Bone Mineral Density in Pseudohypoparathyroidism Type 1a

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Context: The biochemical hallmark of pseudohypoparathyroidism type 1a (PHP1a) is resistance to PTH, but based on tissue-specific imprinting of GNAS, PTH resistance may be limited to the renal cortex. Some studies have shown that bone is responsive to PTH, suggesting that PHP1a patients with chronically elevated PTH levels may have low bone mineral density (BMD).

Setting: This observational study was conducted at the Institute of Clinical and Translational Research, Johns Hopkins Medical Institutions.

Subjects: Twenty-two children and adults with PHP1a were studied.

Main Outcome Measure: The main outcome measure was BMD Z-score at the lumbar spine (LS), total hip, femoral neck, and total body using dual-energy x-ray absorptiometry, relative to height, weight, and pubertal status.

Results: The mean (±sd) Z-score for height was 0.77 ± 1.66 and 1.85 ± 1.15 for BMI. The BMD Z-score at each of the four sites studied was as follows: LS, 0.29 ± 1.08; total hip, 0.27 ± 1.24; femoral neck, 0.02 ± 1.26; and total body, 0.98 ± 1.50. Only two subjects (9%) had BMD Z-scores less than −2, and each had additional risk factors for low BMD. BMD in total body and LS spine corrected for height-for-age Z-score was significantly greater than normal. There was no correlation between PTH level and BMD Z-score or between body mass index and BMD Z-score.

Conclusions: Despite secondary hyperparathyroidism, region-specific BMD is not reduced in patients with PHP1a, and total body BMD is significantly greater than normal. (J Clin Endocrinol Metab 95: 4465–4475, 2010)
allele in pituitary somatotropes, the gonad, and the thyroid in humans (3–6) and in the renal proximal tubule and the gonads in mice (7). GNAS mutations on maternally inherited alleles result in the AHO phenotype plus hormone resistance in cells in which Gαs is imprinted [e.g. renal cortex, thyroid, gonad, somatotropes (1, 2)]. By contrast, similar GNAS mutations on the paternal allele lead to AHO without hormone resistance, a condition termed pseudopseudohypoparathyroidism (PPHP). Some patients with paternal GNAS mutations develop only severe and extensive heterotopic membranous ossifications and are considered to have the related disorder progressive osseous heteroplasia (1, 2, 8).

Patients with the genetically distinct disorder PHP1b lack typical features of AHO and have endocrine dysfunction that is limited to PTH resistance in the renal proximal tubule, although recently an overlap between PHP1a and PHP1b has been described in which some patients manifest brachydactyly (9). Subjects with PHP1b have normal Gαs activity in accessible tissues (10). The coding region of GNAS is normal, but an epigenetic defect switches the maternal GNAS allele to a paternal pattern of methylation (i.e. paternal epigenotype). In most familial cases, the epigenetic defect results from microdeletions in the STX16 gene located approximately 220 kb centromeric of GNAS exon 1A (11, 12) or deletions removing differentially methylated regions of GNAS exon NESP55 and exons 3 and 4 of the antisense transcript (13). Inheritance of a mutation from a female (or spontaneous mutation of a maternally derived allele) abolishes Gαs expression from both alleles in imprinted tissues and results in PTH resistance (14).

The unusual skeletal features of AHO have invited investigation of Gs-dependent signaling in this tissue. PTH, as well as the related gene product PTHrP, binds and stimulates the type I PTH/PTHrP receptor (PTHR1) with equal affinity. Nevertheless, recent studies now suggest that the two ligands may have very different signaling properties because PTHrP1-36 stimulates generation of more cAMP than PTHrP1-36 (15). The basis for this unexpected dichotomy appears to be the prolonged association of PTH1,34 with the PTHR1 and Gαs within a persistently active, internalized ternary complex, whereas PTHrP1,36 action is restricted to the cell membrane and is short lived (15).

Activation of this Gs-coupled receptor on osteoblasts and renal tubule cells is responsible for the classical effects of PTH on calcium and phosphate metabolism and, similarly, mediates the paraneoplastic effects of PTHrP. In skeletal growth plates, PTHrP is expressed in the perichondrium and maturing chondrocytes, whereas its receptor is primarily found in the proximal prehypertrophic layer. Recent studies demonstrated that decreased signaling through Gαs-coupled pathways in the growth plate leads to accelerated differentiation of chondrocytes with consequent premature closure of the growth plates (16–18). Moreover, loss of PTHrP leads to short-limbed dwarfism in mice (19) and brachydactyly type E in humans (20), whereas loss of the PTHR1 leads to Blomstrand chondrodystrophy (21).

Gαs is biallelically expressed in human bone (22) and mouse chondrocytes (17, 18), and thus, haploinsufficiency of Gαs has been implicated as the cause of defective PTHrP signaling in the growth plate. By contrast, skeletal responsiveness to PTH appears intact in patients with PHP1a and PHP1b (23–30). Indeed, hyperparathyroid bone disease has been described in at least some patients with PHP1a (27, 30) and PHP1b (23–25, 29), and patients with PHP1b have been shown to have lower bone mineral density (BMD) than age-, race-, and sex-matched controls (31, 32). By contrast, previously reported studies of BMD in PHP1a have been limited by small numbers of subjects, and thus, definitive conclusions about bone density are lacking (30–34).

The primary objective of this study was to determine BMD, relative to height, weight, and pubertal status, in a large cohort of children, adolescents, and adults with PHP1a. Secondary objectives were to determine the relationships between BMD and PTH levels related to PHP1a.

Subjects and Methods

Subjects

BMD measurements at the lumbar spine (LS), total hip (TH), femoral neck (FN), and total body (TB) were obtained in 22 subjects with PHP1a, 18 of 22 with confirmed mutations in the GNAS gene (data not shown). Bone densitometry, laboratory studies, anthropometric data, and bone age were all obtained during the same evaluation. A diagnosis of PHP1a had been made months to years before enrollment in this study with the exception of subject 13, who was newly diagnosed, and treatment with calcium and/or calcitriol was begun between ages 2 and 7 yr. The criteria for PHP1a included the presence of AHO features combined with multihormone resistance, in particular, resistance to PTH (elevated serum intact PTH, sometimes associated with hypocalcemia and hyperphosphatemia), TSH (elevated TSH with low or normal free T4), GHRH (associated with GH deficiency), or clinical symptoms of GnRH resistance (amenorrhea, oligomenorrhea). Features of AHO included short stature, brachydactyly, round face, sc ossifications, and cognitive impairment. Pubertal Tanner staging was performed by clinical examination. Adults were defined by age 18 yr or older and Tanner stage V.

Two subjects were taking glucocorticoids for chronic medical conditions that were unrelated to PHP1a.

All subjects were evaluated at the Institute for Clinical and Translational Research at the Johns Hopkins Hospital (Baltimore, MD), and all studies were approved by the Institutional Review Board of the Johns Hopkins Medical Institutions. Informed consent
was obtained from all subjects, or a parent of each child, and assent was obtained from children when appropriate.

**Laboratory evaluation**

Laboratory evaluation was performed after an overnight fast of at least 8 h. Serum concentrations of intact PTH (Intact PTH immunoradiometric assay; Nichols, San Juan Capistrano, CA), TSH, free T4, calcium, phosphate, and alkaline phosphatase levels were obtained as described previously (16). GH stimulation testing was performed by both arginine-L-dopa and arginine-GHRH testing for each subject with the assessment of either GH deficiency or sufficiency being consistent with the two methods. The GH stimulation testing and diagnosis of GH deficiency has been described in detail previously (16, 35). The laboratory data presented were obtained at the time of the dual-energy x-ray absorptiometry (DXA) scans; this was the initial visit for 16 of the 22 patients. The other six patients had been followed up by us long term.

**Anthropometric evaluation**

Standing height was assessed using a digital stadiometer (Harpendon; Holtain Ltd., Crymych, Dyfed, UK). Weight was measured using a digital electronic scale (Detecto Scale Co., Webb City, MO). Height measurements were also expressed as SD from the mean (adjusted for age and gender). Body mass index (BMI; kilograms per square meter) was calculated from height and weight measurements, and age- and gender-specific Z-scores in children were calculated using applications from Baylor College of Medicine (www.bcm.edu/bodycomplab/) (36).

**GNAS mutation analysis**

Mutation analysis of the GNAS gene was performed on the 13 coding exons and flanking intronic sequences in the Johns Hopkins DNA Diagnostics Laboratory (Clinical Laboratory Improvement Amendments-approved laboratory) or in our research laboratory at the Johns Hopkins University School of Medicine, as previously described (16, 35). Southern blot analysis was performed to assess large deletions, insertions, or imprinting defects (16).

**Imaging studies**

Study subjects underwent whole-body DXA scan (Hologic QDR 4500, Waltham MA) as well as DXA of the LS (L1–L4), TH, FN, and TB less head in the array mode. In the Institute for Clinical and Translational Research, the coefficient of variation of BMD is less than 1%.

BMD results were expressed as Z-scores, the SD from the mean for age- and gender-matched controls, and were based on chronological age, not bone age. Z-scores were calculated from a Hologic Pediatric Reference Database based on data collected at five centers on white children aged 5–20 yr for the spine (n = 1444) and hip (n = 1047) and children aged 3–20 yr for whole body (n = 1948). For children between the ages of 7 and 17 yr, we also determined BMD Z-scores that were adjusted for height using the height-for-age Z-score (HAZ) (37).

Bone mineral content (BMC) Z-scores for TB were calculated using normative data that are relative to age, gender, and race (www.bcm.edu/bodycomplab/) (36).

Bone age of the left hand and wrist was determined in children and adolescents using the standards of Gruelich and Pyle. The bone age interpretations were conducted by a pediatric radiologist on the faculty at the Johns Hopkins University School of Medicine as well as independently by a pediatric endocrinologist on the faculty (E.L.G.-L.).

**Statistics**

All values are expressed as means ± SD. Data were analyzed by unpaired two-tailed Student t tests. Differences between group Z-scores and normal means (Z-score = 0) were determined by one-group t tests. P < 0.05 was considered significant. A Pearson coefficient was calculated for analysis of correlations except for analysis of alkaline phosphatase and bone density, for which a Spearman correlation was determined.

**Results**

**Subjects**

DXA scans were obtained in 22 subjects with PHP1a, including seven adults and 15 children who ranged in age from 3 to 54 yr. All subjects had features of AHO, including brachydactyly type D and/or E and evidence of PTH and/or TSH resistance. Bone mineral density was measured at the time of the DXA scan or on our evaluation when diagnosed in our clinic. Biochemical data at the time of the DXA scans are shown in Table 1. Tanner stage information along with BMD Z-scores is provided in Table 2.

**Laboratory evaluation**

The biochemical characteristics of study subjects at the time of the DXA scans are shown in Table 1. Fourteen of 22 subjects were receiving calcitriol and calcium at the time that bone densitometry was performed (indicated by an asterisk in Table 1). Intact PTH levels ranged from 6 to 458 pg/ml, with elevated levels reflecting inadequate dosing of calcium and calcitriol vs. noncompliance with medication. No subjects had hypocalcemia, whereas one of the 22 subjects (subject 18) had hyperphosphatemia. Alkaline phosphatase levels were normal for age in all subjects except for subject 14, who had a mild elevation. Serum levels of 25-hydroxy-vitamin D were available for five of 22 subjects and were within the normal range for four of the five subjects (data not shown).

All patients were taking levothyroxine with the exception of patient 13, who was newly diagnosed. TSH levels were elevated in eight subjects; only one subject had a TSH concentration above 9 μIU/ml. Free T4 levels were normal in all subjects, consistent with very mild TSH resistance. Seventeen of 22 (77%) subjects were GH deficient at the time that BMD was measured, consistent with our prior data (15). None of the subjects who had received GH in the past except patient 16 (18.3 yr old) who had received GH for a short period of time (ages 9.5–12.0 yr).
Anthropometric evaluation

We recently described the height, weight, and BMI characteristics of these subjects in detail (38). For the purposes of this study, we calculated BMI Z-scores to evaluate for a relationship between BMI and BMD Z-scores; Z-scores in adults were calculated using the age of 20 yr as the adult BMI standard (38). BMI Z-scores and height SDs are shown in Table 2. The mean BMI Z-score (n = 11005) was 1.86/1.15. Ten of 15 children (67%) had a BMI Z-score greater than 2 SDs. Three of seven adults (43%) had a BMI Z-score greater than 2 SDs. The mean height SD in children (n = 15) was 0.2/1.69, whereas the mean height SD in adults (n = 7) was 1.9/0.87, further supporting our previous finding that children with PHP1a have normal stature until they undergo premature epiphyseal fusion resulting in adult short stature (16, 35, 38).

GNAS mutation analyses

Eighteen of 22 subjects had a confirmed mutation in one of the 13 coding exons of GNAS [data not shown (38)]. Southern blot analysis was performed in three (subjects 1, 5, and 22) of the four subjects (subjects 1, 5, 13, and 22) who did not have a confirmed GNAS mutation and showed normal maternal methylation of exon 1A, thus excluding an imprinting defect.

Bone measures

DXA BMD Z-scores for the LS (L1–L4), TH, FN, and TB are shown in Table 2 and Fig. 1. The mean BMD Z-score for the LS (n = 22) was 0.29/1.08 (range -2.6 to 2.2), which is not significantly different from normal. Moreover, 14 subjects (64%) had Z-scores within ±1, similar to expected for the normative population. We also used a recently developed method that utilized a HAZ to adjust for the effect of stature on bone density to analyze LS BMD in study subjects between the ages of 7 and 17 yr (37). This generated a HAZ-adjusted BMD Z-score of 0.63/0.89, which was significantly (P < 0.003) greater than normal. There is no generally accepted method to adjust for the effect of short stature on BMD in adults. As shown in Fig. 1, the mean BMD Z-score for the total hip (n = 20) was 0.27/1.24 (range -1.5 to 2.5), and the HAZ-adjusted BMD Z-score for children (n = 12) was 0.19/0.91, both not significantly different from normal. Eleven subjects (55%) had Z-scores within ±1: six (30%) between 0 and 1 and five (25%) between 0 and -1. Two subjects (10%) had Z-scores between greater than 1 and 2; four subjects (20%) had Z-scores between less than -1 and -2; three subjects (15%) had Z scores greater than 2; and no subjects had a Z-score of less than -2.

TABLE 1. Biochemical data

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<th>Alk phos (U/liter)</th>
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sCalcium, Serum calcium; sPhosphate, serum phosphate; Alk phos, alkaline phosphatase.

a On treatment with oral calcitriol and/or calcium.
TABLE 2. Bone densitometry and clinical features

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<th>LS BMD\text{max} Z-score</th>
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Mean Z-score \( \pm SD \): 0.29 \((\pm 1.08)\), 0.63 \((\pm 0.89)\), 0.27 \((\pm 1.24)\), 0.02 \((\pm 1.26)\), 0.98 \((\pm 1.50)\), 0.77 \((\pm 1.66)\), 1.85 \((\pm 1.15)\)

Median Z-score \( \pm SD \): 0.5 \((-2.6--2.2)\), 0.9 \((0.3--2.2)\), 0.15 \((-1.5--2.5)\), 0.05 \((-2.1--2.3)\), 0.55 \((-1.2--4.8)\), -0.63 \((-4.5--2.3)\), 2.04 \((-0.73--4.9)\)

BMD Z-scores for each subject for LS (L1–L4), TH, FN, and TB with age, Tanner stage, hand/wrist bone age, height SD, and BMI Z-score at the time of the DXA scan. The mean and median \( \pm SD \) as well as the range are shown for BMD Z-scores at each DXA site and also for the height SD and BMI Z-score.
The mean BMD Z-score for the FN (n = 20) was 0.02 ± 1.26 (range −2.1 to 2.3). Ten subjects (50%) had Z-scores within ±1: five (25%) between 0 and 1; five (25%) between 0 and −1. Three subjects (15%) had Z-scores between greater than 1 and 2; four subjects (20%) between less than −1 and −2; two subjects (10%) had Z-scores greater than 2; and one patient (5%) had a Z-score less than −2.

The mean Z-score for whole-body BMD (n = 20) was 0.98 ± 1.50 (range −1.2 to 4.8), which is significantly (P = 0.006) greater than normal. Nine subjects (45%) had Z-scores within ±1: six (30%) between 0 and 1 and three (15%) between 0 and −1. Five subjects (25%) had Z-scores between greater than 1 and 2; two subjects (10%) had Z-scores between less than −1 and −2; four subjects (20%) had Z-scores greater than 2; and no subjects had Z-scores less than −2.

Overall, only two subjects (9%) had BMD Z-scores less than −2. Five subjects (23%) had BMD-Z scores greater than +2.

Mean total body BMC Z-score was 0.32 ± 0.24 and not significantly different from normal controls.

Correlations

The height Z-score was positively associated with LS Z-score, as expected (r = 0.53, P = 0.01). Age was highly skewed, but the correlation between height Z-score and age showed a Spearman r of −0.5953, P = 0.0035.

BMD Z-scores and BMC Z-scores were highly correlated (r = 0.78). The serum alkaline phosphatase levels correlated with LS Z-scores (r = 0.44, two tailed P = 0.04) but not with bone density measured at other sites. There were no significant correlations of serum phosphate levels and BMD at any sites. In addition, we found no correlations between intact PTH levels and BMD Z-scores at the LS, TH, FN, or TB sites (Fig. 2) for the patient population as a whole as well as for the children and adults when analyzed separately. There were also no correlations between BMI Z-scores and BMD Z-scores at the LS, TH, FN,
and TB (Fig. 3). This was true for the entire group and the children and adults analyzed separately. Finally, we did not find a significant correlation between BMI Z-score and BMC Z-score for the TB (data not shown).

Total fat mass correlated with TB BMC ($r = 0.77$) but not TB BMC Z-score or TB BMD Z-score (data not shown). Similarly, lean tissue mass was highly correlated with TB BMC ($r = 0.92$) but not correlated with TB BMC Z-score or TB BMD Z-score.

Seventeen of 22 subjects (77%) were GH deficient at the time that subjects underwent bone densitometry, but there was no significant difference in mean BMD Z-scores at any site between the GH-deficient and GH-sufficient group (data not shown).

Sex steroids have been shown to play a critical role in the pubertal increase in bone mass (39). The adolescent and adult female subjects included in our study all entered puberty at an appropriate time. However, three of the four adult women had a history of secondary amenorrhea, and the fourth had experienced a premature menopause at age 40 yr. Despite evidence of gonadotropin resistance, two of the three women with secondary amenorrhea had BMD measurements greater than +2 SDs.

**Discussion**

PTH can have both anabolic and catabolic effects on the skeleton. Constant elevation of PTH, as occurs in primary hyperparathyroidism, causes bone loss, with greater effects on cortical than cancellous bone (40). By contrast, intermittent elevations of PTH can increase bone mass, primarily at skeletal sites that are enriched for cancellous bone (40, 41). Finally, a lack of PTH, as occurs in hypoparathyroidism, is associated with both increased bone density and reduced bone remodeling, which can result in either beneficial or potentially adverse effects, respectively (33, 42–44).

Despite significant secondary hyperparathyroidism, we found that the subjects with PHP1a in our study had significantly increased whole-body bone density when compared with healthy control subjects of the same age and gender. Moreover, BMD Z-scores were normal in the LS, TH, and FN. Total body BMC was also not reduced.

Our results contrast with previous studies that suggest that BMD is decreased in PHP1a (20, 27, 28). However, these previous studies were limited by small sample size (30–32, 34, 43), did not discriminate between PHP1a and PHP1b, and were confounded by widely variable BMD Z-scores (33, 34). For example, the largest series, reported by Breslau et al. (31), had only three PHP1a subjects. Our analysis of BMD in 22 subjects with PHP1a demonstrates that bone mass is either normal or increased at the four sites studied (LS, TH, FN, TB).

This study has several technical limitations. First, bone density is greatly influenced by pubertal status. Although pubertal maturation is often delayed in patients with PHP1a, we felt it would be inappropriate to apply an adjustment for bone age in our subjects because PHP1a children experience premature fusion of epiphyses and have markedly advanced bone ages from an early age (16).
addition, they exhibit inconsistencies between bone age in the hand/wrist and that in the lower extremity (16, 35).

Second, although areal BMD (grams per square centimeter) as determined by DXA is the preferred method to assess and monitor bone disease in adults, this technique has important shortcomings when used in growing children. In particular, DXA is greatly influenced by bone size (45, 46) because areal bone density is determined by only two rather than three dimensions. Thus, smaller patients, with smaller bones, have lower BMD measurements when DXA is used to assess bone density. The International Society of Clinical Densitometry has therefore recommended that spinal and total body (less head) BMC and areal BMD results for children with linear growth or maturational delay be adjusted for absolute height or height age, or compared with pediatric reference data that provide Z-scores that are specific for age, sex, and height (47).

Accordingly, several corrections have been proposed to adjust BMD Z-scores in children who are short for age or who have a delayed puberty because mineral accrual in childhood is linked more closely to pubertal and skeletal maturation than to chronological age (39). Although there are no accepted guidelines on how to adjust areal BMD for absolute height, we used a recently developed technique for adjustment of LS BMD by HAZ that provides significant advantages over the height-age correction that is commonly used (37). Although the application of this correction methodology to our LS data may be of limited validity because we did not use the same normative reference database that was used to develop the technique, this analysis showed that HAZ-adjusted BMD for the LS spine (LS BMD_{HAZ}) was significantly greater in PHP1a subjects aged 7 through 17 yr than in normal subjects, thus confirming the results of the total body bone densitometry.

The World Health Organization criteria for assessing BMD is limited to postmenopausal women and relies on T-scores (SDs from mean peak bone mass) to establish a diagnosis of osteopenia or osteoporosis. Thus, T-scores, and these criteria, cannot be used when studying children, adolescents, and young adults who have not yet reached peak bone mass (39). We therefore followed recommendations of the International Society for Clinical Densitometry and used Z scores to interpret DXA measurements and defined low BMD on the basis of age-adjusted Z-scores less than −2.0 (39). Using this standard, only two subjects (9%) met the criteria for low BMD; the FN BMD Z-score in patient 5 (child) and the LS BMD Z-score in patient 21 (adult). Both subjects had additional risk factors for low BMD; patient 5 had limited mobility due to joint problems in addition to frequently requiring oral glucocorticoid steroids for reactive airway disease; patient 21 had undergone early menopause at age 40 yr without subsequent hormone replacement in addition to requiring long-term glucocorticoid therapy for chronic lung disease.

PHP1a patients who are undertreated or noncompliant with calcium and calcitriol therapy typically have elevated PTH levels. We measured intact PTH at the same time that we measured bone density, and in 18 patients (82%), the PTH values at the time of the DXA scan correlated with their chronic parathyroid homeostasis over the prior years (many of the patients who were referred had received inadequate treatment). We expected that high PTH levels would be associated with low BMD Z-scores, especially at sites with a greater proportion of cortical vs. trabecular bone. As depicted in Fig. 2, no correlation was found between BMD Z-scores and PTH levels at any of the BMD sites studied. Both subjects with BMD Z-scores of less than −2 (subjects 5 and 21) had normal PTH levels (66 and 29 pg/ml, respectively). Furthermore, patient 18 had the highest total body Z-score of 4.8 with a PTH level of 253 pg/ml.

There are several potential explanations for increased bone density in PHP1a. First, haploinsufficiency of Gaq in bone cells may reduce PTH-dependent bone remodeling similar to that which occurs in rodents (48, 49) and humans with hypoparathyroidism (44, 50, 51). Although the presence of brachydactyly in patients with PHP1a implies defective PTHr1 signaling in the growth plate (19, 20), other studies have shown apparently normal PTH responsiveness in vitro of bone cells from patients with PHP1a (30) as well as the development of osteitis fibrosa cystica in some patients with PHP1a (27, 30). Thus, it is conceivable that increased bone density in PHP1a results from the short half-life of calcitriol, which can cause fluctuations in serum calcium levels and thereby induce changes in serum PTH that resemble those achieved by intermittent administration of PTH.

Recent clinical studies indicated that daily administration of recombinant human PTH 1-34 (40, 41) and recombinant human PTH1-84 (40, 41, 52–54) significantly increases BMD, particularly at skeletal sites that are enriched for cancellous bone and reduces fracture risk. However, sites enriched for cortical bone show smaller increases (e.g. total hip) or actual decreases (distal radius) in BMD (54). By contrast, we found that whole-body BMD, which represents predominately cortical bone, was significantly increased in patients with PHP1a and cancellous BMD was only modestly increased. These observations are inconsistent with the typical anabolic effects of PTH on the skeleton (55).

Alternatively, increased total-body BMD in patients with PHP1a may reflect the effect of altered Gaq signaling in the brain. Recent work suggests the existence of a neural control mechanism for bone mass that uses both positive and negative mediators of bone formation and resorption.
One control pathway involves leptin signaling, and one of the mediators expressed in hypothalamic neurons that leptin uses to inhibit osteoclast differentiation and thereby bone resorption is cocaine- and amphetamine-regulated transcript. Cocaine- and amphetamine-regulated transcript expression is also increased in both humans (56) and mice (57) lacking only one allele of the melanocortin 4 receptor (MC4R), a Gs-coupled receptor that is highly expressed in hypothalamic neurons and that controls energy intake and energy expenditure. Remarkably, deficiency of MC4R leads to not only obesity but also decreased bone resorption and high bone mass (57). The recent observation (58) that mice with mutations in maternal Gnas (i.e. replicating PHP1a) that are limited to the central nervous system have some of the same features as those of MC4R mutations (obesity, reduced sympathetic nervous system activity and energy expenditure, and impaired glucose metabolism) suggests that increased bone density in PHP1a may derive from disordered hypothalamic signaling rather than defective PTH action on the skeleton. In this situation one would expect that patients with paternal GNAS mutations (i.e. PPHP) would not develop increased bone density similar to their lack of abnormal weight gain (38).

Because low BMD is associated with an increased fracture risk in both adults (59) and children (60, 61), our findings may have important clinical implications. Specifically, because increased whole-body BMD represents an increase predominately in cortical bone, which contributes to overall mechanical strength of the skeleton, PHP1a patients may have a reduced risk for fracture. Future studies of BMD in patients with PPHP will be needed to assess the relative effects of elevated levels of PTH and Gαs, haploinsufficiency on the skeleton.

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**References**

1. Levine MA, Germain-Lee E, Jan de Beur SM 2003 Genetic basis for resistance to parathyroid hormone. Horm Res 60(Suppl 3):87–95
microdeletions within or upstream of the GNAS locus. Ann NY Acad Sci 1068:250–255
34. Ahmed SF, Russell S, Rashid R, Beattie TJ, Murphy AV, Ramage IJ, Maxwell H 2005 Bone mineral content, corrected for height or bone area, measured by DXA is not reduced in children with chronic renal disease or in hypoparathyroidism. Pediatri Nephrol 20:1466–1472
41. Thomas T 2006 Intermittent parathyroid hormone therapy to increase bone formation. Joint Bone Spine 73:262–269


57. Ahn JD, Dubern B, Lubrano-Berthelier C, Clement K, Karsenty G 2006 Cart overexpression is the only identifiable cause of high bone mass in melanocortin 4 receptor deficient. Endocrinology 147:3196–3202


