001), 0.27 ± 0.21 at 3 months (*P* < 0.001), and 0.61 ± 0.23 at 12 months (*P* < 0.001).

### Serum PTH, calcium, and phosphate

The 24-h mean PTH concentration decreased significantly after 1 (4.14 ± 0.04 pmol/liter) and 3 (4.10 ± 0.04 pmol/liter) months of GHR compared with baseline (4.54 ± 0.04 pmol/liter; *P* < 0.001), with a further decrease at 6 months (3.87 ± 0.04 pmol/liter) compared with the previous three visits (*P* < 0.001) that was maintained at 12 months (4.00 ± 0.04 pmol/liter; *P* < 0.001 compared with 0 and 1 months and *P* < 0.05 compared with the 3 month visit; Fig. 1A).

The 24-h mean adjusted serum calcium significantly increased at 1 month (2.32 ± 0.002 mmol/liter) and was maximal at 3 months (2.36 ± 0.002 mmol/liter) compared with baseline (2.31 ± 0.002 mmol/liter; *P* < 0.001), followed by a significant decrease to levels below baseline after 6 (2.28 ± 0.002 mmol/liter) and 12 (2.28 ± 0.002 mmol/liter; *P* < 0.001; Fig. 1B) months.

Similarly, the 24-h mean serum phosphate concentration increased significantly after 1 month (1.17 ± 0.006 mmol/liter) and was maximal at 3 months (1.27 ± 0.006 mmol/liter) compared with baseline (1.09 ± 0.006 mmol/liter; *P* < 0.001), followed by a significant decrease at 6 (1.20 ± 0.006 mmol/liter) and 12 (1.20 ± 0.006 mmol/liter) months compared with 3 months (*P* < 0.001), but remained significantly higher than baseline (*P* < 0.001; Fig. 1C).

### Urine biochemistry

NcAMP values significantly increased at 1 month (17.59 ± 0.77 nmol/liter GFR; *P* < 0.001) and 3 months (15.66 ± 0.77 nmol/liter GFR; *P* < 0.05) compared with baseline (13.31 ± 0.77 nmol/liter GFR), followed by a significant decrease at 6 (12.37 ± 0.79 nmol/liter GFR) and 12 (11.76 ± 0.77 nmol/liter GFR) months, with values decreasing below baseline (*P* < 0.001; Fig. 1D).

The 24-h urinary calcium excretion demonstrated a non-significant decrease at 1 month (2.59 ± 0.40 mmol/liter; *P* = NS) and increase at 3 months (3.06 ± 0.42 mmol/liter; *P* = NS), followed by a significant decrease at 6 (1.71 ± 0.37 mmol/liter; *P* < 0.001) and 12 (1.58 ± 0.26 mmol/liter; *P* < 0.001) months compared with baseline (2.99 ± 0.46 mmol/liter; Fig. 1E).

Similarly, a significant decrease in 24-h urinary phosphate excretion was observed after 6 (10.4 ± 2.2 mmol/liter) and 12 (2.7 ± 0.4 mmol/liter) months of GHR compared with baseline (15.9 ± 2.3 mmol/liter; *P* < 0.001; Fig. 1F). Concomitantly, TmPO<sub>4</sub>/GFR progressively increased at 1 (1.03 ± 0.02

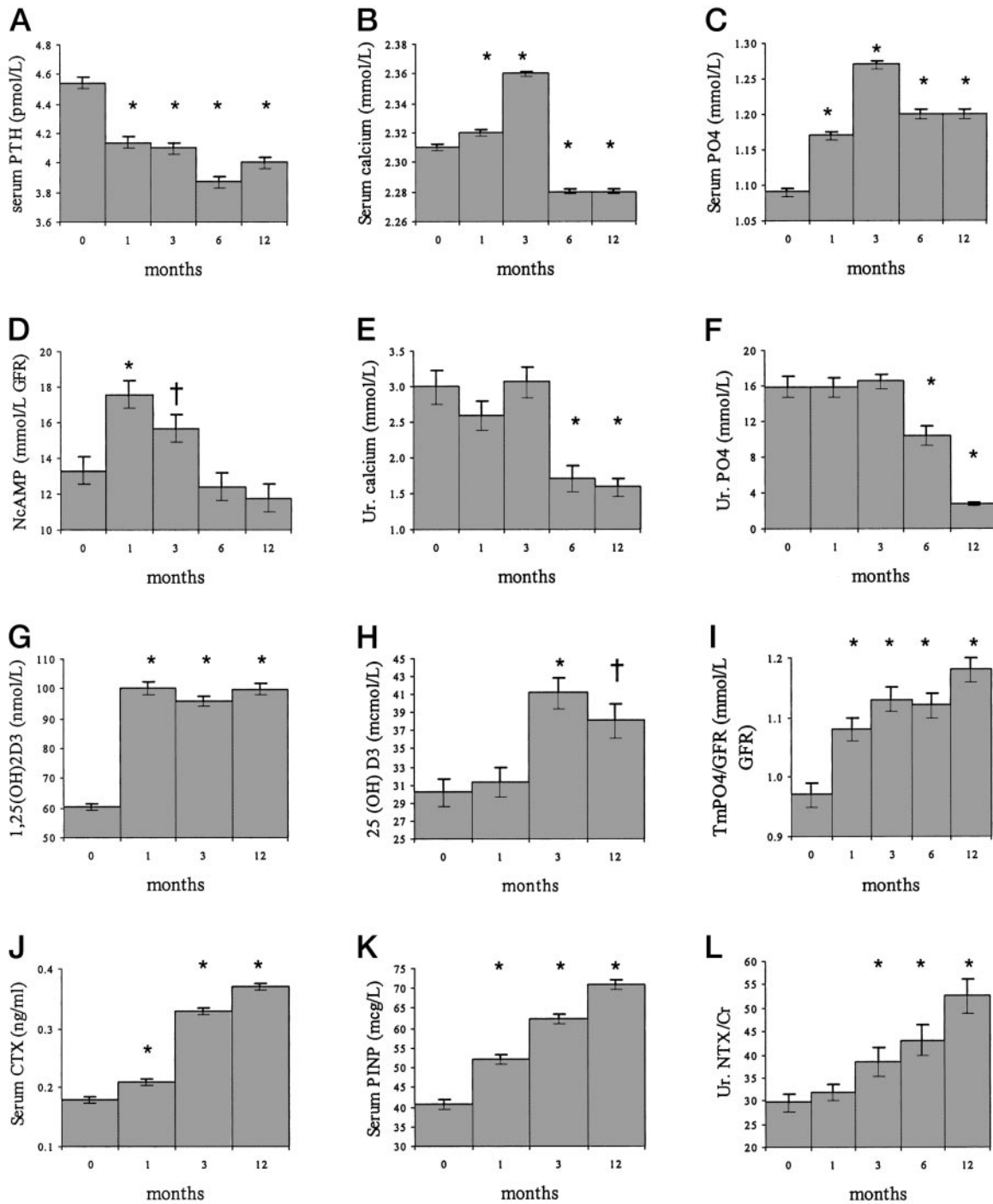


FIG. 1. Twenty-four-hour mean  $\pm$  SEM for serum PTH (A), serum calcium (B), serum phosphate (C), NcAMP excretion (D), urinary calcium excretion rate (E), urinary phosphate excretion rate (F), serum 1,25-(OH)<sub>2</sub>D<sub>3</sub> (G), 25(OH)D<sub>3</sub> (H), TmPO<sub>4</sub>/GFR (I), serum CTx (J), serum PINP (K), and urinary NTx/Cr (L), before GHR at 0 months and then at 1, 3, 6, and 12 months of GHR. \*,  $P < 0.001$ ; †,  $P < 0.05$ .

mmol/liter GFR), 3 ( $1.08 \pm 0.02$  mmol/liter GFR), 6 ( $1.07 \pm 0.02$  mmol/liter GFR), and 12 ( $1.13 \pm 0.02$  mmol/liter GFR) months compared with baseline ( $0.92 \pm 0.02$  mmol/liter GFR;  $P < 0.001$  compared with each visit; Fig. 1I).

*Serum vitamin D metabolites*

There was a significant increase in 1,25-(OH)<sub>2</sub>D<sub>3</sub> after 1 month of GHR ( $100.25 \pm 4.24$  nmol/liter;  $P < 0.001$ ), and

these levels were maintained at 3 ( $95.63 \pm 3.43$  nmol/liter) and 12 ( $99.81 \pm 3.86$  nmol/liter) months compared with baseline ( $60.56 \pm 2.23$  nmol/liter;  $P < 0.001$ ; Fig. 1G). There were no significant changes in 1,25(OH)<sub>2</sub>D<sub>3</sub> concentrations between 1, 3, and 12 months. Serum 25-hydroxyvitamin D<sub>3</sub> increased significantly after 3 months ( $41.13 \pm 3.26$   $\mu$ mol/liter;  $P < 0.001$ ) and was maintained after 12 months ( $38.13 \pm 3.83$   $\mu$ mol/liter;  $P < 0.01$ ) of GHR compared with baseline

( $30.19 \pm 3.01 \mu\text{mol/liter}$ ), with no significant differences between pretreatment and 1 month ( $31.25 \pm 3.17 \mu\text{mol/liter}$ ;  $P = \text{NS}$ ) values (Fig. 1H).

#### Bone markers

The changes in 24-h serum CTX and PINP as well as NTX/Cr are presented in Fig. 1, J–L. The 24-h mean CTX increased significantly at 1 month ( $0.21 \pm 0.005 \text{ ng/ml}$ ;  $P < 0.001$ ) and increased further at 3 ( $0.33 \pm 0.005 \text{ ng/ml}$ ;  $P < 0.001$ ) and 12 months ( $0.37 \pm 0.005 \text{ ng/ml}$ ;  $P < 0.001$ ) compared with baseline ( $0.18 \pm 0.005 \text{ ng/ml}$ ).

Similarly, there was a significant increase in PINP at 1 month ( $52.01 \pm 1.25 \mu\text{g/liter}$ ;  $P < 0.001$ ) that increased further at 3 ( $62.32 \pm 1.25 \mu\text{g/liter}$ ;  $P < 0.001$ ) and 12 ( $70.82 \pm 1.27 \mu\text{g/liter}$ ;  $P < 0.001$ ) months compared with baseline ( $40.76 \pm 1.23 \mu\text{g/liter}$ ).

The 24-h urinary NTX/Cr (nanomoles of bone collagen equivalent per millimoles of creatinine) progressively increased after GHR and was significant at 3 months ( $38.44 \pm 6.01$ ;  $P < 0.001$ ), then increased further at 6 ( $43.03 \pm 6.78$ ,  $P < 0.001$ ) and 12 ( $53.53 \pm 7.57$ ,  $P < 0.001$ ) months compared with baseline ( $29.58 \pm 3.80$ ).

#### Circadian rhythms

*PTH, calcium, and phosphate.* All individuals demonstrated significant circadian rhythms for PTH and phosphate ( $P < 0.001$ ), with no rhythmicity detected for adjusted serum calcium ( $P = \text{NS}$ ). Cosinor-derived population mean circadian rhythm parameter estimates are presented in Fig. 2. There was a significant decrease in PTH MESOR at 3 months ( $4.13 \pm 0.44 \text{ pmol/liter}$ ;  $P < 0.05$ ) compared with baseline ( $4.56 \pm 0.41 \text{ pmol/liter}$ ) that was maintained at 12 months ( $4.01 \pm 0.31 \text{ pmol/liter}$ ;  $P < 0.05$ ). The amplitude decreased significantly at 6 months compared with baseline ( $0.62 \pm 0.09$  vs.  $0.87 \pm 0.10 \text{ pmol/liter}$ ;  $P < 0.05$ ), with no significant change in acrophase.

The phosphate MESOR, similar to the 24-h phosphate mean, showed a significant increase at 1 month compared with baseline ( $1.16 \pm 0.02$  vs.  $1.08 \pm 0.02 \text{ mmol/liter}$ , respectively;  $P < 0.001$ ) that increased further at 3 months ( $1.26 \pm 0.05 \text{ mmol/liter}$ ;  $P < 0.001$  compared with baseline;  $P < 0.05$  compared with 1 month), and although there was a nonsignificant decrease at 6 ( $1.18 \pm 0.03 \text{ mmol/liter}$ ) and 12 ( $1.21 \pm 0.03 \text{ mmol/liter}$ ) months compared with 3 months, the values remained significantly higher than baseline ( $P < 0.001$ ). The amplitude increased at 1 month ( $0.12 \pm 0.01 \text{ mmol/liter}$ ;  $P < 0.05$ ) and remained significantly higher at 3 months ( $0.13 \pm 0.01 \text{ mmol/liter}$ ;  $P < 0.05$ ), 6 months ( $0.12 \pm 0.01 \text{ mmol/liter}$ ;  $P < 0.05$ ), and 12 months ( $0.14 \pm 0.02 \text{ mmol/liter}$ ;  $P < 0.001$ ) compared with baseline ( $0.09 \pm 0.01 \text{ mmol/liter}$ ). There was a nonsignificant backward shift in acrophase from 0217 h at baseline to 0031 h after 12 months.

Although PTH circadian rhythmicity was maintained in AGHD patients, both before and after GHR, we observed a sustained increase in PTH secretion between 1400–2200 h, with a less pronounced nocturnal rise in untreated AGHD compared with treated AGHD patients (Fig. 2). We therefore analyzed the difference in the percent increase in PTH secretion during the periods 1400–2200 h and 2230–0800 h

between visits. The percent increase in PTH secretion between 1400–2200 h was 19.3% before commencing GHR, then significantly decreased to 8.1%, 6.4%, 12.9%, and 6.6% after 1, 3, 6, and 12 months of GHR, respectively ( $P < 0.001$ ). Concomitantly, nocturnal PTH secretion between 2230–0800 h increased from 9.0% before GHR to 9.4% ( $P = \text{NS}$ ), 10.1% ( $P = \text{NS}$ ), 15.8% ( $P < 0.001$ ), and 16.4% ( $P < 0.001$ ) after 1, 3, 6, and 12 months of GHR, respectively. These significant changes may explain the change in PTH amplitude observed after GHR and possibly suggest that GHR in AGHD patient plays an important role in the PTH secretory pattern. As most previous studies using single time point measurements would have invariably measured PTH between 0800–1700 h, we analyzed the difference in PTH concentration at each time point between 0800–1700 h after GHR. We found no significant differences in PTH concentrations at each time point. Further analysis demonstrated that the PTH concentration in blood samples drawn between 0800–0930 h was significantly higher than that in samples drawn between 1000–1130 and 1200–1330 h ( $P < 0.001$ ) and significantly lower than that from samples drawn between 1400–1530 and 1600–1730 h. It was also apparent from our data that most changes in PTH occur during the evening and early morning after GHR (Fig. 2).

*Bone markers.* All individuals demonstrated a significant circadian rhythm for CTX and PINP, both before and after GHR ( $P < 0.001$ ). Cosinor-derived population mean circadian rhythm parameter estimates for CTX and PINP are presented in Fig. 2. There was a significant increase in the CTX ( $0.21 \pm 0.01 \text{ ng/ml}$ ;  $P < 0.01$ ) and PINP ( $52.1 \pm 5.0 \mu\text{g/liter}$ ;  $P < 0.01$ ) MESORs after 1 month of GHR compared with baseline ( $0.18 \pm 0.01 \text{ ng/liter}$  and  $40.8 \pm 3.2 \mu\text{g/liter}$ , respectively); these progressively increased after 3 ( $0.33 \pm 0.02 \text{ ng/ml}$  and  $62.5 \pm 4.9 \mu\text{g/liter}$ , respectively;  $P < 0.001$ ) and 12 ( $0.37 \pm 0.02 \text{ ng/liter}$  and  $70.8 \pm 6.4 \mu\text{g/liter}$ , respectively;  $P < 0.001$ ) months. There was a significant increase in CTX amplitude after 1 month of GHR ( $0.10 \pm 0.01 \text{ ng/ml}$ ;  $P < 0.05$ ) that increased further at 3 ( $0.13 \pm 0.01 \text{ ng/ml}$ ;  $P < 0.001$ ) and 12 ( $0.15 \pm 0.01 \text{ ng/ml}$ ;  $P < 0.001$ ) months compared with baseline ( $0.07 \pm 0.01 \text{ ng/ml}$ ), with no significant differences between 3 and 12 months. PINP amplitude increased significantly after 3 months ( $5.9 \pm 0.5 \mu\text{g/liter}$ ;  $P < 0.01$ ) of GHR compared with baseline ( $2.6 \pm 0.6 \mu\text{g/liter}$ ) and was maintained after 12 months ( $5.4 \pm 0.6 \mu\text{g/liter}$ ;  $P < 0.01$ ), with no significant differences between 3 and 12 months. There were no significant differences in acrophase between visits for either parameter.

#### Discussion

Our data demonstrate not only a significant decrease in PTH concentration, but also a change in the PTH secretory pattern with a concomitant increase in NcAMP after GHR, suggesting an increase in PTH target cell sensitivity (34). These changes were associated with an increase in  $1,25\text{-(OH)}_2\text{D}_3$  and a simultaneous increase in serum calcium that may be the result of increased intestinal calcium absorption (35) and renal tubular calcium reabsorption (6). The increase in serum phosphate and  $\text{TMPO}_4/\text{GFR}$  observed may reflect a direct antiphosphaturic effect of GH (36, 37) or augmented

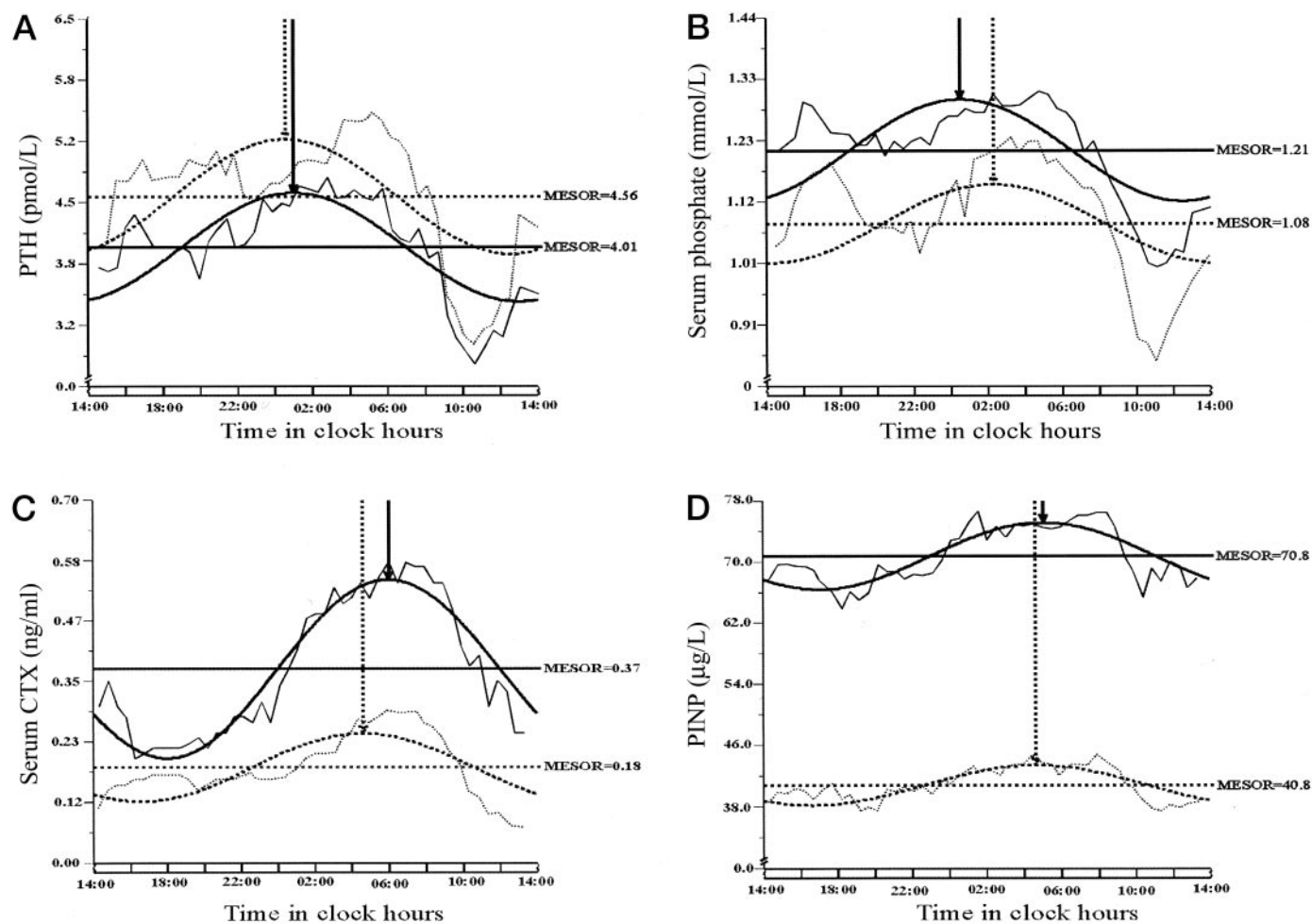


FIG. 2. Cosinor-derived circadian rhythmometry (CHRONOLAB) for PTH (A), serum phosphate (B), CTX (C), and PINP (D) before (dotted lines) and after 12 months of GHR (bold lines). Arrows represent the acrophase.

intestinal absorption mediated by an increased  $1,25\text{-(OH)}_2\text{D}_3$  production after GHR (38). The simultaneous increases in bone resorption and bone formation markers suggest significantly increased bone turnover that may also have contributed to the increase in serum calcium and phosphate. These changes were maximal at 3 months, with serum PTH, calcium, phosphate, and NcAMP excretion decreasing to pretreatment levels, possibly reflecting a new equilibrium state with further contribution from the increased calcium and phosphate uptake by bone, as bone turnover continued at 6 and 12 months. Target IGF-I was achieved in all patients within 3 months and was maintained at 12 months of GHR.

There remains controversy about the mechanism and effects of GHR on phosphocalcium and bone mineral metabolism. PTH, which is mainly regulated by calcium, phosphate, and  $1,25\text{-(OH)}_2\text{D}_3$ , has been reported to decrease (21, 22), not change (24, 34, 35), or increase (39) after GHR. Similarly, reports on the effects of GHR on  $1,25\text{-(OH)}_2\text{D}_3$  vary from a decrease (35), to no change (21, 24, 40), to an increase (22, 34) after therapy. Most previous studies have consistently shown an increase in serum calcium and phosphate after GHR (21, 23, 24, 40). GH administered for 3 d in postmenopausal women resulted in increased in calcium excre-

tion and calcitriol levels, with an increase in serum phosphate observed after 5 wk of GH, whereas calcitriol levels returned to baseline, and PTH showed no changes (41). Other studies that have shown an increase in serum calcium and phosphate after GHR, with variable effects on PTH and  $1,25\text{-(OH)}_2\text{D}_3$ , have suggested the changes to be either due to increased bone turnover and enhanced mobilization of skeletal calcium or increased calcium absorption (21, 23, 24, 40). None of these studies (21, 23, 24, 35, 40) measured NcAMP, which reflects the circulating activity of PTH in both physiological and pathophysiological states and is a reliable index of PTH function (29). In contrast, Burstein *et al.* (34) reported an increase in  $1,25\text{-(OH)}_2\text{D}_3$ , with no significant change in PTH or serum calcium in GHD patients, and a significant increase in NcAMP excretion after GHR, suggesting a possible increase in renal sensitivity to PTH after GHR.

Our findings differ from each of these studies and probably provide a rationalization for the disparity between the results obtained. We have demonstrated a decline in PTH concentration, with a reciprocal increase in NcAMP excretion after 1 and 3 months of GHR. These changes were associated with a simultaneous increase in serum  $1,25\text{-(OH)}_2\text{D}_3$ , calcium, and phosphate, followed by a decrease in 24-h urinary

calcium and phosphate excretion after 6 months of GHR. As NcAMP excretion parallels changes in PTH secretion (42), a reciprocal increase in NcAMP excretion with decreasing PTH concentration, as observed in our study, would suggest increased renal sensitivity to the effects of PTH, as suggested by Burstein *et al.* (34). The increased renal sensitivity to PTH would result in increased  $1\alpha$ -hydroxylase activity with increased  $1,25\text{-(OH)}_2\text{D}_3$  production (41, 43, 44). This, in turn, leads to increased intestinal calcium absorption (35) and renal calcium reabsorption as a direct effect of PTH (6) due to restoration of renal sensitivity (34). This would in part explain the increase in serum calcium observed in our study. Renal insensitivity to PTH in AGHD is further supported by the high 24-h urinary calcium excretion in the presence of a relatively high PTH concentration before GHR that is reduced significantly after 6 months of treatment. The relatively high urinary calcium excretion at 1 and 3 months is in agreement with previous studies (40) and reflects a higher filtered calcium load due to increasing serum calcium observed at concurrent visits. It is unlikely that the observed changes in serum calcium and PTH are a result of relative intestinal insensitivity to  $1,25\text{-(OH)}_2\text{D}_3$  (35), as we would expect to see a decline in  $1,25\text{-(OH)}_2\text{D}_3$  concentrations with restoration of sensitivity, which is clearly not the case in our study.

GH has been shown to support phosphate retention by increasing the renal threshold for phosphate excretion (45, 46), and this effect occurs independently of PTH, vitamin D, or urinary cAMP and is mediated by IGF-I (45, 47). Such an effect derives from an increase in  $\text{TMPO}_4/\text{GFR}$  (45, 46), which is found to be in the lower limit of normal (46) or reduced (48) in GHD and increases with GHR (46, 48). In this regard, the increases in  $\text{TMPO}_4/\text{GFR}$  and serum phosphate concentration may reflect a direct antiphosphaturic effect of GH (36, 37), which is supported by the decrease in 24-h urinary phosphate excretion. The increase in serum phosphate is also mediated by increased intestinal absorption (45), which may be due to the increased production of  $1,25\text{-(OH)}_2\text{D}_3$  (38).

Bone remodeling requires bone resorption and formation, which is reliably assessed by measuring biochemical markers of bone resorption and formation (49). A negative equilibrium between bone resorption and formation has been suggested to explain the low bone mass in AGHD patients (50). GHR simultaneously increases markers of resorption and formation, demonstrating that GH reactivates bone remodeling in AGHD patients (40, 50). It is still unclear whether the changes in bone turnover markers and bone mineral density (BMD) are a direct GH effect or an indirect effect mediated via changes in PTH and  $1,25\text{-(OH)}_2\text{D}_3$ . PTH increases renal tubular reabsorption of calcium, bone resorption, and  $1,25\text{-(OH)}_2\text{D}_3$  production, factors important for the positive bone remodeling during GH treatment (6). Inconsistent reports of changes in PTH concentrations, possibly due to the single time point PTH measurements used in studies (18, 40, 51) have led to the suggestion that changes in bone turnover and BMD are associated with a direct GH effect or an effect via IGF-I. We have previously demonstrated decreased bone and renal target cell sensitivity to the effects of PTH, with significantly lower NcAMP and higher PTH concentration in

untreated AGHD patients compared with healthy controls leading to decreased bone resorption and formation, decreased  $1,25\text{-(OH)}_2\text{D}_3$  production, and increased calcium excretion (8). Our present data support our concept of decreased end-organ sensitivity to the effects of PTH in untreated AGHD patients, which is restored after GHR, resulting in a simultaneous increase in bone turnover markers,  $1,25\text{-(OH)}_2\text{D}_3$ , and serum calcium absorption/reabsorption that will contribute to the previously reported increase in BMD after GHR (18).

Circadian rhythm is known to exist for many hormones (52, 53), and the changes in normal concentrations appear to be important for their physiological and pathophysiological effects. Detailed analysis of the PTH rhythms in our study demonstrated a sustained increase between 1400–2200 h, with a reduced nocturnal rise in untreated AGHD, a pattern similar to that observed in osteoporotic women (10). After GHR, PTH secretion decreased significantly between 1400–2200 h, with a pronounced increase in nocturnal PTH secretion, a pattern previously observed in healthy individuals and nonosteoporotic women (9, 12, 42). These findings may suggest a possible role for GH regulation of PTH secretory pattern. Increased PTH secretion with reduced NcAMP is characteristic of patients with pseudohypoparathyroidism, who have target tissue unresponsiveness to the biological actions of PTH (54). It is possible that decreased target tissue responsiveness to PTH, as suggested by our present data and in part by Burstein *et al.* (34), is responsible for the observed sustained increase in PTH secretion in untreated AGHD. Restoration of the PTH secretory pattern may be an important factor contributing to increased bone remodeling and BMD observed in AGHD after GHR (18). However, it is important to recognize that changes observed after exogenous PTH administration may not necessarily be the same as those occurring in response to enhanced skeletal sensitivity.

As PTH is secreted in a circadian pattern with significant day-night variability (9, 42), the inconsistency in previous reports regarding changes in PTH (21–23) may reflect the single time point sampling methodology used in the studies. We have shown that variability in the time of sampling by 2 h, as may be the case in previous studies using single time point measurements, would result in significant differences in PTH concentration. Our data show that most changes in PTH occur during the evening and early morning after GHR, whereas single PTH measurements would have been performed between 0800–1700 h. These observations emphasize that single time point PTH measurements may not be appropriate to detect changes in PTH and help explain the previously reported PTH variability.

In conclusion, our results suggest that AGHD leads to both renal and bone insensitivity to the effects of PTH. These changes may explain the underlying mechanisms that lead to decreased BMD in untreated AGHD patients and eventually osteoporosis. GHR restores renal and bone sensitivity to PTH, resulting in increased bone turnover and the increase in BMD reported in previous studies. We suggest that future studies investigating the effects of GHR on PTH should use frequent blood sampling to accurately report the changes in this fluctuant hormone.

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