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Bone Density in Amenorrheic Women with and without Hyperprolactinemia*

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ABSTRACT. To determine whether decreased bone density in patients with PRL-secreting pituitary tumors is specifically related to hyperprolactinemia or is a consequence of disordered pituitary-gonadal function common to all amenorrheic states, we measured the bone mineral content of the radius by photon absorptiometry in normal subjects, in women with amenorrhea and normal serum PRL levels, and in women with PRL-secreting pituitary tumors. The women did not differ significantly in mean age, height, weight, or serum calcium, phosphorous, gonadotropin, testosterone, vitamin D, or PTH concentrations, and all had normal renal and thyroid function.

The bone mineral content in women with amenorrhea and normal serum PRL levels (0.91 ± 0.02 g/cm) was not significantly different from that in control subjects (0.88 ± 0.01 g/cm). Patients with PRL-secreting tumors studied 2–5 yr after transsphenoidal surgery had significantly diminished bone mineral content whether they were cured (0.82 ± 0.02 g/cm) or had persistent amenorrhea and hyperprolactinemia (0.81 ± 0.02 g/cm). Serum estradiol concentrations did not differ significantly in the four groups, and there was no correlation between estradiol concentration and bone mineral content or between PRL concentration and bone mineral content in the amenorrheic women.

The presence of decreased bone mineral content in hyperprolactinemic patients suggests that PRL may have a direct effect on bone and may be another indication for early treatment of PRL-secreting pituitary tumors. (*J Clin Endocrinol Metab* **56:** 1120, 1983)

PRL-SECRETING pituitary tumors are responsible for a distinctive clinical syndrome of amenorrhea, galactorrhea, and infertility. Until recently it appeared that the deleterious effects of the elevation in serum PRL were limited to its effects on the reproductive system. Evidence that hyperprolactinemia may have other systemic effects has been reported by Klibanski and coworkers (1), who found that amenorrheic women with PRL-secreting pituitary tumors have decreased bone density. The decreased bone density in hyperprolactinemic patients has been attributed to the decreased concentrations of serum estradiol occasionally seen in these patients. To determine whether this reduction in bone mineral content is specifically related to hyperprolactinemia and hypoestrogenemia or is a consequence of disordered pituitary-gonadal function common to all amenorrheic states, we measured the bone mineral content of the radius by direct photon absorptiometry in normal women, in amenorrheic women with normal serum PRL concentrations, and in women with histologically proven PRL-secreting pituitary tumors.

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Subjects and Methods

We examined bone density by photon absorptiometry in 38 women with histologically proven PRL-secreting pituitary tumors. The women were studied 2–5 yr after transsphenoidal surgery. Fifteen of them, who had regular menses and normal serum PRL postoperatively, were designated cured (group I), while 23 women who remained amenorrheic and had persistent hyperprolactinemia postoperatively were designated noncured (group II). Twenty-nine healthy volunteers with no history of menstrual irregularity were used as control subjects (group III). We also studied 14 women with amenorrhea and normal serum PRL concentrations (group IV). Seven of these women had idiopathic amenorrhea, 3 carried the diagnosis of polycystic ovarian disease, 3 had primary amenorrhea, and 1 had late-onset congenital adrenal hyperplasia. All of the women were caucasian, all had normal renal and thyroid function, and none were receiving estrogens, corticosteroids, or other medications at the time of this study. We also used measurements from 25 postmenopausal women (aged 56–80 yr) with radiographic evidence of osteopenia.

Each subject in groups I–IV underwent a detailed hormonal evaluation, which included measurement of serum PRL, estradiol, testosterone, LH, and FSH. We considered the serum PRL and estradiol concentrations to be the mean of 3 morning samples obtained at 30-min intervals. In addition, serum from each patient was analyzed for calcium, phosphorous, C-terminal PTH, and alkaline phosphatase. Serum from 10 patients in groups I–III was analyzed for 25-hydroxyvitamin D (25OHD)

and 1,25-dihydroxyvitamin D [1,25-$(OH)_2D$]. Serum PRL, LH, FSH, estradiol, and testosterone concentrations were measured by RIA using agents distributed by the National Pituitary Agency (2–5). 25OHD and 1,25-$(OH)_2D$ were measured by competitive protein binding assays and high pressure liquid chromatography by Nichols Institutes (Los Angeles, CA). PTH was measured using a kit supplied by Immunonuclear Corp. (Stillwater, MN).

All samples for PRL, estradiol, testosterone, LH, FSH, PTH, and vitamin D measurements were analyzed in single assays. The assay sensitivity was 2 ng/ml for PRL, 10 ng/ml for FSH 2 ng/ml for LH, 0.2 ng/ml for testosterone, 10 pg/ml for estradiol, 0.1 ng/ml for PTH, 5 ng/ml for 25OHD, and 10 pg/ml for 1,25-$(OH)_2D$. Intraassay coefficients of variation for PRL and PTH were concentration dependent and ranged from 6% and 9%, respectively. The intraassay coefficient of variation was 12% for estradiol, 5% for LH, 11% for FSH, 8% for testosterone, 7% for 25OHD, and 10% for 1,25-$(OH)_2D$.

Direct photon absorptiometry was performed using a Norland bone mineral analyzer (Norland Corporation, Fort Atkinson, Wisconsin) with ^{125}I as the radionuclide source. The measurement site was the junction of the proximal two thirds and the distal one third of the nondominant radius. Four measurements were made with the arm in one position. The coefficient of variation for this technique was 4%. The statistical method employed was one-way analysis of variance.

Results

Table 1 shows the clinical characteristics of the normal subjects and the women with amenorrhea. The subjects ranged in age from 23–58 yr, and mean height and weight were comparable in all groups. Patients with persistent amenorrhea postoperatively (group II) had a mean (±SEM) duration of amenorrhea (8.1 ± 1.2 yr) that was slightly longer than in the other women with amenorrhea, but the difference was not statistically significant ($P = 0.1$, by one-way analysis of variance). Only women with persistent postoperative amenorrhea had hyperprolactinemia (PRL, 77 ± 14 ng/ml). The women in each group reported similar smoking histories and duration of oral contraceptive use.

All subjects had normal serum concentrations of calcium, phosphorous, alkaline phosphatase, and C-terminal PTH (Table 2). Mean 25OHD and 1,25-$(OH)_2D$ concentrations in 10 women with pituitary tumors were not different from mean vitamin D measurements in control subjects. Mean serum estradiol concentrations in amenorrheic patients did not differ significantly from those in normal subjects (Table 3), and there was no correlation between estradiol and serum PRL ($r = -0.14$) or between estradiol and duration of amenorrhea ($r = -0.39$). Mean serum testosterone and gonadotropin concentrations were not significantly different in the four groups (Table 3).

The bone mineral contents of the radial shaft in the four groups of patients are shown in Fig. 1. Amenorrheic subjects with normal serum PRL had a mean bone mineral content of 0.91 ± 0.02 g/cm, which did not differ significantly from the bone mineral content in normal subjects (0.88 ± 0.01 g/cm). In contrast, women with

TABLE 1. Clinical characteristics of normal women and women with amenorrhea

Group	Age (yr)	Ht (cm)	Wt (kg)	Duration of amenorrhea (yr)
I	30.7 ± 2.2	165 ± 2	65 ± 3	4.8 ± 0.06
II	33.1 ± 1.7	164 ± 1	74 ± 4	8.1 ± 1.2
III	28.6 ± 1.2	167 ± 1	63 ± 2	
IV	26.4 ± 1.2	165 ± 2	68 ± 5	5.5 ± 1.1

Values are the mean ± SEM.

TABLE 2. Mean serum calcium, phosphorous, PTH, and vitamin D levels in healthy women and women with amenorrhea

Group	Ca_2 (mg/dl)	PO_4 (mg/dl)	PTH (ng/ml)	25OHD (ng/ml)	1,25-$(OH)_2D$ (pg/ml)
I	9.5 ± 0.04	3.3 ± 0.09	0.80 ± 0.03	22 ± 2	63 ± 4
II	9.6 ± 0.07	3.3 ± 0.07	0.83 ± 0.03	24 ± 4	64 ± 8
III	9.5 ± 0.07	3.4 ± 0.14	0.91 ± 0.06	22 ± 8	56 ± 4
IV	9.6 ± 0.08	3.4 ± 0.15	0.88 ± 0.03	ND	ND

Values are the mean ± SEM. ND, Not determined.

TABLE 3. Mean serum PRL, estradiol (E_2), testosterone (Test), and gonadotropin concentrations in normal women and women with amenorrhea

Group	PRL (ng/ml)	E_2 (pg/ml)	Test (ng/ml)	LH (ng/ml)	FSH (ng/ml)
I	21 ± 3	68 ± 15	1.2 ± 0.08	106 ± 16	141 ± 39
II	77 ± 15[a]	67 ± 16	1.2 ± 0.08	124 ± 36	181 ± 36
III	19 ± 2	77 ± 12	1.2 ± 0.06	182 ± 49	280 ± 52
IV	9 ± 1	54 ± 16	1.4 ± 0.01	225 ± 103	226 ± 61

Values are the mean ± SEM.

[a] $P < 0.001$, by one-way analysis of variance.

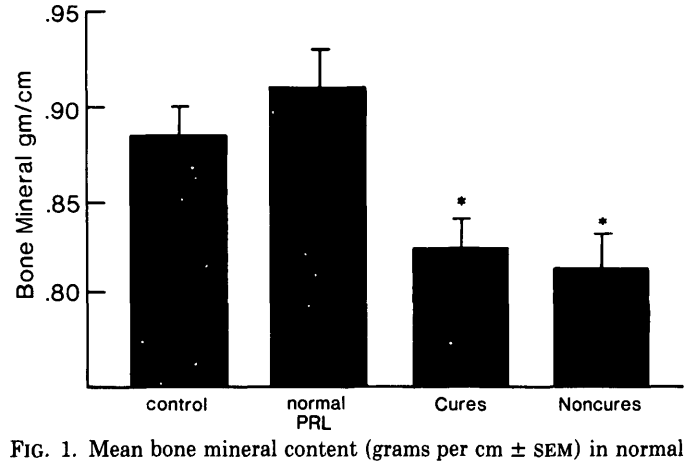

FIG. 1. Mean bone mineral content (grams per cm ± SEM) in normal women and women with amenorrhea. *, $P = 0.009$, by one-way analysis of variance.

PRL-secreting pituitary tumors had significantly decreased bone density regardless of surgical outcome ($P = 0.009$, by one-way analysis of variance). There was no correlation between serum PRL and bone density ($r = -0.35$) or between estradiol and bone density ($r = 0.8$).

To determine whether the decreased bone density in amenorrheic women was related to the decreased estradiol concentration, we subdivided the patients with amenorrhea into those with low (<25 pg/ml) and normal (>25 pg/ml) estradiol concentrations (Table 4). In both groups, patients with serum estradiol concentrations less than 25 pg/ml had bone mineral contents that were not significantly different from those in amenorrheic patients with estradiol concentrations greater than 25 pg/ml (Table 4). In contrast, the bone mineral content in elderly postmenopausal women (0.64 ± 0.07 g/cm) was significantly less than that in women with hyperprolactinemic amenorrhea ($P < 0.001$, by one-way analysis of variance).

Discussion

There are several factors that could explain the difference in bone density in hyperprolactinemic and normoprolactinemic amenorrhea. These include duration of oral contraceptive use, smoking and exercise history, hypoestrogenemia, and abnormalities in calcium regulatory hormones. None of our patients had exercise-induced amenorrhea, and all groups had comparable smoking and oral contraceptive use histories.

Klibanski *et al.* (1) demonstrated reduced bone density in young women with hyperprolactinemia and found that the decrease in bone mineral content was most prominent in women with the lowest serum estradiol levels. Our results demonstrate that factors other than the estrogen status of the patient may also be important. Patients with PRL-secreting tumors do not uniformly have hypoestrogenemia (6–8), and many of the women with hyperprolactinemic amenorrhea in this study had estradiol levels comparable to those in normal subjects.

TABLE 4. Mean estradiol concentrations and bone mineral contents in normal women and women with amenorrhea

	Estradiol (pg/ml)	Bone mineral (g/cm)
Group II		
n = 5	19 ± 4	0.78 ± 0.03[a]
n = 18	80 ± 17	0.82 ± 0.02[a]
Group IV		
n = 4	16 ± 3	0.93 ± 0.01
n = 10	65 ± 23	0.90 ± 0.03
Group III (n = 29)	77 ± 12	0.88 ± 0.01

Values are the mean ± SEM. n, Number of subjects in each group or subgroup.

[a] $P = 0.03$, by one-way analysis of variance.

Mean estradiol levels in the groups did not differ, and in amenorrheic patients, individuals with serum estradiol levels less than 25 pg/ml had bone density not significantly different from that of individuals with normal estradiol concentrations.

In light of comparable estradiol concentrations, we wondered if other tests of gonadal function would distinguish between hyperprolactinemic and normoprolactinemic amenorrhea and account for the difference in bone density. While determinations of bone mineral content by photon absorptiometry in patients with amenorrhea and relative androgen excess have not been made, persistent elevations in plasma androgen levels might account for the higher bone density seen in amenorrheic patients with normal serum PRL concentrations. While 3 of 14 patients with normoprolactinemic amenorrhea carried a diagnosis of polycystic ovarian disease, and 1 was known to have late-onset congenital adrenal hyperplasia, mean serum testosterone concentrations were not higher in that group, nor did these 4 individuals have significantly higher testosterone concentrations. Similarly, mean serum gonadotropin concentrations were comparable in all four groups.

It also seemed possible the PRL might exert an effect on calcium regulatory hormones to cause the decrease in bone density seen in hyperprolactinemic women. In animals, PRL has been reported to increase renal 1α-hydroxylase activity (9). Pregnant and lactating women have elevated levels of 1,25-$(OH)_2$D (10), and normal to slightly increased 1,25-$(OH)_2$D levels have been reported in patients with PRL-secreting pituitary tumors (11–13). The lack of quantifiable abnormalities in serum calcium, phosphorous, PTH, and vitamin D measurements in this study and that of Klibanski *et al.* (1) suggests that PRL has no effect on calcium regulatory hormones and may act directly on bone to cause the diminished bone mineral content found in hyperprolactinemic women. This hypothesis is supported by recent work of Pahuja and DeLuca (14), who have demonstrated that PRL has bone-resorptive capabilities in pregnant, vitamin D-deficient rats.

Measurement of radial bone mineral content by photon absorptiometry affords an estimate of the degree of mineralization of trabecular bone, but these measurements do not correlate well with the mineral content in the axial skeleton (15). Because the weight-bearing axial skeleton loses bone more rapidly than trabecular bone in the radius (16), the decreased trabecular radial bone mineral content in hyperprolactinemic patients could be even more significant in the spine.

The presence of diminished bone mineral content in hyperprolactinemic women suggests that these women may be at risk for the development of osteoporosis. If these findings are confirmed by long term studies, pro-

gressive bone demineralization might be considered a new indication for early treatment of PRL-secreting pituitary tumors. The diminished bone mineral content in hyperprolactinemic amenorrhea also suggests that PRL might play a previously unrecognized role in the development of osteoporosis.

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