Parathyroid responsiveness to hypocalcemic and hypercalcemic stimuli in adult growth hormone deficiency after growth hormone replacement

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Extracellular ionized calcium concentration is maintained near constancy via a complex homeostatic mechanism, which includes the calcium-sensing receptors found in the parathyroid glands (15), and by an effector system with specialized cells in the kidneys, bone, and intestines that responds to calciotropic hormones with changes in the transport of mineral ions so as to restore calcium and phosphate concentrations toward normal (13). The parathyroid gland calcium-sensing receptor cell is remarkably sensitive to alterations in extracellular calcium concentration, and an inverse sigmoidal relationship has been shown between parathyroid hormone (PTH) release and extracellular calcium concentration in normal and abnormal parathyroid tissue (12).

Extracellular ionized calcium concentration is generally regarded as the principal regulator of PTH secretion in vivo (34). However, it has been suggested that changes in maximal PTH secretion, slope of the PTH secretory curve at the calcium set point (the calcium concentration at which the rate of PTH secretion is one-half of its maximal value) (17), and the calcium set point and maximum suppressibility of PTH release might all contribute to PTH secretion (12). On a quantitative basis, changes in calcium set point have been shown to produce the largest alteration in PTH secretory rate for a given change in the value of a parameter (12). Inherited mutations in the calcium-sensing receptors can produce clinical disorders of mineral ion metabolism, such as familial hypocalciuric hypercalcemia (30), by changing the calcium set point (6, 25).

Elevations in set point are typically seen in hyperparathyroid states (12), implying varying degrees of calcium “resistance” of the parathyroid calcium-sensing mechanism in these conditions that may be the greater contributor to the PTH hypersecretion seen in hyperparathyroidism.

Untreated AGHD has been shown to be associated with an increased prevalence of osteoporosis (7, 22). We have previously demonstrated significantly higher PTH concentration and alterations in PTH secretory pattern with significantly lower nephrogenous cAMP (NcAMP) excretion in untreated adult growth hormone deficiency (AGHD) patients compared with healthy age- and gender-matched controls (2). These findings were associated with low bone turnover and changes in phosphocalcium metabolism in untreated AGHD patients, suggesting decreased end-organ sensitivity to the effects of PTH (2). In a more recent study (4), we demonstrated that growth hormone replacement (GHR) significantly decreases PTH concentration with changes in PTH secretory pattern and a significant increase in NcAMP. In addition, we observed a significant increase in bone turnover and changes in phosphocalcium metabolism, suggesting an increase in end-organ sensitivity to PTH. These abnormalities may, in part, be due to changes in end-organ PTH sensitivity (4) as well as possible “resistance” of the parathyroid calcium-sensing mechanism, as seen in abnormal parathyroid glands (13).

Few studies have assessed parathyroid gland function in aging and osteoporotic patients by use of stimulative tests. Postmenopausal women were found to have a much smaller PTH response to oral phosphate stimulus compared with age-matched control subjects (35). Normal premenopausal women also demonstrated a less exuberant response than normal postmenopausal women, leading to the belief that a greater PTH increment was needed to maintain 1,25(OH)2D3 production (35). Untreated osteoporotic women have shown less suppression of PTH-(1–84) in response to human (h)PTH-(1–34) at all
calcium concentrations tested, compared with nonosteoporotic postmenopausal women (19). There are as yet no such stimulation studies in AGHD patients, who have an increased incidence of osteoporosis.

Because alterations in the sensitivity of the parathyroid gland calcium-sensing receptors to perturbations in calcium concentrations may be partially responsible for skeletal changes in age-related and primary osteoporosis, we investigated parathyroid gland response to hypocalcemia induced by EDTA infusion, hypercalcemia induced by calcium gluconate, and the response to PTH-(1–34) infusion in AGHD patients before and 1, 3, 6, and 12 mo after GHR.

MATERIALS AND METHODS

Patients

Eighteen AGHD patients were recruited from the outpatient clinic at the Royal Liverpool University Hospital. Patients were randomized to one of three groups, receiving EDTA infusion (group 1, n = 6), calcium infusion (group 2, n = 6), or PTH-(1–34) infusion (group 3, n = 6). The random allocation was done by equal probability of random selection method. Eighteen patients were each assigned a serial number from 1 to 18. Eighteen random numbers were generated with a personal computer. Six patients according to the first 6 random numbers were randomized to group 1, the next six patients according to the next 6 random numbers were randomized to group 2, and a third batch of six patients were randomized to group 3.

AGHD was defined as a peak GH response of <9 mU/l (3 μU/l) to insulin-induced hypoglycemia (blood glucose <2.2 mmol/l) (5). Isolated AGHD was diagnosed by means of two provocative tests in one patient. Eleven patients in the study had a peak GH response of <0.5 mU/l to provocative tests, with the peak GH response in 7 patients between 0.5–5.0 mU/l. The mean age ± SD was 51.2 ± 10.7 yr in group 1, 55.5 ± 17.6 yr in group 2, and 53.8 ± 11.2 yr in group 3 [P = not significant (NS)]. The mean time ± SD from diagnosis to recruitment was 10.0 ± 7.8, 8.8 ± 4.6, and 12.7 ± 4.0 yr in groups 1, 2, and 3, respectively. The original diagnoses were nonfunctioning pituitary adenoma in 13 patients, prolactinoma in 4 patients, and craniopharyngioma in 1 patient. All patients required additional pituitary hormone replacement, were receiving optimal doses at recruitment, and were stable for >3 yr before recruitment. None of these patients previously received GH therapy, and none was receiving calcium, phosphate, or vitamin D supplements. All patients gave informed written consent. The study was approved by the local ethics committee.

Infusion Protocols

EDTA infusion (group 1). Patients randomized to group 1 were admitted to our investigation unit, after an overnight fast, at 0800 for a period of 10 h before commencement of GHR. An intravenous cannula was inserted into each arm, one for calcium gluconate infusion and one attached to a heparin lock for phlebotomy. One basal urine sample and five basal half-hourly blood samples were collected starting at 0900. At 1130, serum calcium concentrations were increased over 2 h by means of calcium gluconate infusion in 500 ml of 5% dextrose, which provided 146 μmol elemental calcium/kg body wt·h⁻¹, and half-hourly blood sampling continued until 1800. Urine samples were collected at 3-h intervals and aliquots taken from each sample. Patients were discharged home after the 1800 sample and returned the following morning, again in the fasting state, for the 24-h blood sample and return of their 24-h urine collection.

PTH-(1–34) infusion (group 3). Patients randomized to group 3 were admitted to our investigation unit at 1300 for a period of 24 h before commencement of GHR. An intravenous cannula was inserted into each arm, one for PTH-(1–34) infusion and one attached to a heparin lock for phlebotomy. One basal urine sample and one basal blood sample were collected before commencement of the infusion. Recombinant hPTH-(1–34) (Rhone-Poulenc Rorer, Montreal, Canada) was dissolved in normal saline and infused at a rate of 0.55 U/kg·h⁻¹ over 24 h, commencing at 1400. Half-hourly blood samples were collected throughout the infusion period for 24 h. Urine samples were collected at 3-h intervals for the 24 h, except between 2300 and 0800, while patients were sleeping.

The same investigative procedures were then repeated for all infusion protocols after 1, 3, 6, and 12 mo of GHR commencement. Light activity was allowed during their stay, and each patient received the same standard hospital diet at each visit. A single-lead electrocardiogram was monitored continuously during each infusion, and blood pressure was determined every 15 min using oscillometric SPACELABS 90207 ambulatory blood pressure monitoring equipment (SPACELABS, Redmond, WA).

GH Doses

Patients were instructed in subcutaneous self-administration of recombinant human GH with a pen device [somatropin (Genotropin) pen, Pharmacia & Upjohn, Milton Keynes, UK]. GH (Genotropin) was commenced at a low dose of 0.4 IU/day after completion of baseline blood sampling. GH dosage was then titrated at 4 weekly intervals, by increments of 0.4 IU/day, to achieve an IGF-I standard deviation score (IGF-I SDS) within a range between median and the upper limit of reference.

Assays

Serum GH concentration was estimated using the Nichols Advantage Chemiluminescence System (Nichols Institute Diagnostics, Nijmegen, The Netherlands), as previously described (3). Serum IGF-I was measured by an RIA method described elsewhere (3), and an IGF-I SDS was then calculated from these values (8).

Biochemistry

Plasma. Serum calcium, magnesium, phosphate, creatinine, and albumin were measured on all samples by the standard automated method (Hitachi 747, Roche, Lewes, UK). Serum calcium was adjusted for albumin (21). Serum-adjusted calcium correlates strongly with ionized calcium and has been found to be precise in subjects with calcium and albumin within the reference range (21, 26, 39). Serum intact PTH-(1–84) was measured on all samples with a commercial immunometric sandwich assay (Nichols Institute, San Juan Capistrano, CA) with a detection limit of 0.5 pmol/l and interassay and intra-assay coefficients of variance (CVs) of <5% across the range (1–40 pmol/l).

Plasma cAMP (P_cAMP) was measured using an RIA method (BIO-TRAC cAMP, Amersham Pharmacia Biotech, Little Chalfont, UK). The intra-assay CV was <8% and the interassay CV <10% across the working range with a detection limit of 5 nmol/l. N_cAMP, which
reflects the circulating activity of PTH (11), was determined according to the formula
\[ N_{\text{AMP}} = \frac{[S_{\text{C}} \times (U_{\text{AMP}}/U_{\text{C}})]}{P_{\text{AMP}}}, \]
where \( N_{\text{AMP}} \) is expressed as nmol/l GFR, \( S_{\text{C}} \) is serum creatinine in \( \mu \)mol/l, \( U_{\text{AMP}} \) is urinary cAMP in \( \mu \)mol/l, \( U_{\text{C}} \) is urinary creatinine in \( \mu \)mol/l, and \( P_{\text{AMP}} \) is plasma cAMP in \( \mu \)mol/l.

Serum 1,25(OH)\(_2\)D\(_3\) was extracted by acetonitrile, purified through a C\(_{18}\)-OH reverse-phase column, and measured by RIA kit (Nichols Institute) with titrated recovery on each sample. Intra-assay CV was <9% and interassay CV <12% across the working range, with a detection limit of 15 nmol/l.

Serum concentrations of type I collagen C-telopeptide (CTX), which is a bone resorption marker, were measured on all samples using ElectroChemiLuminescence ImmunoAssay (ECLIA) (ELEC-SYS, Roche Diagnostics, Penzberg, Germany). The intra-assay CV was <4% and the interassay CV <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 ECLIA (ELEC-SYS, Roche Diagnostics). The intra-assay CV was <2% and the interassay CV <2.5% across the working range, with a detection limit of 4 ng/l.

Urinary creatinine, calcium (U\(_{\text{Ca}}\)), and phosphate (U\(_{\text{PO}_4}\)) were measured on all samples using ElectroChemiLuminescence ImmunoAssay (VITROS ECI, Johnson & Johnson, Amersham, UK) and expressed per millimoles of excreted creatinine (NTX/U\(_{\text{Ca}}\)). The intra-assay CV was <4% and the interassay CV <10% across the working range, with a detection limit of 10 nmol of bone collagen equivalent. Serum and urine samples for all assays were processed as a single batch to obviate the interassay variability.

**Statistical Analysis**

Calcium set point was defined as the serum calcium concentration at which the serum PTH level was midway between the maximum value achieved during hypercalcemia and the minimum value attained during hypocalcemia (12). The numerical value for set point was obtained by linear extrapolation of PTH release at calcium concentrations between those immediately higher than and lower than the set point (14, 16). We used ANOVA to analyze the changes in PTH-(1–84) and serum calcium in response to the different infusions used in each group at each visit and between visits. Pairwise comparisons by Student’s t-test were then performed to determine the significance of changes observed, and Bonferroni’s correction was applied to allow for the multiple comparisons. Values are expressed as means ± SE. A P value of <0.05 was considered significant.

**RESULTS**

**GH Doses and IGF-I**

The GH dose increased significantly from 0.4 IU/day at baseline to 1.06 IU/day at 12 mo in group 1, to 1.13 IU/day in group 2, and 1.00 IU/day in group 3 (P < 0.05, all groups). Target IGF-I was achieved within 3 mo of commencing GHR in all groups and remained within range at 12 mo, with IGF-I increasing from 8.6 ± 1.1 \( \mu \)mol/l at baseline to 38.2 ± 2.5 \( \mu \)mol/l at 12 mo (P < 0.001) in group 1, from 7.6 to 41.3 \( \mu \)mol/l (P < 0.001) in group 2, and from 9.2 to 40.0 \( \mu \)mol/l (P < 0.001) in group 3.

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**Basal Biochemistry**

For each patient, the first five basal values from 0900 to 1100, which were taken before the commencement of the infusion, were averaged to determine any significant difference between them and basal values after GHR. Serum PTH demonstrated a significant decrease (P < 0.01) in all groups (Table 1), with a concomitant increase in \( N_{\text{AMP}} \) excretion after 12 mo on GHR compared with baseline, i.e., before GHR (P < 0.01). A significant increase was observed in serum calcium concentrations after GHR (P < 0.01) in all groups, with a significant decrease observed in the urinary calcium-to-creatinine ratio (P < 0.05). We observed a significant increase in serum phosphate concentrations (P < 0.01) in all groups after GHR, with no significant differences in the urinary phosphate-to-creatinine ratio in either group (P = NS). There was, however, a significant increase in TmPO\(_4\)/GFR after 12 mo on GHR in all patients (P < 0.05). Both the bone resorption marker CTX and the bone formation marker PINP increased significantly after 12 mo of GHR in all groups (P < 0.001). Serum 1,25(OH)\(_2\)D\(_3\) demonstrated a significant increase after 12 mo of GHR (P < 0.001).

**Table 1. Changes in basal plasma and urinary bone minerals**

<table>
<thead>
<tr>
<th>Group 1</th>
<th>0 mo</th>
<th>12 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH, pmol/l</td>
<td>4.66±0.17</td>
<td>3.41±0.16*</td>
</tr>
<tr>
<td>Serum calcium, mmol/l</td>
<td>2.282±0.012</td>
<td>2.451±0.012*</td>
</tr>
<tr>
<td>Serum phosphate, mmol/l</td>
<td>0.82±0.024</td>
<td>0.95±0.02*</td>
</tr>
<tr>
<td>(N_{\text{AMP}}), mmol/l</td>
<td>19.48±5.59</td>
<td>44.62±6.84</td>
</tr>
<tr>
<td>Urine calcium/creatinine</td>
<td>0.12±0.09</td>
<td>0.26±0.11*</td>
</tr>
<tr>
<td>Urine phosphate/creatinine</td>
<td>1.62±0.40</td>
<td>2.56±0.49</td>
</tr>
<tr>
<td>TmPO(_4)/GFR, mmol/l</td>
<td>0.78±0.12</td>
<td>0.98±0.12*</td>
</tr>
<tr>
<td>Serum CTX, ng/ml</td>
<td>0.098±0.006</td>
<td>0.166±0.010*</td>
</tr>
<tr>
<td>Serum PINP, µg/l</td>
<td>16.39±1.76</td>
<td>25.43±2.236</td>
</tr>
<tr>
<td>Serum 1,25(OH)(_2)D(_3), µmol/l</td>
<td>60.40±2.97</td>
<td>91.20±4.962</td>
</tr>
</tbody>
</table>

**Group 2**

| PTH, pmol/l | 5.20±0.14 | 3.74±0.14*|
| Serum calcium, mmol/l | 2.267±0.008 | 2.430±0.008*|
| Serum phosphate, mmol/l | 0.97±0.02 | 1.24±0.02*|
| \(N_{\text{AMP}}\), mmol/l | 13.59±4.62 | 38.81±4.62*|
| Urine calcium/creatinine | 0.70±0.14 | 0.37±0.14*|
| Urine phosphate/creatinine | 1.59±0.41 | 2.17±0.41|
| TmPO\(_4\)/GFR, mmol/l | 0.94±0.13 | 1.24±0.14*|
| Serum CTX, ng/ml | 0.268±0.030 | 0.610±0.07|
| Serum PINP, µg/l | 39.32±4.99 | 76.97±8.904|
| Serum 1,25(OH)\(_2\)D\(_3\), µmol/l | 58.60±4.06 | 92.60±6.82|

**Group 3**

| PTH, pmol/l | 4.28±0.12 | 3.53±0.11*|
| Serum calcium, mmol/l | 2.278±0.005 | 2.568±0.005*|
| Serum phosphate, mmol/l | 1.11±0.01 | 1.23±0.01*|
| \(N_{\text{AMP}}\), mmol/l | 13.04±3.45 | 21.12±3.46*|
| Urine calcium/creatinine | 0.40±0.05 | 0.22±0.05*|
| Urine phosphate/creatinine | 1.36±0.30 | 1.20±0.27|
| TmPO\(_4\)/GFR, mmol/l | 0.99±0.08 | 1.28±0.10*|
| Serum CTX, ng/ml | 0.257±0.070 | 0.466±0.076|
| Serum PINP, µg/l | 41.25±8.56 | 110.55±2.198|
| Serum 1,25(OH)\(_2\)D\(_3\), µmol/l | 62.66±3.02 | 95.50±3.673|

Values are expressed as means ± SE. PTH, parathyroid hormone; \(N_{\text{AMP}}\), norphrogenous cAMP; TmPO\(_4\)/GFR, renal threshold for maximum tubular phosphate reabsorption rate; CTX, collagen C-telopeptide; PINP, procollagen immunosassay type I amino-terminal propeptide; 1,25(OH)\(_2\)D\(_3\), vitamin D. *P < 0.05; †P < 0.01; ‡P < 0.001.
Serum Calcium, PTH, and Phosphate Response to Infusion Protocols

Group 1. After EDTA infusion, significant hypocalcemia (expressed as the maximum percentage decrease) was achieved before GHR (23.8%) and at 1 (22.3%), 3 (21.0%), 6 (20.0%), and 12 mo (18.0%) on GHR, with no significant difference in the percent decrease between visits (P = NS), Fig. 1A. The maximum PTH stimulation occurred at a calcium concentration (percent decrease at which maximum PTH stimulation occurred) of 1.79 mmol/l (23.8%) before GHR. After GHR, maximum PTH stimulation occurred at a calcium concentration of 1.92 mmol/l (17.2%) at 1 mo, 1.93 mmol/l (18.9%) at 3 mo, 2.05 mmol/l (15.6%) at 6 mo, and 2.16 mmol/l (12.9%) at 12 mo (P < 0.05; see Fig. 4A). There was no significant difference in the maximum PTH response, which was a 365% increase before and 326% increase after 12 mo on GHR (P = NS; Fig. 1B). Serum magnesium concentration did not change significantly during the EDTA infusions at 0 mo (preinfusion 0.84 ± 0.08 vs. 0.74 ± 0.05 mmol/l postinfusion, P = NS), at 1 mo (0.83 ± 0.07 vs. 0.74 ± 0.05 mmol/l, P = NS), at 3 mo (0.81 ± 0.05 vs. 0.73 ± 0.04 mmol/l, P = NS), at 6 mo (0.81 ± 0.06 vs. 0.72 ± 0.04 mmol/l, P = NS), and at 12 mo (0.80 ± 0.07 vs. 0.71 ± 0.03 mmol/l, P = NS). These findings suggest increased parathyroid gland sensitivity to smaller changes in serum calcium after GHR.

The calcium set point progressively increased from 2.08 ± 0.04 mmol/l before GHR to 2.18 ± 0.04 mmol/l at 1 mo (P = 0.004), 2.20 ± 0.04 mmol/l at 3 mo (P < 0.001), 2.22 ± 0.04 mmol/l at 6 mo (P < 0.001), and 2.35 ± 0.04 mmol/l at 12 mo on GHR (P < 0.001; see Fig. 4A). A significant decrease in serum phosphate concentrations occurred during the EDTA infusion, which increased back to baseline within 4–5 h at all visits, with the maximum percent decrease of 35% observed in untreated AGHD patients compared with an 18% decrease from baseline after 12 mo on GHR (Fig. 1C). There was no significant difference in the mean percent decrease between 0 mo (26.5 ± 2.0%) and 1 (23.7 ± 2.0%), 3 (22.8 ± 2.0%), and 6 mo (22.2 ± 2.0%), but the percent decrease was significantly less after 12 mo (16.0 ± 2.0%) on GHR (P < 0.01).

Group 2. There was a significant percent increase in serum calcium during the calcium gluconate infusion at all visits, with a simultaneous percent decrease in serum PTH concentrations (Fig. 2A). After calcium gluconate infusion, maximum PTH suppression occurred at a calcium concentration (percent increase) of 2.79 mmol/l (18.9%) at 0 mo, 2.96 mmol/l (20.6%)
at 1 mo, 2.92 mmol/l (17.1%) at 3 mo, 2.81 mmol/l (16.2%) at 6 mo, and 2.84 mmol/l (14.7%) at 12 mo after GHR (P < 0.01 compared with 0 mo; see Fig. 4B). The maximum PTH suppression was 75% before and 82% after 12 mo on GHR (P = NS), which was in response to a significantly lower calcium increase, Fig. 2B. The calcium set point significantly increased from 2.34 ± 0.04 mmol/l at 0 mo to 2.47 ± 0.04 mmol/l at 1 mo (P < 0.05), 2.57 ± 0.04 mmol/l at 3 mo (P < 0.01), 2.59 ± 0.04 mmol/l at 6 mo (P < 0.001), and 2.61 ± 0.04 mmol/l at 12 mo after GHR (P < 0.001; Fig. 4B).

The mean percent increase in serum phosphate was significantly lower during the calcium gluconate infusion after 12 mo (5.3 ± 2.7%) on GHR compared with the baseline visit (18.5 ± 2.7%, P < 0.01), Fig. 2C. The percent increase in serum phosphate remained lower after GHR 4 h after the completion of the infusion.

Group 3. After PTH-(1–34) infusion, the calcium concentration began to increase after 12 h in untreated AGHD patients, whereas after 12 mo on GHR the calcium concentration began to increase after 6 h (Fig. 3A), a finding that was similar to the previously reported responses in healthy individuals (19). After 12 mo on GHR, the percent increase in calcium concentration at 6 mo was 3.2 ± 0.6 vs. 0.8 ± 0.7% in untreated AGHD patients (P < 0.05), and it remained significantly higher over the 24 h of hPTH-(1–34) infusion in GH-replaced patients (17.8 ± 2.7 vs. 12.6 ± 2.2%, P < 0.05), Fig. 3A. The maximum PTH-(1–84) suppression occurred at a calcium concentration (percent increase) of 2.63 mmol/l (16.2%) at 0 mo, 2.65 mmol/l (15.1%) at 1 mo, 2.69 mmol/l (16.6%) at 3 mo, 2.72 mmol/l (17.1%) at 6 mo, and 3.01 mmol/l (18.1%) at 12 mo after GHR (P = NS), Fig. 4C. The maximum PTH-(1–84) suppression was 78 ± 2.4% before and 79 ± 2.5% after 12 mo on GHR (P = NS), Fig. 3B. The calcium set point significantly increased from 2.38 ± 0.02 mmol/l at 0 mo to 2.46 ± 0.02 mmol/l at 1 mo (P < 0.05), 2.50 ± 0.02 mmol/l at 3 mo (P < 0.01), 2.58 ± 0.02 mmol/l at 6 mo (P < 0.001), and 2.74 ± 0.02 mmol/l at 12 mo (P < 0.001) after GHR, Fig. 4C.

At all visits, serum phosphate demonstrated a significant percent decrease after ~5 h that was maximal at 20 h after the commencement of the PTH-(1–34) infusion (Fig. 3C), after which it began to increase toward the baseline. There were no significant differences observed in the mean percent decrease in serum phosphate between visits during the period of maximal change from 20 to 24 h after commencement of PTH-(1–34) infusion.

DISCUSSION

Our results have demonstrated a significant decrease in basal serum PTH in all groups, with a concomitant increase in basal N\text{\textsubscript{cAMP}} excretion and serum calcium, phosphate, 1,25(OH\textsubscript{2})\text{D\textsubscript{3}}, CTX, and PINP concentrations, and a significant reduction in
urinary calcium/creatinine excretion after 12 mo of GHR compared with baseline. The maximum PTH-(1–84) response to both hypocalcemic and hypercalcemic stimuli was similar in terms of percent change, with the maximum PTH-(1–84) response occurring at significantly higher calcium concentrations and in response to significantly smaller increments in calcium concentration. We also observed a significant increase in calcium set point after GHR in all groups. These findings suggest increased end-organ sensitivity to the effects of PTH, resulting in increased N\textsubscript{AAMP} excretion, an increase in 1,25(OH)\textsubscript{2}D\textsubscript{3} and bone turnover, as well as increased parathyroid gland calcium-sensing receptor sensitivity to changes in serum calcium concentration. To maintain calcium concentration within the very narrow range, despite wide variations in short-term availability, calcium-regulated PTH secretion must exhibit a rapid response, as well as exquisite sensitivity to changes in the calcium concentration. The parathyroid gland must also be capable of adaptive responses that enable it to achieve larger than normal responses to severe and long-term changes in calcium concentration. Alteration in parathyroid gland function and changes in the calcium-sensing receptor (15) response to perturbations in calcium concentration may alter mineral ion homeostasis. Changes in the sensitivity of the parathyroid gland to hypocalcemic stimuli have been suggested to be primarily responsible for the skeletal changes observed in patients with osteoporosis (19). A decrease in sensitivity of the parathyroid gland in estrogen-treated osteoporotic women to increases in calcium concentration and hypocalcemic stimuli has previously been shown, suggesting an estrogen-induced resistance (18, 19). However, in previous studies the percentage of PTH changes were not considered, which may have corrected for the significant differences in baseline calcium (19) and PTH concentrations between groups (18). In our present study, we have analyzed the percent changes in serum calcium and PTH-(1–84) and demonstrated that the parathyroid gland is capable of similar suppressibility and stimulation in response to rapid changes in calcium concentrations before and after GHR. However, it is evident from our data that to produce such a level of PTH response requires a significantly larger change in calcium concentration in untreated patients than in AGHD patients, suggesting a decrease in the calcium-sensing receptor sensitivity of the parathyroid gland or resistance to changes in calcium concentration in untreated AGHD patients.

The calcium-sensing receptor plays, independently of PTH, an important role in directly regulating renal calcium reabsorption, i.e., inhibiting tubular reabsorption of calcium when the level of peritubular calcium increases (24). The increase in basal serum calcium and in the calcium concentration at which maximum PTH-(1–84) response was achieved may in part be explained by the increase in calcium-sensing receptor set point that we have observed in AGHD patients after GHR. The increase in basal serum calcium may also be partly explained by the increased bone turnover and increased vitamin D levels, in addition to increased calcium reabsorption and decreased urinary calcium observed in this study.

PTH-dependent regulation of mineral ion homeostasis is largely mediated through the PTH/parathyroid hormone-related peptide type I receptor, which is coupled to adenylate cyclase and phospholipase C (1, 23) and is most abundantly expressed in the target tissues for PTH action (i.e., kidney and bone) (33). In the kidney, PTH stimulates the reabsorption of calcium, inhibits reabsorption of phosphate, and enhances the synthesis of 1,25(OH)\textsubscript{2}D\textsubscript{3}. Administration of PTH leads to the release of calcium from a rapidly turning over pool of calcium near the surface of bone (36, 37), and the calcium is resorbed more efficiently when stimulated by PTH. PTH also enhances the production of 1,25(OH)\textsubscript{2}D\textsubscript{3} over hours, which in turn increases serum calcium concentration via increased intestinal calcium absorption. We observed a significant delay in the calcemic response to PTH-(1–34) infusion in untreated AGHD patients, which may be due to decreased end-organ sensitivity to the effects of PTH, resulting in the lack of rapid turnover of calcium from the bone surface and the resorption from the kidneys. After GHR, the increase in serum calcium concentration was significantly more rapid, suggesting restoration of target tissue, i.e., bone and renal responsiveness to the effects of PTH. The parathyroid response [PTH-(1–84)] to increments in calcium after exogenous PTH-(1–34) infusion, however, has to be viewed cautiously, as it may not account for the complicating effects of PTH-(1–34) on phosphate and 1,25(OH)\textsubscript{2}D\textsubscript{3}.

Phosphate is normally reabsorbed from the glomerular filtrate in both the proximal and distal tubules, and at both sites reabsorption is inhibited by PTH (9, 31). The marked decrease and increase in serum phosphate after hypo- and hypercalcemic stimuli, observed in this study, would in part be due to the increase and decrease in serum PTH concentration observed during the period of the infusion. The significant difference in the percent change in serum phosphate in response to hypocalcemic and hypercalcemic stimuli after 12 mo on GHR may in part be due to the GH antiphosphaturic effect (28, 32), negating some of the PTH-induced renal loss. The decrease in serum phosphate concentration observed after 10–12 h of PTH-(1–34) infusion in AGHD patients before and after GHR may also, in part, be explained by the PTH effects on renal tubules. It is, however, interesting to note that serum phosphate concentration began to increase after 20 h of PTH-(1–34) infusion and continued to do so until the end of the infusion at 1400. This may be due to the effects of PTH on the vitamin D axis, which is not immediate, because the stimulation of 1,25(OH)\textsubscript{2}D\textsubscript{3} production occurs over several hours and requires the synthesis of new proteins (20, 27). A low serum phosphate concentration also markedly increases the synthesis of this metabolically active vitamin D metabolite. This stimulation of 1,25(OH)\textsubscript{2}D\textsubscript{3} synthesis in the kidney would lead to an enhanced mobilization of phosphorus from the bone and a hypophosphatemic-induced increase in TmPO\textsubscript{4}/GFR (10), as demonstrated in results. The exact mechanism of this effect is unknown. The increased circulating concentration of 1,25(OH)\textsubscript{2}D\textsubscript{3} also leads to an increase in intestinal absorption of phosphate (10). The net result of this sequence of adjustments is a return of serum phosphate concentration to normal, which would explain the significant decrease in serum phosphate, followed by an increase in phosphate concentration, while these patients were still receiving PTH-(1–34) infusion.

On the basis of our findings, we suggest a new model to explain the pathophysiology of bone loss in AGHD patients, which may also be valid for some other causes of osteoporosis. We propose that GHD results in renal, bone, and intestinal target cell insensitivity or resistance to the effects of PTH, leading to a mild “pseudohypoparathyroid state” or “PTH-resistant syndrome.” This would result in relatively increased...
PTH secretion, decreased N\textsubscript{cAMP}, decreased vitamin D production, decreased calcium reabsorption and absorption, and decreased bone cell activation and bone turnover, which in turn would lead to bone loss. In addition, GHD leads to resistance or decreased responsiveness of the calcium-sensing receptors in the parathyroid gland to the changes in calcium concentration with lowering of the calcium set point, which further contributes to the increased PTH secretion and decreased calcium concentration observed in our AGHD patients as well as reported in osteoporotic men and women.

In summary, GHD leads to a PTH-resistant syndrome at both the effector level, i.e., renal, bone, and intestine, and the calcium-sensing receptor level in the parathyroid gland, resulting in alterations in phosphocalcium and bone metabolism, which in part may contribute to the development of osteoporosis in GHD adults. This model rationalizes most of the previous findings and may provide a pathophysiological explanation for the development of GHD-related osteoporosis and a possible link between other causes of osteoporosis.

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