Bone turnover during inpatient nutritional therapy and outpatient follow-up in patients with anorexia nervosa compared with that in healthy control subjects¹–⁴

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ABSTRACT

Background: Osteopenia and osteoporosis are among the most frequent and severe complications in adolescents with anorexia nervosa.

Objective: The aim of this study was to assess the influence of nutritional therapy on bone metabolism during adolescent anorexia nervosa.

Design: We studied 19 anorectic patients aged 14.1 ± 1.4 y (x ± SD) with a body mass index (BMI; in kg/m²) of 14.2 ± 1.4 and 19 age-matched control subjects aged 15.1 ± 2.3 y with a BMI of 20.8 ± 1.9 for 1 y. Blood samples were taken for the measurement of bone markers, insulin-like growth factor I (IGF-I), and leptin.

Results: BMI rose significantly from 14.2 ± 1.4 at baseline to 17.4 ± 0.6 (P < 0.0001) at week 15. Compared with concentrations in the control subjects, concentrations of the bone formation markers procollagen type I propeptide (PICP) and bone alkaline phosphatase (bAP) in the anorectic patients were lower at baseline (PICP: P = 0.0071; bAP: P = 0.0012), increased with nutritional therapy (PICP: P = 0.0060, bAP: P = 0.0147), and were no longer significantly different (P > 0.05) during the follow-up period. Concentrations of IGF-I and leptin were significantly lower (P < 0.0001 for both) in the anorectic patients than in the control subjects at baseline. IGF-I increased with nutritional therapy but was still significantly lower (P = 0.0036) than that in the control group and decreased again during the follow-up period (P = 0.0126). In contrast, serum C-telopeptide decreased with nutritional therapy (P = 0.0446).

Conclusion: Nutritional therapy improves concentrations of bone formation markers in adolescent patients with anorexia nervosa. 


KEY WORDS Adolescence, anorexia nervosa, nutritional therapy, weight gain, leptin, insulin-like growth factor I, IGF-I, bone turnover markers

INTRODUCTION

Anorexia nervosa is a severe eating disorder defined by an inability to maintain a body weight ≥85% of that expected for age and height. Grinspoon et al (1) showed that bone mineral density (BMD) was lower than normal by ≥1.0 SD at one or more skeletal sites in 92% of young women with anorexia nervosa and by ≥2.4 SD in 38% of patients with anorexia nervosa. Malnutrition plays an important role in this reduction because patients achieve low body weight and reductions in fat mass by maintaining a restrictive diet or fasting. The restrictive eating pattern is also associated with profound metabolic complications, including prolonged amenorrhea, growth hormone resistance, elevated plasma concentrations of cortisol, low production of insulin-like growth factor I (IGF-I) (2, 3), and decreased serum leptin concentrations (4, 5). Long-term studies lasting up to 10 y show that in most anorectic patients, the disorder takes a protracted course, with recurring periods of relapse (6, 7). In 85% of anorexia nervosa patients who are considered partly recovered and in whom menses has resumed and weight rehabilitation to within 10% of ideal body weight is attained, a BMD deficiency persists (8).

The vast majority of peak bone mass is built during adolescence (9–12). Bailey et al (13) have shown that the age range of peak calcium accretion is 10.5–14.6 y in girls and 12.0–15.9 y in boys. During this period, sufficient nutrient supply and physical exercise play a very important role in the buildup of peak bone mass (14). Thus, malnutrition during adolescence may be an important factor in the development of osteopenia or osteoporosis in later life (15–17).

Anorexia nervosa is highly prevalent during adolescence, and it seems that the average age of onset of this disease is decreasing (18). As mentioned above, adolescence is the most important period of peak bone mass buildup in both girls and boys. Thus, it is very likely that deficient nutrient intakes lead to retardation of bone growth and reduced serum concentrations of pubertal hormones, such as 17-β estradiol, IGF-I, and leptin. Because the onset of anorexia nervosa occurs at a time when maximal peak bone mass is usually reached, nutritional therapy and weight

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rehabilitation during these years may play an essential role in preventing patients with anorexia nervosa from developing osteopenia or osteoporosis in later life.

In this study, we prospectively investigated the effects of an inpatient nutritional therapy program on bone turnover in a group of adolescents with anorexia nervosa in comparison with that in a group of age-matched healthy control subjects. A secondary outcome measure was the change in serum leptin during nutritional therapy and at 1 y of follow-up.

SUBJECTS AND METHODS

Patients and control subjects

Nineteen female adolescent inpatients [SD: age, 14.2 ± 1.4 y (range: 11.5–17.4 y); body weight, 39.3 ± 5.4 kg (range: 27.4–47.3 kg); body mass index (BMI; in kg/m^2), 14.2 ± 1.4 (range: 11.4–17.4)] with a diagnosis of anorexia nervosa [according to DSM-IV (Diagnostic Statistical Manual, 4th ed)] took part in our study. Fifteen of the patients were characterized as having “restricting type” and the remaining 4 as having “purging type” anorexia nervosa. In the restricting type, weight loss is mainly accomplished by dieting or fasting, whereas in the purging type, the person has regularly engaged in binge eating, vomiting, or both with or without laxative misuse. All patients and control subjects were evaluated on the basis of a structured interview for the assessment of eating disorders (19). As a control group, 19 age-matched adolescents [age, 15.1 ± 1.4 y (range: 13.0–19.6 y); body weight, 56.3 ± 1.8 kg (range: 44.5–68.5 kg); BMI, 20.8 ± 0.4 (range: 16.5–24.0)] participated in the study. Age-matched volunteers were enrolled as a control group because, obviously, healthy adolescents do have higher body weights. A careful history of the healthy control subjects was taken to ensure that they had neither a history of an eating disorder nor other medical illnesses known to affect bone health. Control subjects with eating disorders were excluded from the study. The mean duration of the patients’ disease was 11.4 ± 1.6 mo (range: 3–27 mo) before admission to the Department of Child and Adolescent Psychiatry and Psychotherapy of the Technical University of Aachen, Germany. Thirteen patients had had secondary amenorrhea for >6 mo. Six patients had never menstruated. All of the control subjects were postmenarchal and menstruating regularly.

Study design

The investigation took place at the Department of Child and Adolescent Psychiatry and Psychotherapy of the Technical University of Aachen, Germany. The study was approved by the Ethics Committee of the University of Aachen and was in accordance with the current revision of the Helsinki declaration. All of the patients and healthy volunteers, and their parents, gave written informed consent.

The study period was divided into 2 parts: part 1 was the inpatient treatment phase that ended, in most cases, by week 15. Part 2 was a follow-up period that lasted for up to 1 y. Except for the measurements of body composition, the patients were studied immediately after admission and during weeks 3, 7, 11, 15, 23, 32, 41, and 50 of inpatient and outpatient treatment. Baseline body-composition data were collected during week 3 after a diet-stabilization period. Results (PTH, bone turnover markers, IGF-I, and leptin) from the patient group during the first 11 wk of inpatient treatment were published elsewhere (20).

During the follow-up outpatient period, the BMIs of 13 of the 19 patients fell to <17.5. Three of the relapsed patients were rehospitalized in the same department and continued the study in the hospital. In this case they adhered to the same treatment regimen as in part 1. The other relapsed patients received ambulatory psychiatric therapy plus continuous supplementation with calcium and vitamin D.

The corresponding control group was studied at baseline and during weeks 7, 15, 32, and 50 of treatment. At each time point, body weight and BMI were measured. Lean body mass, body cell mass (BCM), and fat mass of 18 of the 19 patients and of all 19 of the control subjects were analyzed by bioelectrical impedance analysis (BIA), starting at week 3. Because many anorexia nervosa patients are dehydrated after admission, we decided to take the baseline measurements for body composition after a 3-wk stabilization period. A blood sample was drawn at 0800, while the patients were in a fasting state, for the measurement of serum calcium and phosphate, PTH, 17-β estradiol, dehydroepiandrosterone sulfate (DHEAS), cortisol, IGF-I, procollagen type I propeptide (PICP), bone alkaline phosphatase (bAP), serum C-telopeptide (CTX), and leptin concentrations. Concentrations of 25-hydroxyvitamin D were analyzed at baseline, week 15, and week 50.

During the inpatient treatment, the patients were physically active but were not allowed to engage in excessive exercise. In the first week, the patients were allowed an activity level of light physical workload. After they had been in the hospital for 6–8 wk, the patients’ activity level was comparable with a medium muscular workload. The control group maintained their normal activity level, which consisted of ≈2 h of sports activities per week at school plus some activity in their leisure time. None of the control subjects participated in competitive sports. During treatment and the follow-up period (up to 1 y), the patients did not vomit or use laxatives on the basis of regular, carefully conducted interviews. In the follow-up period, 2 of the patients took oral contraceptives for 6–7 mo. Three volunteers took oral contraceptives during the entire study period.

Diet

Anorexia nervosa patients

After a short observation period of 2–3 d, the patients received an individual diet of 7.8 ± 0.6 MJ/d for week 1. Energy intake was gradually increased to 9.1 ± 1.1 MJ/d by week 3. The caloric intake further increased to 10.7 ± 0.9 MJ/d in week 7 and stayed at that level until the patients were discharged from the hospital. Dietary protein intakes increased from 70 ± 13 g/d in the first week to 84 ± 15 g/d until discharge from the hospital. Fat intakes increased from 58 ± 23 g/d in week 1 to 103 ± 24 g/d during inpatient treatment. Carbohydrate intakes also increased continuously from 262 ± 61 g/d in week 1 to 324 ± 50 g/d in the hospital. Calcium intakes were 1565 ± 392 mg/d in week 1 and increased to 2218 ± 370 mg/d in week 3. This high level was maintained until discharge from the hospital. During outpatient treatment, the patients were asked to maintain their energy intake and to increase their consumption of milk and dairy products. In addition, they took calcium supplements (Calcium Sandoz forte; Novartis, München, Germany) to reach the required high intake of ≥2000 mg/d.
control subjects

The control group maintained their normal eating pattern during the 1-y study and were asked to increase their consumption of milk and dairy products. To reach the high calcium intake of 2000 mg/d, they took calcium supplements (Calcium Sandoz forte). To avoid any changes in serum vitamin D concentrations, we administered 400 IU ergocalciferol/d as a multivitamin tablet (Osspulvit S forte; Madaus, Köln, Germany). The tablet consisted of 400 IU ergocalciferol (vitamin D<sub>2</sub>), 5000 IU retinol acetate (vitamin A), 10 mg α-tocopherol (vitamin E), 5 mg thiamine (vitamin B-1), 5 mg riboflavin (vitamin B-2), 0.5 mg pyridoxine hydrochloride (vitamin B-6), 50 mg ascorbic acid (vitamin C), 15 mg niacinamide, and 493 mg calcium hydrogen phosphate.

The control group maintained their normal eating pattern during the 1-y study, which was assessed by regular interviews; however, they were also asked to increase their consumption of milk and dairy products. To reach the high calcium intake of 2000 mg/d, they also took calcium supplements (Calcium Sandoz forte). In addition, they received 400 IU ergocalciferol/d as a multivitamin tablet (Osspulvit S forte) (20).

Blood

Blood samples were taken from the antecubital vein with a short catheter and serum Monovettes (Sarstedt, Germany). Immediately thereafter, the contents of the Monovettes were divided into 2 parts. One part was immediately taken to the laboratory for centrifugation (5 °C, 2100 × g, 10 min) and the measurement of serum calcium, 17-β estradiol, DHEAS, and progesterone. The other part of the sample was immediately centrifuged (5 °C, 2100 × g, 10 min). Serum was distributed into small tubes, which were immediately frozen at −20 °C until analyzed for serum phosphate, PTH, IGF-I, PICP, bAP, leptin, and vitamin D (25-hydroxyvitamin D). The methods used for analyses were published elsewhere (20).

Body composition

BMI was calculated as body weight (kg) divided by body height squared (m) at the time of admission and during weeks 3, 7, 11, 15, 23, 32, 41, and 50 for the patients and at baseline and during weeks 7, 15, 32, and 50 for the control group. A BIA device (DATA INPUT software, BIA 2000-M; Nutri 4, Hofheim, Germany) was used to determine lean body mass, BCM (as an indicator of nutritional status), and fat mass in 18 of the 19 patients during weeks 3, 7, 11, 15, 23, 32, 41, and 50, and in the control group at baseline and weeks 7, 15, 32, and 50. Electrodes were placed on the right hand and wrist and on the right foot and ankle. Four frequencies (100, 50, 5, and 1 kHz) were measured to analyze lean body and fat masses. The calculations were performed by using the commercially available software provided by the manufacturer (DATA INPUT; Nutri 4). BCM was derived from lean body mass as follows: lean body mass × phase angle × constant.

Statistical analysis

The data presented as means ± SDs. Except for the body-composition data, all data were collected immediately after the subjects were admitted to the hospital. Because many anorexia nervosa patients are dehydrated after admission, we decided to take the baseline measurements for body composition after a 3-wk stabilization period. Statistical analyses were performed with the statistical analysis software SAS (21). Because multiple measurements were made per test subject, and because age and menstrual status were assumed to affect the variables, repeated-measures analysis of covariance (ANCOVA) was performed to assess differences between the patients and the control subjects; the values were adjusted for age and menstrual status. The overall alpha level was set at 0.05. If a significant interaction existed between group and time, post hoc analysis was used to evaluate differences between the 2 study groups at baseline and the effect of nutritional rehabilitation during the nutritional therapy and follow-up period. To compensate for multiple tests per variable, Bonferroni’s correction was applied.

RESULTS

Nutritional therapy increased the patients’ serum leptin concentrations significantly during the study period (P < 0.0001). At baseline, the serum leptin concentration was significantly lower in the patients than in the control group (P < 0.0001; Figure 1). During the first 15 wk of inpatient nutritional therapy, the patients’ serum leptin concentration rose and was no longer significantly lower than the concentration in the control group (P > 0.05; Figure 1). From weeks 15 to 50, the patients’ serum leptin concentrations tended to decrease but were not significantly lower than those of the control group (P > 0.05).

Serum IGF-I concentrations of the patients significantly increased during the study period (P = 0.0004). At baseline the patients’ serum IGF-I concentrations were significantly lower than those of the control group (P < 0.0001; Figure 1). In the first 15 wk of treatment, IGF-I concentrations increased but were still significantly lower than those of the control subjects (P = 0.0036; Figure 1). From weeks 15 to 50, the serum IGF-I concentrations of patients remained significantly lower than those of the control group (P = 0.0126).

The patients’ body weight also significantly increased during the study period (P < 0.0001). Baseline body weight was significantly lower in the patients than in the control group (P < 0.0001; Table 1). During the inpatient treatment period, the patients’ body weight increased significantly but was still lower than the body weight of the control group (P < 0.0001). During weeks 15–50, the mean body weight of the patients tended to decrease but was not significantly different (P > 0.05) from that of the control group. However, 13 of the 19 patients lost body weight during the outpatient treatment period.

Nutritional therapy increased BMI significantly during the study period (P < 0.0001). At baseline, BMI, lean body mass, BCM, and fat mass were significantly lower in the patients than in the control group (P < 0.0001; Table 1). During the inpatient treatment period, the patients’ body weight increased significantly but was still lower than the body weight of the control group (P < 0.0001). During weeks 15–50, the mean body weight of the patients tended to decrease but was not significantly different (P > 0.05) from that of the control group (P > 0.05). However, in 13 of 19 patients, BMI fell again to <17.5.

Lean body mass also increased significantly in the patients during the study period (P = 0.0215). At baseline, lean body mass tended to be lower in the patients than in the control group (P = 0.0522; Table 1). Lean body mass did not change significantly during the inpatient or outpatient treatment period (baseline to week 15: P > 0.05; week 15 to 50: P > 0.05). In the first 15 wk of inpatient treatment, BCM and fat mass increased in the
patients, but values were still significantly (both $P < 0.0001$) lower than those of the control group. During the outpatient treatment up to 1 y after inpatient treatment, BCM decreased again in the patients and remained significantly lower than the BCM of the control group ($P = 0.0402$). Development of fat mass also remained significantly lower in the patients than in the control group ($P = 0.0447$).

Nutritional therapy increased serum PICP concentrations significantly during the study period ($P = 0.0071$). The results for the bone formation markers PICP and bAP are shown in Figure 2. At baseline, the serum concentration of PICP was significantly lower in the patient group than in the control group ($P < 0.0001$). During the inpatient treatment, the mean serum PICP concentration increased in the patients, whereas the concentration in the control subjects did not change significantly ($P = 0.0060$). From week 15 to week 50, serum PICP in the patients was no longer significantly different from that of the control subjects ($P > 0.05$). A similar pattern was seen for bAP concentrations. Nutritional therapy increased serum bAP concentrations significantly during the study period ($P = 0.0048$). Baseline bAP concentrations were also significantly lower in the patient group than in the control group ($P = 0.0012$). In the first 15 wk, the mean serum bAP concentration increased in the patients but remained significantly unchanged in the control group ($P = 0.0147$). From week 15 to week 50, serum bAP concentrations in the patient and control groups were no longer significantly different ($P > 0.05$).

Serum concentrations of the bone resorption marker serum CTX are shown in Figure 3. Serum CTX concentrations decreased significantly in the patients during the study period ($P = 0.0044$), but were not significantly different from concentrations in the control group at baseline, during weeks 1 to 15, or during weeks 15 to 50 of the dietary treatment ($P > 0.05$).

Serum concentrations of calcium, PTH, cortisol, 17β-estradiol, DHEAS, and creatinine are shown in Table 1. Mean serum calcium and PTH concentrations in the patients were not significantly different ($P > 0.05$ for both) from those in the control group (Table 1). Mean serum cortisol concentrations in the patients were also comparable with those in the control group ($P > 0.05$).

Nutritional therapy increased serum concentrations of 17-β estradiol significantly during the study period ($P = 0.0101$). At baseline, the mean serum concentration of 17-β estradiol was significantly lower in the patient group than in the control group ($P < 0.0001$; Table 1). In the first 15 wk of treatment, 17-β estradiol concentrations increased in the patients but were still significantly lower than those in the control group ($P = 0.0255$). However, during the following weeks, mean serum 17-β estradiol concentrations in the 2 groups were no longer significantly different ($P > 0.05$).

Mean serum DHEAS and creatinine concentrations were not significantly different between the patients and the control group at any time during the study ($P > 0.05$; Table 1).

**DISCUSSION**

To the best of our knowledge, we were the first to compare prospectively the kinetics of bone turnover changes during 15 wk of inpatient nutritional therapy and over the following 9 mo between adolescent anorexia nervosa patients and an age-matched control group. As the patients gained and lost weight during this long observation period, we were able to evaluate the effects of weight change–associated metabolism on bone turnover. During the 15 wk of inpatient nutritional therapy, serum concentrations of the bone formation markers in the patients increased to concentrations in the control group. At week 15, the patients’ serum leptin and IGF-I concentrations nearly reached concentrations in the control group. However, during the following outpatient treatment for up to 1 y, IGF-I concentrations and BMI decreased again, whereas serum PICP, bAP, and CTX concentrations did not change significantly. Weight loss in the patients after the clinical phase was associated with a decrease in IGF-I concentrations and resulted from a voluntary reduction in energy intakes.
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<tr>
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<th>Anorexia nervosa patients</th>
<th>Control subjects</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>Week 15</td>
<td>Week 50</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>38.9 ± 5.0</td>
<td>47.9 ± 4.4</td>
<td>46.3 ± 7.2</td>
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<tr>
<td>BMI (kg/m$^2$)</td>
<td>14.2 ± 1.4</td>
<td>17.4 ± 0.6</td>
<td>16.5 ± 1.6</td>
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<td>Lean body mass (kg)</td>
<td>36.5 ± 3.7</td>
<td>38.1 ± 3.4</td>
<td>36.7 ± 4.3</td>
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<td>Body cell mass (kg)</td>
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<td>18.2 ± 1.6</td>
<td>17.4 ± 2.0</td>
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<td>Fat mass (kg)</td>
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<td>Calcium (mmol/L)</td>
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<td>PTH (pg/mL)</td>
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<td>Cortisol (nmol/L)</td>
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<td>499.8 ± 152.8</td>
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<td>17-β-Estradiol (pmol/L)</td>
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<td>135.0 ± 103.0</td>
<td>196.4 ± 200.1</td>
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<td>DHEAS (μmol/L)</td>
<td>4.49 ± 2.78</td>
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<td>Creatinine (mg/L)</td>
<td>9.62 ± 1.84</td>
<td>8.51 ± 1.36</td>
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1 PTH, parathyroid hormone; DHEAS, dehydroepiandrosterone.

2 Analysis of covariance with age and menstrual status as covariates was performed to assess the global interaction term ($P < 0.05$) and differences between the patients and the control subjects. Post hoc comparison was significant at $P < 0.05$ (Bonferroni corrected).

3 $\bar{x} \pm$ SD (all such values).
During the 15 wk of inpatient treatment, the individually calculated high energy intakes resulted in an increase in body weight, fat mass, and BCM in the patients. In contrast with an increase in BCM, lean body mass did not change during that period. Lean body mass consists of a cellular component, BCM, and extracellular mass. Because anorexia nervosa patients are often dehydrated, extracellular mass was most likely low during the baseline measurement. During treatment, BCM increased, whereas the extracellular mass decreased. In total, lean body mass in the patients did not change significantly. However, the tendency of patients to lose mean body weight and mean BMI after leaving the hospital suggests that 68% of the patients were in danger of relapsing and were thus unable to supply an adequate energy intake when they were on their own at home. This finding is in line with the findings of several studies in anorectic patients, who often lose body weight after discharge from hospital treatment and several of whom have relapsed (7, 22, 23).

In more recent studies, it was found that leptin—which is synthesized by adipocytes—plays an important role in bone remodeling, eg, promotes osteoblastic differentiation and inhibits osteoclast generation (24–27). In our study, baseline serum leptin concentrations in the control subjects were 4 times those in the patients. This finding is in line with the findings of other studies (28–30). As expected, during the 15-wk of inpatient treatment, the patients’ leptin concentrations increased with increasing body weight and fat mass and reached the concentrations of the healthy age-matched control group. However, the patients still had a significantly lower body weight and fat mass than did the control subjects (31). Serum concentrations of leptin tended to decrease, whereas IGF-I decreased significantly during the outpatient treatment—possibly because of the patients’ reduced energy intakes. This finding suggests that leptin might also be a marker for the nutritional status of subjects.

It has been shown by several groups (32–34), and was confirmed by our findings, that extremely malnourished adolescent patients with anorexia nervosa have very low serum concentrations of PICP and bAP. However, the rise in serum leptin in our study was accompanied by increases in serum concentrations of IGF-I and the bone formation markers PICP and bAP, which almost reached the concentrations of the control group at the end of the inpatient period. This suggests that the increase in fat mass and the concomitant leptin synthesis and increase in IGF-I concentrations may have had a stimulating effect on osteoblasts, which induced bone formation. The stimulating effect of IGF-I on bone turnover in patients with profound osteopenia due to anorexia nervosa was also shown by Grinspoon et al (4) in a short-term (6 d) and a 9-mo longitudinal (35) study. Our finding also supported by data from Matkovic et al (36), who observed a positive correlation between leptin and bone density.

The trend of increasing serum leptin concentrations, together with the increase in bone formation markers during refeeding, suggests a positive effect of leptin on bone. This finding is in contrast with results published by Ducy et al (37), who identified leptin as a potent inhibitor of bone formation in mice. It seems
that leptin acts on bone growth in 2 ways (38). First, it stimulates the release of an undefined hypothalamic osteoblast-inhibiting factor or factors (37, 39, 40). Second, it is a bone anabolic factor that directly promotes bone growth by stimulating osteoblasts to make IGF-I and inhibit osteoclast generation (24, 38). Our results suggest that the anabolic factor that is active during nutrition therapy in patients with adolescent anorexia nervosa is more dominant than are osteoblast-inhibiting factors.

At the end of the observation period of the outpatient treatment, the patients’ body weight and serum leptin concentrations were again lower than those of the control group. However, the markers of bone turnover did not decrease significantly. Weight loss in the relapsed patients probably led to a significantly lower mean serum leptin concentration than that observed in the control group. Concurrently, the patients’ serum concentrations of bone formation markers tended to decrease but were not significantly different from those of the control subjects. On one hand, this may have resulted because of a delayed response of osteoblast activity to lower serum leptin concentrations. On the other hand, because the patient group included both recovered and non-recovered anorectics, measurements from these patients were characterized by a large SD. Thus, during this observation period, serum concentrations of bone formation markers in patients tended to decrease without becoming significantly different from the markers in the control subjects.

The baseline serum CTX concentrations of our patient group were comparable with those published by Caillot-Augusseau et al (32). However, in the current study, serum CTX concentrations were almost identical in the patient group and the control group at the beginning of the study. Nutrition therapy led to a transient decrease in the bone resorption activity of the patients. At mean ages of 14 and 15 y, respectively, the patients and the control subjects were at the ages in which peak bone mass buildup occurs. Peak bone mass is accompanied by a high bone turnover rate, including high osteoclast activity (41). This would explain the high serum CTX concentrations observed in the control group. The high serum CTX concentrations observed in the patients indicates that osteoclast activity also increases in malnourished persons, although osteoblast activity decreases. Stefanis et al (34) hypothesized that the elevated bone resorption observed in anorexia nervosa patients results because bone collagen is used as an amino acid source for energy metabolism, especially for the heart muscles. The elevated creatinine excretion observed at baseline in the current study and the tendency for serum CTX to decrease with increasing energy intakes supports this hypothesis.

The finding that, during the first 11 wk of nutritional therapy, bone resorption and bone formation processes were uncoupled in our 19 patients with anorexia nervosa was published previously (20) and corroborated the findings of several other investigators (4, 32, 42). This pattern of reduced bone formation and increased bone resorption is similar to the pattern that occurs during physical inactivity (43–45). Reduced bone formation might be secondary to a reduction in serum IGF-I, which takes place in both malnourished and physically inactive subjects. In patients with anorexia nervosa, an increase in bone resorption might be caused by severe malnutrition, which is characterized by insufficient protein sources. Bone collagen might then be used as a protein source, which would result in increased bone resorption, as proposed by Stefanis et al (34).

The nutritional therapy we provided to the patients in the current study was individually tailored to provide high energy intakes at the beginning of treatment and to provide the patient and control groups with high calcium intakes and vitamin D supplementation throughout the study. Many published studies have shown that high calcium intakes plus vitamin D supplementation during adolescence result in greater bone mass than do low calcium intakes and no vitamin D supplementation (13, 46–50). Although the healthy control subjects in the current study also increased their calcium intake to >2000 mg/d and received vitamin D supplementation (400 IU/d), no significant changes in concentrations of bone formation or bone resorption markers were observed over the 1-y treatment. We therefore speculate that our control group already had sufficiently high calcium intakes. Because calcium intakes reach a threshold level, increases from sufficient to very high calcium intakes (>2000 mg/d) may not induced further increases in bone formation in healthy adolescents.

As mentioned before, 2 of the patients and 3 of the control subjects took oral contraceptives during the outpatient treatment period (patients) or the entire study period (control subjects). However, changes in the individual bone formation markers in those who took oral contraceptives were not significantly different from those in the subjects who did not take oral contraceptives. Neither the patients nor the control subjects had higher concentrations of bone formation markers that were due to oral contraceptive use, which is in line with data from other publications (51–53). Therefore, our finding support that of other studies (51–53), ie, oral contraceptive use does not have a significant effect on bone formation.

One might argue that a deficiency in 17 β estradiol induced an increase in bone resorption activity in our patients. This may have been true before inpatient treatment; however, 17 β estradiol concentrations were no longer significantly different from those in the control group at the end of the study. In contrast, bone resorption activity was at the same level as at baseline.

In summary, this prospective controlled study provides additional evidence that nutritional therapy followed by weight gain has a profound beneficial effect on bone formation in patients with anorexia nervosa. Thus, increasing energy intake seems to be the first important step in building up bone mass in patients with anorexia nervosa. Especially in light of the high risk of osteoporosis in these patients, additional studies are necessary to examine the effects of delivering energy and specific nutrients to prevent osteopenia or osteoporosis in later life.

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MH designed the study, performed the experiment, collected and analyzed the data, and wrote the manuscript. CM designed the study, performed the experiment, and collected the data. IG performed the experiment and collected the biochemical data. NH designed the study and performed the statistical analysis of the data. BH-D designed the study and analyzed the data. None of the authors had any financial support from or personal interest in any company or organization sponsoring the research, including advisory board affiliations.

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