Treatment of anorexia nervosa is associated with increases in bone mineral density, and recovery is a biphasic process involving both nutrition and return of menses1–3


ABSTRACT
Background: Recovery from osteoporosis in anorexia nervosa (AN) is uncertain.
Objective: The purpose of this study was to understand the changes in bone mineral density (BMD) in women with AN and the mechanisms of recovery from osteopenia.
Design: We studied BMD and markers of bone formation and resorption, osteocalcin and N-telopeptide (NTX), in patients with AN (n = 28) who were following a behavioral weight-gain protocol.
Results: Anorexic patients experienced significant percentage increases in BMD (4.38 ± 7.48% for spine; 3.77 ± 8.8% for hip; P < 0.05 for both) from admission until recovery of 90% ideal body weight, achieved over 2.2 mo. NTX concentrations were higher in patients with AN at admission than in healthy control subjects (n = 11; 69.0 ± 31.09 and 48.3 ± 14.38 nmol/mmol creatinine, respectively; P < 0.05) and in reference control subjects (n = 30; 69.0 ± 31.09 and 37.0 ± 6.00 nmol/mmol creatinine, respectively; P < 0.001). In weight-recovered subjects with AN, osteocalcin increased (from 8.0 ± 3.05 to 11.2 ± 6.54 ng/mL; P < 0.05), whereas NTX remained elevated (from 69.0 ± 31.09 to 66.7 ± 45.5 nmol/mmol creatinine; NS). A decrease in NTX (from 70.7 ± 40.84 to 45.9 ± 22.72 nmol/mmol creatinine; NS) occurred only in the subgroup of subjects who regained menses with weight recovery.
Conclusions: Nutritional rehabilitation induces a powerful anabolic effect on bone. However, a fall of NTX and a shift from the dominant resorptive state, which we postulate involves full recovery, may involve a hormonal mechanism and require a return of menses. Nutritional rehabilitation appears to be critical to bone recovery and may explain the ineffectiveness of estrogen treatment alone on BMD in the cachectic state.

KEY WORDS
Anorexia nervosa, bone mineral density, osteopenia, amenorrhea, bone markers

INTRODUCTION
Amenorrhea is a known risk factor for osteopenia, and it occurs with weight loss in persons with anorexia nervosa (AN). Women with AN are at high risk of fractures and severe osteoporosis at menopause. Studies of the efficacy of hormone therapy or oral contraceptives (OCPs) in increasing the bone mass of women with AN have not consistently shown positive results (1–5). The mechanism by which the osteopenia develops and reverses is thought to be nutritionally mediated, but it has not yet been well defined. The role of nutrition in the recovery of bone has been underestimated. Indeed, therapy consisting of OCPs or estrogen replacement was associated with continued fractures or bone loss (6, 7).

Some studies of indexes of bone turnover in women with AN have found an uncoupling of bone homeostasis, characterized by a decrease in osteoblastic function (bone formation) and an increase in osteoclastic function (bone resorption), although results have been inconsistent (6–12). The mechanisms by which bone homeostasis is disrupted and recovered in women with AN are poorly understood. Osteocalcin and N-telopeptide (NTX) are established biochemical markers of bone formation and resorption, respectively, and may be used as reliable measures of bone metabolism (13).

In this longitudinal study, we studied women with AN before and after their weight was normalized. We then compared them with healthy female control subjects and a previously studied reference population to better understand the changes in bone mineral density (BMD) and the mechanism of recovery (14; L Audi, personal communication, 23 November 2004).

SUBJECTS AND METHODS
Subjects
We studied 28 patients with AN and 11 control subjects. In addition, we compared our data with those from 30 reference control subjects (14; L Audi, personal communication, 23 November 2004). Patients were women with AN who were hospitalized for treatment at the Eating Disorder Research Unit at the New York State Psychiatric Institute, Columbia University Medical Center. All met DSM-IV criteria for AN from the 4th edition.
of the *Diagnostic and Statistical Manual of Mental Disorders*, except for amenorrhea in one subject who maintained regular cycles despite low weight. Patients had a mean (±SD) length of illness of 98.0 ± 59.4 mo. Subjects were recruited from referrals by physicians and mental health workers or by direct contact with the clinic. The patients with AN were 18–35 y old at hospital admission (x ± SD age: 23.0 ± 3.98 y). All patients were screened before entering the study. Patients with primary amenorrhea, polycystic ovarian syndrome, or prolactin-secreting tumors; those who were taking medications known to affect bone metabolism or reproductive function and hypothalamic-pituitary-ovarian axis or hypothalamic-pituitary-adrenal axis, including estrogen or OCPs; or those who were pregnant were excluded by physical examination and hormonal profiles. Formal exercise was not permitted during hospitalization, but no effort was made to control for previous exercise load.

The 11 healthy control subjects were recruited from the New York City area and the Columbia University campus by public advertisements. All were healthy, did not have significant medical or psychiatric histories, and were matched with patients according to age and percentage of ideal body weight (IBW) range after recovery (90–100% IBW). Their ages ranged from 18 to 36 y (x ± SD age: 24.7 ± 5.10 y). None of the control subjects had a history of an eating disorder. Potential control subjects with a history of psychiatric or medical illness or who were receiving hormonal or other medications known to affect reproductive function or bone metabolism were excluded. All of the control subjects exercised <3 h/wk and had regular menstrual cycles.

We also compared our data with reference control subjects from a study done in 2002 (14; L Audi, personal communication, 23 November 2004). That study examined 30 healthy, postpubertal white girls (x ± SD age: 18.2 ± 2.5 y) who were of Spanish descent. We used that reference group as a comparison for our subjects, because the body mass index (BMI; in kg/m2) and body composition were similar.

All subjects gave written informed consent. The study protocol was approved by the Institutional Review Boards of the New York State Psychiatric Institute, Columbia University, and St Luke’s–Roosevelt Hospital Center, and procedures were followed according to approved ethical guidelines.

**Medical and menstrual histories and hormonal evaluation**

Patients were evaluated at the initiation of hospitalization for history of eating disorder and previous treatment (age at onset of AN, prior treatment type and duration, lowest and highest adult BMI, and menstrual history), current symptoms of eating disorder, general activity, and nutritional profile. A full medical history was taken, and a physical examination was performed by a physician of the Eating Disorders Unit staff. Venous blood and urine samples were taken for hormone profile analysis. All patients with secondary amenorrhea fit the criteria for hypothalamic amenorrhea: normal concentrations of prolactin, testosterone, and dehydroepiandrosterone sulfate and low-to-normal concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Patients were interviewed monthly to assess menstrual status.

Each healthy control subject completed a medical questionnaire designed to assess general medical and menstrual history and was given a brief physical examination by a study physician. Healthy control subjects were also given a take-home ovulation test kit to confirm ovulatory cycles and were asked to keep records of menstrual periods. All studies were done during the follicular phase of the menstrual cycle (days 3–10) in menstruating subjects.

**Treatment**

All patients underwent an inpatient, behavioral weight-gain treatment at the New York State Psychiatric Institute with cognitive, supportive, family, nutritional, and psychoeducational group elements aimed at restoring weight to a minimum of 90% IBW according to the 1959 Metropolitan Life Insurance Tables (15). Mean BMI at recovery was 20.5 ± 1.13. On admission, patients were fed a standard hospital diet of 1800 kcal (=55% of energy from carbohydrates, 15% of energy from protein, and 30% of energy from fat), prescribed as 3 meals/d plus a snack. Patients were expected to eat 100% of the food prescribed and were observed during and for 1 h after each meal. If they were unable to gain weight, calories were increased in 400-kcal increments in food or liquid nutritional supplement (Ensure Plus; Abbott Laboratories, Abbott Park, IL). After a brief medical stabilization period (1–2 wk), patients began the active weight-gain phase of treatment, which continued until the patients reached 90% IBW. The minimum expected rate of weight gain was 1 kg/wk, although, on average, patients tended to gain 1.6 kg/wk. At the peak of weight gain, caloric intake was about 3700 kcal, with 3000 kcal as food and the remainder as supplement.

The weight-gain phase of treatment was followed by a 4–6-wk period of weight maintenance with increasing independence and transition to outpatient care. Mean caloric intake on discharge was 2600 kcal. For the patients in this study, the duration of inpatient treatment ranged from 26 to 112 d (x ± SD treatment duration: 65.7 ± 20.7 d). No vitamin D or calcium supplements were given.

**Biochemical analyses**

Serum osteocalcin was measured with the use of a human immunoradiometric assay (Immunotopics International, San Clemente, CA) with a sensitivity of 0.5 ng/mL and an interassay CV of 5.5–6.7%. Urine NTXs were measured with the use of an enzyme-linked immunosassay (Ostex International Inc, Seattle, WA) with a detection limit of 20 nmol bone collagen equivalent and an interassay CV of 4.1%. Blood and urine were collected twice from patients for measurements of bone markers and hormones, once at admission and once after maintenance of 90% IBW for ≥2 wk. Bone markers were measured once in control subjects.

Estradiol, FSH, and LH were measured in serum by using a solid-phase chemiluminescence immunoassay (Immulite; Diagnostic Products Co, Los Angeles, CA). Assay sensitivity was 20 pg/mL for estradiol, 0.1 mU/mL for FSH, and 0.1 mU/mL for LH. The intraassay and interassay CVs for estradiol were 9.3% and 10.5%, respectively. The intraassay and interassay CVs for FSH were 1.9% and 5.0%, respectively. The intraassay and interassay CVs for LH were 3.6% and 5.0%, respectively.

**Bone density and body composition**

Total-body dual-energy X-ray absorptiometry (DXA) was used to measure bone mass and bone density. The reports from the DXA-L scanner (GE Systems, Madison, WI) were analyzed with the use of version 3.6 software and were used to determine regional BMD of the hip and spine, total body bone mineral.
content, and total percentage of body fat. The CVs for BMD measurements ranged from 0.5% to 1.0% (16). When measured by DXA, percentage of body fat is independent of BMD, because this value is measured directly by recognized standard means in fat depots at sites where bone is not present (17).

Statistical analysis

We used multiple t tests and adjusted the probability level with the use of the Bonferroni correction to compare patients, those with amenorrhea, and those who regained menses. We used independent and dependent t tests to measure differences between groups. To examine percentage increases, we performed one-sample t tests. Multiple comparisons were made with the use of the Bonferroni method, available in SPSS software (version 12; SPSS Inc, Chicago, IL). Initially, the group of patients was analyzed as one group with the use of independent t tests; later, the menstrual status of the patients was taken into account, and one group was categorized as remaining amenorrheic and the other categorized as regaining menses. For comparisons with the reference control subjects, 2-factor repeated-measures analysis of variance with Bonferroni correction was conducted.

The power calculation was based on the changes in BMD found in hypothalamic amenorrhea resulting from weight loss. On the basis of earlier data from amenorrheic dancers, 5 patients for spine BMD (t difference: 0.127 ± 0.077) would be necessary for a power of 0.80 and a level of statistical significance of 0.05 (18). We do not have data on hip BMD, but data on athletic amenorrhea treated with OCPs (19) indicated that 12 subjects are necessary to show a significant difference (SD: ± 4.05; effect size: 3.55%) over 1 y in Ward’s triangle. These numbers of subjects are necessary for a power of 0.80 and a level of significance of 0.05.

RESULTS

Thirty-seven patients and 12 healthy control subjects entered the study. Twenty-eight patients and 11 healthy control subjects completed the study. Of the 28 study patients, 8 patients regained normal menstruation at 90% IBW.

Anthropometric measures, age, and data on the onset of the eating disorder in these subjects are shown in Table 1. No significant difference was observed in age between patients at 90% IBW and control subjects. However, the patients were significantly older than the reference control subjects (age at admission: 23.0 ± 3.9 y for patients; age for reference control subjects: 18.2 ± 2.5 y; P < 0.001). No significant difference was observed in weight, BMI, or lean body mass between patients at 90% IBW and normal control subjects. BMIs of patients at 90% IBW did not differ significantly from the BMIs of the reference control subjects (ie, 20.6 ± 2.1).

We divided the patients into 2 groups according to menstrual status at the time of treatment after 90% IBW testing. Anthropometric measures and descriptive data for the patients who regained menses during recovery (n = 8) and those who remained amenorrheic (n = 20) compared with healthy control subjects are shown in Table 2. No significant differences were observed in age, weight, BMI, lean body mass, or percentage body fat between the group with regained menses and the amenorrheic group at admission or after weight gain. Both groups had similar weight gain (regained menses group: 27.0 lb; amenorrheic group: 27.1 lb). After weight gain, neither group differed significantly from control subjects. The mean length of illness with AN before the time of admission was 98.0 ± 59.36 mo for all patients, and no significant difference was observed between the duration of illness for patients who regained menses than for those who remained amenorrheic.

An analysis of bone markers showed that patient osteocalcin concentrations at admission did not differ significantly from those of our healthy control subjects (Table 3). The osteocalcin concentrations were significantly higher in patients with AN (8.0 ± 3.05 ng/mL) than in the reference control subjects (6.2 ± 1.90 ng/mL; P < 0.05). A significant increase of 47.93 ± 73.38% (P < 0.01) was observed in serum osteocalcin concentrations in patients with AN from low weight (8.0 ± 3.05 ng/mL) to 90% IBW (11.2 ± 6.54 ng/mL; P < 0.05), an increase that surpassed the values for the reference control group (6.2 ± 1.90 ng/mL).

Urine NTX concentrations of patients with AN (69.0 ± 31.09 nmol/mmol creatinine) were higher at admission than those of healthy control subjects (48.3 ± 14.38 nmol/mmol creatinine; P < 0.05) and reference control subject (37.0 ± 6.00 nmol/mmol creatinine; P < 0.001). After nutritional rehabilitation, there was
a decrease in NTX concentrations so that the NTX concentrations of patients with AN who reached 90% IBW (66.7 ± 45.48 nmol/mmol creatinine) did not differ from those of control subjects, although they remained higher than those of reference control subjects (37.0 ± 6.00 mmol/mmol creatinine, P < 0.001). Bone density analysis showed that, on treatment, patients gained a significant 4.38 ± 7.48% (P < 0.05) increase in spine BMD and a 3.77 ± 8.8% (P < 0.05) increase in hip BMD after weight gain from low weight to 90% IBW in just 2.2 mo. Despite the large increases, the bone density of the patients with AN did not reach the values of control subjects and was still significantly different at 90% IBW (Table 3).

Patients on admission had significantly lower estradiol (24.4 ± 7.11 pg/mL compared with 36.0 ± 33.92 pg/mL; P < 0.05) and FSH (1.8 ± 2.15 IU/mL compared with 3.7 ± 1.34 IU/mL; P < 0.05) concentrations than did control subjects (Table 3), and those concentrations remained lower despite weight rehabilitation. When we examined the change in hormone concentrations only, estradiol concentrations increased significantly in patients with AN from admission (24.4 ± 7.11

### TABLE 3

<table>
<thead>
<tr>
<th>Bone marker, bone density, and hormone data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with AN (n = 28)</td>
</tr>
<tr>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Admission</td>
</tr>
<tr>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Osteocalcin (ng/mL)</td>
</tr>
<tr>
<td>Urine NTX (nmol/mmol Cr)</td>
</tr>
<tr>
<td>BMD spine (g/cm²)</td>
</tr>
<tr>
<td>BMD hip (g/cm²)</td>
</tr>
<tr>
<td>Total BMD (g/cm²)</td>
</tr>
<tr>
<td>LH (mIU/mL)</td>
</tr>
<tr>
<td>FSH (mIU/mL)</td>
</tr>
<tr>
<td>Testosterone (ng/dL)</td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
</tr>
<tr>
<td>Prolactin (ng/mL)</td>
</tr>
<tr>
<td>DHEAS (μg/dL)</td>
</tr>
</tbody>
</table>

1 All values are x ± SD. Control subjects were at 90-100% IBW. AN, anorexia nervosa; IBW, ideal body weight; NTX, N-telopeptide; BMD, bone mineral density; LH, luteinizing hormone; FSH, follicle-stimulating hormone; DHEAS, dehydroepiandrosterone sulfate.  
2 Significant difference between patients with AN at admission and at 90% IBW, P < 0.05 (dependent t test).  
3 Significant difference between patients with AN at admission and control subjects, P < 0.05 (independent t test).  
4 Significant difference between patients with AN at admission and at 90% IBW, P < 0.01 (dependent t test).  
5 Significant difference between patients with AN at admission and control subjects, P < 0.05 (independent t test).  
6 Significant difference between patients with AN at admission and at 90% IBW, P < 0.001 (dependent t test).  
7 Significant difference between patients with AN at admission and control subjects, P < 0.001 (independent t test).  
8 Significant difference between patients with AN at admission and control subjects, P < 0.001 (independent t test).
TABLE 4
Bone marker, bone density, and hormonal data by menstrual status

<table>
<thead>
<tr>
<th></th>
<th>Regained menses at 90% IBW (n = 8)</th>
<th>Amenorrheic at 90% IBW (n = 20)</th>
<th>Control subjects (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Admission</td>
<td>90% IBW</td>
<td>Admission</td>
</tr>
<tr>
<td>Osteocalcin (ng/mL)²⁻⁵</td>
<td>5.9 ± 1.49</td>
<td>9.5 ± 3.84 (69.8 ± 89.55)</td>
<td>8.8 ± 3.15</td>
</tr>
<tr>
<td>Urine NTX (nmol/mmol Cr)²⁻⁶</td>
<td>70.7 ± 40.84</td>
<td>45.9 ± 22.72 (–28.2 ± 27.4)</td>
<td>68.2 ± 27.52</td>
</tr>
<tr>
<td>BMD spine (g/cm²)⁷⁻¹⁰</td>
<td>0.959 ± 0.141</td>
<td>1.003 ± 0.132 (4.59 ± 6.38)</td>
<td>0.896 ± 0.149</td>
</tr>
<tr>
<td>BMD hip (g/cm²)⁷⁻¹⁰</td>
<td>0.972 ± 0.085</td>
<td>1.002 ± 0.083 (3.08 ± 2.35)</td>
<td>0.910 ± 0.135</td>
</tr>
<tr>
<td>Total BMD (g/cm²)⁷⁻¹¹</td>
<td>1.092 ± 0.027</td>
<td>1.095 ± 0.029 (0.27 ± 7.41)</td>
<td>1.048 ± 0.089</td>
</tr>
<tr>
<td>LH (IU/mL)⁷⁻¹²</td>
<td>3.9 ± 5.36</td>
<td>3.5 ± 3.25</td>
<td>0.49 ± 0.83</td>
</tr>
<tr>
<td>FSH (IU/mL)⁷⁻¹²</td>
<td>3.0 ± 2.86</td>
<td>2.4 ± 1.67</td>
<td>1.3 ± 1.66</td>
</tr>
<tr>
<td>Testosterone (ng/dL)⁷⁻¹³</td>
<td>86.8 ± 34.60</td>
<td>89.0 ± 31.82</td>
<td>71.7 ± 38.58</td>
</tr>
<tr>
<td>Estradiol (pg/dL)⁷⁻¹³</td>
<td>29.3 ± 10.32</td>
<td>47.5 ± 15.74</td>
<td>22.5 ± 4.49</td>
</tr>
<tr>
<td>Prolactin (ng/mL)</td>
<td>8.5 ± 3.47</td>
<td>11.2 ± 9.34</td>
<td>8.7 ± 3.66</td>
</tr>
<tr>
<td>DHEAS (µg/dL)</td>
<td>183.6 ± 30.14</td>
<td>150.2 ± 59.27</td>
<td>147.7 ± 68.58</td>
</tr>
</tbody>
</table>

¹ All values are ± SD; percentage of change in parentheses. Control subjects were at 90-100% IBW. AN, anorexia nervosa; IBW, ideal body weight; NTX, N-telopeptide; BMD, bone mineral density; LH, luteinizing hormone; FSH, follicle-stimulating hormone; DHEAS, dehydroepiandrosterone sulfate. Analysis involved multiple t tests, and probability levels were adjusted by using the Bonferroni corrections.
² Significant difference between the regained-menses group at admission and the amenorrheic group at admission, P < 0.05 (independent t test).
³ Percentage of change from admission to 90% IBW was calculated by using a one-sample t test with Bonferroni correction.
⁴ The percentage of change did not differ significantly between the amenorrheic and the regained-menses groups.
⁵ Significant difference between the amenorrheic group at admission and at 90% IBW, P < 0.05 (independent t test).
⁶ Significant difference in percentage of change between the regained-menses group and the amenorrheic group at 90% IBW, P < 0.05 (Mann-Whitney test).
⁷ Significant difference between the amenorrheic group at admission and at 90% IBW, P < 0.05 (dependent t test).
⁸ Significant difference between the amenorrheic group at admission and control subjects, P < 0.01 (independent t test).
⁹ Significant difference between the amenorrheic group at 90% IBW and control subjects, P < 0.05 (independent t test).
¹⁰ Significant difference between the amenorrheic group at admission and at 90% IBW, P < 0.001 (dependent t test).
¹¹ Significant difference between the amenorrheic group at admission and control subjects, P < 0.05 (independent t test).
¹² Significant difference between the amenorrheic group at admission and control subjects, P < 0.001 (independent t test).
¹³ Significant difference between the regained-menses group and the amenorrheic group at 90% IBW, P < 0.05 (independent t test).

pg/mL to 90% IBW (35.0 ± 14.45 pg/mL; P < 0.001). Although the increase was significant, at 90% IBW, the patients (35.0 ± 14.45 pg/mL) still differed significantly (P < 0.01) from control subjects (56.0 ± 33.92 pg/mL).

Data on the return of menstrual function are shown in Table 4. No significant differences were observed in spine, hip, and total BMDs between the 2 patient groups at admission and upon reaching 90% IBW. Only the group that remained amenorrheic after treatment had significantly lower spine (P < 0.05), hip (P < 0.001), and total BMDs compared with control subjects.

As seen in the subanalysis of menstrual status, osteocalcin concentrations rose with weight gain in both groups (Figure 1; Table 4). The rise in osteocalcin did not differ significantly between the regained menses group and the amenorrheic group. However, the amenorrheic group continued to have higher values than did our reference control subjects (P < 0.02), which indicated a powerful effect of weight gain in this group (11.9 ± 7.33 ng/mL compared with 6.2 ± 1.90 ng/mL; P < 0.001). The higher NTX concentrations seen in subjects with persistent amenorrhea (75.0 ± 49.94 nmol/mmol creatinine) did not differ significantly from those in healthy control subjects, but they were significantly higher than the reference control population even after weight gain (37.0 ± 6.00 nmol/mmol creatinine; P < 0.02) (Figure 2). As expected, patients with return of menses at 90% IBW showed a fall in NTX (from 70.7 ± 40.84 to 45.9 ± 22.72 nmol/mmol Cr; NS) that reached into the ranges in healthy control subjects (45.9 ± 22.72 compared with 48.3 ± 14.38 nmol/mmol Cr; P < 0.09) (Table 4) and the reference control subjects (45.9 ± 22.72 compared with 37.0 ± 6.00 nmol/mmol Cr; P = 1.00) (Figure 2). None of these changes were statistically significant because of the large SD and the small number of subjects. No significant time-by-group interaction was observed for either group. However, a significant percentage fall in NTX was observed in the group that regained menses (–28.2 ± 27.4%; P < 0.05) (Table 4).

FIGURE 1: Mean (± SD) changes in osteocalcin concentrations from admission for treatment of anorexia nervosa until recovery of 90% of ideal body weight (IBW) for subjects who regained menses and subjects who remained amenorrheic. Bold lines represent ± 2 SDs from the mean osteocalcin value of 6.20 ± 1.90 ng/mL for reference control subjects (14; L Audi, personal communication, 23 November 2004). The error bars represent ± 2 SDs. Two-factor repeated-measures ANOVAs with Bonferroni corrections were used for comparisons to the reference control subjects. Time-by-group interaction was not significant; values in the amenorrheic group were compared with those in the reference control group (P < 0.02). Mean age was 18.2 ± 2.5 y, and mean BMI (in kg/m²) was 20.6 ± 2.1.
which may explain why they did not regain menses in 2.2 mo.

In the menstrual status subanalysis (Table 4), LH and FSH concentrations differed significantly \( (P < 0.001) \) at baseline, with lower concentrations in the amenorrheic group than in the healthy control subjects. In the amenorrheic group, a nonsignificant rise in LH and FSH concentrations was observed.

With respect to menstrual status (Table 4), the patients who regained menses did show an increase of estradiol into the normal range and did not significantly differ from the healthy control subjects \( (47.5 \pm 15.74 \text{ compared with } 56.0 \pm 33.92 \text{ pg/mL}; \text{NS}) \). Those who remained amenorrheic had a significant increase in estradiol between admission \( (22.5 \pm 4.49 \text{ pg/mL}) \) and the achievement of 90% IBW \( (30.0 \pm 10.63 \text{ pg/mL}; \text{P} < 0.05) \) (Table 4), but, at 90% IBW, their estradiol concentrations remained significantly lower than those in the recovered-menses group \( (30.0 \pm 10.63 \text{ pg/mL} \text{ and } 47.5 \pm 15.74 \text{ pg/mL}, \text{respectively}) \). This suggests, along with the lower gonadotrophs at baseline, that the group that remained amenorrheic was more profoundly suppressed in terms of hormone concentrations, which may explain why they did not regain menses in 2.2 mo.

**DISCUSSION**

This is the first longitudinal study to show significant percentage increases in BMD \( (4.38 \pm 7.48\% \text{ for spine}; \text{7.77} \pm 8.8\% \text{ for hip}; 0.09 \pm 5.81\% \text{ for total}; \text{P} < 0.05 \text{ for all}) \) with nutritional therapy over a period of 2.2 mo in women with AN, which suggests a powerful anabolic effect of nutritional therapy. This is also the first longitudinal study to show that mildly depressed osteocalcin concentrations increase with weight gain, whereas elevated NTX concentrations fall into the normal range only with resumption of menses. Although the osteocalcin concentrations are not significantly depressed, they do not increase parallel to the increase in resorption (NTX concentrations) until nutritional rehabilitation is achieved. These findings suggest that the recovery of bone metabolism is biphasic, involving a primary nutritional mechanism that stimulates bone formation and a hormonal mechanism that decreases bone resorption.

Although no differences in BMD improvement were observed between the patients who resumed menses and those who did not, and although the 2 groups gained weight to a similar level, we postulate that, in the former group, larger increases in BMD would be seen with a longer period of observation because of normalization of NTX and thus bone resorption. These studies were done with regional BMD measurements; site-specific measurements could provide more accurate data.

Our findings may also explain the lack of effect of estrogen \( (3) \) and OCP treatments \( (4) \) on bone metabolism in the cachectic state, because bone metabolism may not recover fully until a normal nutritional state has been established. However, larger studies are needed to confirm and extend these observations, because our control group was small.

Most studies support the notion that the mechanism of osteopenia in AN is nutritionally related \( (9, 20, 21) \). Our data are consistent with studies of healthy women that show a depression of bone formation indexes with as little as 5 d of nutritional deprivation. Rapid increases in indexes of bone resorption are also seen with more severe nutritional restriction \( (22) \). The changes in markers of bone resorption exactly parallel our findings \( (22) \). Conversely, nutritional rehabilitation of women with AN confirms the fact that recovery of bone formation occurs first, and that it is followed by suppression of resorption \( (10, 23) \). This chronology suggests that therapy with antiresorptives such as estrogen may not be effective without appropriate nutritional therapy. It also may explain the continued fractures in women taking OCPs that are reported in the literature \( (6, 7) \) and the lack of effect of estrogen on bone density in AN \( (3) \). Antiresorptive therapy may, however, have a therapeutic role after weight gain but before return of menses when bone formation has resumed.

Our data are also consistent with other studies indicating that, despite recovery from AN, osteopenia persists \( (6, 10, 14, 24) \). In the present study, however, we were surprised by increases in spine and hip bone densities of 3–4.4% over 2.2 mo, a greater and more rapid response than was seen in previous studies \( (10, 25) \). The current study used a regional analysis of total BMD, in which the defined regions of interest are less precise than are dedicated hip and spine BMD measurements. However, these methods have been used with good precision to evaluate regional BMD in athletes \( (26, 27) \). Because our studies used the same machines and operators in a longitudinal fashion, we consider the results to be highly significant.

Weight gain and changes in body composition may affect the accuracy of DXA scans, although, in a detailed study of this problem that used measurements made by a Lunar machine, the changes were not clinically significant \( (28) \). Because of a spurious increase in bone area, these studies show an increase in bone mineral content with weight gain, whereas BMD decreases. Thus, our findings may be slightly attenuated by weight gain, although there is no method for compensating for this.

To further understand the mechanism of persistent osteopenia in AN, hormones and markers of bone turnover were examined. Our results indicate that, at low weight, patients with AN have normal-to-increased bone formation, as indicated by osteocalcin concentrations, which appears to be insufficient to match the amount of bone resorption, as evidenced by increased NTX. This results in an imbalance in bone turnover and leads to osteopenia. These data are similar to findings from previous studies \( (6, 11, 12, 14, 20, 21) \). Grinspoon et al \( (12) \) compared patients with AN with patients with hypothalamic amenorrhea and healthy control subjects in a cross-sectional study. Both patients with AN and patients with hypothalamic amenorrhea had higher bone resorption indexes than did control subjects, but the indexes in patients with AN were significantly higher. Unlike the present study, that...
study showed lower serum osteocalcin concentrations in patients with AN than in healthy control subjects.

It is interesting that rapid increases in bone density were seen in both groups of anorexic patients—those who regained menses and those who remained amenorrheic. Our hormone data suggest that the group that remained amenorrheic had greater suppression of ovarian function, even at baseline, than did the group that regained menses, as evidenced by significantly lower LH concentrations at baseline and a lack of estradiol rise into the normal range. LH suppression would be expected to compromise ovarian stimulation and, in turn, estradiol secretion. Because estradiol decreases bone resorption, its suppression would favor an increase in NTX, which is consistent with our data. Followed over a longer period of time, the subjects who remained amenorrheic would likely have a greater degree of osteopenia than would those who regained menses.

The difference manifested at baseline between the 2 groups may explain the lack of significance of the time-by-group interaction, because the groups were defined by outcome and not by treatment. There may be underlying physiologic differences at baseline that affect their response and that have not yet been determined because this was not a controlled intervention.

Some studies have implicated insulin-like growth factor 1, a nutritionally dependent bone trophic factor, as the major factor contributing to osteopenia in AN (9, 10). One study showed a modest increase in spine bone density in patients treated with recombinant insulin-like growth factor 1 and OCPs compared with that in patients taking placebo plus OCPs (4). It is interesting that bone density increased 1.8% after 9 mo of therapy (4), a significantly lower increase than we saw in our patients after 2.2 mo of nutritional therapy. With reversal of amenorrhea, studies have shown a 6–25% increase in bone density (6, 7, 23, 29). This is in contrast to studies that have shown a 4.1–4.9% increase in BMD with bisphosphonates (30).

Given the high prevalence of osteopenia in women with AN, the major clinical concern is the progression to osteoporosis and pathologic fractures (31). One study estimated a fracture rate of 7 times normal in women and men with AN (25). A long-term cohort study that followed 208 women with AN for a mean of 12.9 y showed a 57% cumulative risk of fracture at 40 y after diagnosis (32). Fractures are a serious complication from osteopenia in AN and are associated with significant morbidity.

AN affects young women at a critical time for the development of peak bone mass, and it is often a chronic disease with a high relapse rate. As a result, the complication of osteopenia is often profound. A better understanding of the pathogenesis of osteopenia in AN will allow the development of prophylactic or therapeutic treatments to prevent pathologic fractures. Our data suggest that nutritional therapy is critical and necessary for optimal effect of other therapies such as estrogen replacement or other antiresorptives. Nutritional therapy also exerts an anabolic effect, which appears to be more powerful than the use of insulin-like growth factor 1 with OCPs. Treatment with antiresorptives, including estrogen replacement and OCPs, should be reexamined, because these agents do not improve bone formation and may not be effective unless bone formation is stimulated. Our observations may be important to an understanding of the mechanism of possible reversal of osteoporosis in AN, for which there is as yet no effective treatment.

We thank Laura Audi (Hospital Vall d’Hebron Unidad Investigacion Endocrinologia Pediatrica, Barcelona, Spain) for her generosity in allowing the use of her normal data for comparison with our subjects.

The authors’ responsibilities were as follows—JD: collected data and wrote the manuscript; LG: collected and analyzed data; SSG: researched the literature and collected data; LM: conducted clinical trial on patients with anorexia; SFE: assisted in conducting clinical trial, including refeeding of subjects with anorexia; BTW: supervised the treatment of subjects with anorexia nervosa; JW: conducted bone mineral density measurements; RP: conducted bone mineral density and body composition measurements; MPW (primary investigator): organized and conducted the clinical trial, organized and reviewed the data analyses and the literature research, and wrote the manuscript. None of the authors had a personal or financial conflict of interest.

REFERENCES