Moderate energy restriction increases bone resorption in obese postmenopausal women[1–3]

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ABSTRACT

Background: Weight reduction reduces bone mineral density (BMD) and increases the risk of osteoporosis.

Objective: We investigated whether bone is mobilized in postmenopausal women during energy restriction and whether hormones regulate bone turnover and mass.

Design: Twenty-seven obese postmenopausal women with a mean (±SD) age of 55.9 ± 7.9 y and body mass index (in kg/m^2) of 33.0 ± 3.8 completed the 6-mo study. Fourteen women followed a moderate energy-restricted diet (WL group) and 13 control subjects maintained their body weight (WM group). Body weight, bone turnover markers, serum parathyroid hormone (PTH), and dietary intake were measured throughout the study. Total-body BMD, sex hormone binding globulin, leptin, and estrone were measured at baseline and at week 25.

Results: In the WL group, body weight decreased by 10.2 ± 5.5% ($P < 0.001$), body fat mass decreased by 18.7 ± 11.3% ($P < 0.001$), and total-body BMD decreased by 1.2 ± 1.2%; these changes were significantly different from those in the WM group ($P < 0.05$). Serial measurements showed chronically elevated rates of bone resorption and formation during energy restriction that were greater than in the WM group ($P < 0.05$). Serum sex hormone binding globulin increased and leptin decreased with weight loss ($P < 0.05$). Serum PTH tended to increase in the WL group but not in the WM group ($P < 0.06$). The reduction in fat mass with weight loss was directly associated with a decrease in serum estrone ($P < 0.01$, $R^2 = 0.50$).

Conclusions: Moderate energy restriction increases bone turnover in obese postmenopausal women and may be regulated in part by alterations in serum PTH and estrone. *Am J Clin Nutr* 2001;73:347–52.

KEY WORDS Bone mineral density, bone resorption, estrone, parathyroid hormone, postmenopausal women, sex hormone binding globulin, energy restriction, weight loss, obesity

INTRODUCTION

Women with low body weight or those who lose weight have an increased risk of osteoporosis (1, 2). Epidemiologic data show that fracture risk increases in older women who lose ≥10% of their body weight (3, 4). Bone mineral density (BMD) increases with obesity (5); intervention studies showed a significant reduction in BMD with weight loss (6–10). The mechanisms underlying a reduction in BMD and the effects of a reduced-energy diet on bone turnover are unclear. Studies have shown that markers of bone resorption tend to increase with weight loss (7, 8), whereas markers of bone formation both increase (8) and decrease (7). Interpretation of markers of bone turnover is limited by their variability. Inconsistent findings in previous studies may have been due to single measurements taken only before and after the weight-loss period (7, 8). In the present study we measured bone turnover during moderate energy restriction. Additionally, unlike in previous weight-loss and bone studies, we controlled for measurement variability by including parallel measurements in a weight-maintenance group.

After menopause, estrogen concentrations decrease markedly. However, it is thought that adipose tissue production of estrone is greater in obese than in nonobese persons (11) and that estrone is protective against bone loss. Estrone is interconvertible with 17β-estradiol, and the principal source of estrone production is derived from aromatization of androgens in peripheral fat. Some researchers showed that obese postmenopausal women have both higher concentrations of sex hormones and higher bone densities than do nonobese women (5, 12). The reduction that occurs in both estrogen and testosterone with weight loss (13) may be due to loss of body fat. Additionally, sex hormone binding globulin (SHBG), which increases during weight loss (14), was shown to be inversely associated with free circulating testosterone concentrations (13). To our knowledge, there are no previous data to show that a reduction in serum estrone occurs in response to weight loss. Bone loss that occurs with weight loss may be regulated, in part, by a decrease in circulating concentrations of sex steroids.

Serum leptin concentrations increase with increases in body weight, body mass index (BMI), and fat mass (15, 16) and

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decrease with weight loss (17). In addition, leptin may regulate osteoblast differentiation (18) and bone formation (19). It is possible that the marked decrease in circulating leptin with energy restriction (17) influences the rate of bone turnover.

The mechanisms underlying a reduction in BMD and changes in bone turnover during weight loss have not been investigated adequately. In the present controlled trial, we examined whether bone turnover markers and bone-regulating hormones can explain changes in BMD due to weight loss in obese postmenopausal women during moderate energy restriction.

SUBJECTS AND METHODS

Subjects

Thirty-four obese postmenopausal women (> 3 y since menopause) with a BMI (in kg/m^2) ranging from 28 to 39 were recruited for a weight-loss (WL) or a weight-maintenance (WM; control) group. Women who were taking any medication (including hormone replacement therapy) or had a disease known to influence bone metabolism were excluded. Subjects had to have been weight stable in the previous 3 mo and women with a BMI > 40 were excluded because of a reduced sensitivity to bone densitometry measurements. This study was approved by the institutional review boards of Rutgers University (New Brunswick, NJ) and St Lukes–Roosevelt Hospital (New York). A subset of the WL group was used as a control group in a previous study (20).

Study design

Spot urine and blood samples were collected between 0830 and 1000 after subjects had fasted overnight. Body weight, markers of bone resorption (urinary pyridinium cross-links) and formation (serum osteocalcin), and serum parathyroid hormone (PTH) were measured at baseline and at 1–5, 7, 10, 13, 16, 20, and 25 wk in the WL group and at 1–4, 7, 13, 16, and 20 wk in the WM group. At baseline and at the end of the study, serum was collected to measure SHBG, 25-hydroxyvitamin D [25(OH)D], estrone, and leptin concentrations. Twenty-four–hour urine samples were collected to measure calcium excretion. The waist-to-hip ratio (WHR) was measured by using anthropometric methods, and body composition (including total-body bone mineral mass) was measured by dual-energy X-ray absorptiometry (DXA) before and after weight loss.

Diet

Subjects in the WL group (n = 14) were told to consume less than their usual food intake according to a standard behavior-modification, nutrition-education, weight-loss program. The WM group (n = 13) maintained their usual intake to maintain body weight. Subjects in both groups consumed their usual intake of dairy products and were instructed to take no vitamin-mineral supplements 2 wk before and during the study period. Twenty-four–hour dietary recalls were examined at baseline and during the study period (NUTRITIONIST IV; First Data Bank, San Bruno, CA). Dietary vitamin D and serum 25(OH)D were measured to determine vitamin D status. All subjects maintained their typical exercise routine for the duration of the study.

Laboratory methods

Total urinary pyridinium cross-links—pyridinoline and deoxypyridinoline—were measured by HPLC after submitting hydrolyzed samples to a prefractionation procedure (21). Peaks were detected by fluorescence (22), quantified by external standards (courtesy of S Robins), and expressed per mmol creatinine (Sigma, St Louis). The CVs for pyridinoline and deoxypyridinoline were 4% and 6%, respectively. Urinary calcium was measured by a fluorometric assay (no. 587-A; Sigma). Serum osteocalcin (intact and fragments) and estrone were determined by radioimmunoassay (Diagnostic Systems Laboratory, Webster, TX) and had CVs <9%. Serum 25(OH)D was measured by radioimmunoassay (CVs < 15%; DiaSorin, Stillwater, MN), as was serum leptin (CVs < 10%; LINCO Research, Inc, St Charles, MO). Serum intact PTH and SHBG were measured by 2-site immunoradiometric assay (CVs < 12%; Diagnostic Systems Laboratory). Total-body BMD and bone mineral content, fat mass, and lean soft tissue mass were measured by DXA (CV of 0.9%; DPX; Lunar Corp, Madison, WI).

Statistics

A two-way repeated-measures analysis of variance (ANOVA) was used to assess variables over time and to make comparisons between the WL and WM groups (STATVIEW; SAS Institute Inc, Cary, NC). For measurements before and after weight loss, we performed a two-way ANOVA using group and treatment as the main effects. If a main effect or interaction was significant, linear contrast analysis (Scheffe's test) was performed. Values were compared before and after treatment within a group with Student's paired t tests. To determine relations between nutrients or hormones and bone indexes (turnover and BMD), simple linear and stepwise multiple regression analyses were performed. Values were considered significant at $P < 0.05$.

RESULTS

Twenty-seven of the 32 recruited women completed the study. Two women dropped out for personal reasons, 2 were excluded because of previously undiagnosed diabetes, and 1 was excluded because of a markedly elevated rate of bone turnover at baseline. Five women in the WL group who did not lose a significant amount of weight (<0.3 kg after 6 wk) agreed to participate in the WM group. Thus, 14 women (11 whites and 3 African Americans aged 55.0 ± 8.0 y; 10.7 ± 10.6 y since menopause) successfully completed the weight-loss program and 13 women (10 whites and 3 African Americans aged 57.2 ± 8.3 y; 12.6 ± 5.9 y since menopause) maintained their body weight (**Table 1**). At baseline, the clinical characteristics of the WL and WM groups were not significantly different.

Nutrient intake

Nutrient intakes at baseline were not significantly different between groups, whereas during energy restriction, reported intakes of energy, protein, calcium, and sodium during energy restriction were lower in the WL than in the WM group (**Table 2**). At baseline, calcium and vitamin D intakes were below the recommended intakes of 1200 mg/d and 400 IU (10 μg)/d (23), respectively. During energy restriction, calcium intake decreased further by 16 ± 28.4% ($P < 0.05$; Student's paired t test), whereas vitamin D intake remained unchanged. Dietary sodium also decreased during energy restriction ($P < 0.01$). Nutrient intake did not change significantly in the WM group.

TABLE 1
Body composition and bone turnover values at baseline and the percentage changes after weight loss or maintenance[1]

	Weight-loss group (n = 14)		Weight-maintenance group (n = 13)	
Variable	Baseline	% Change	Baseline	% Change
Body weight (kg)	87.3 ± 9.9	−10.2 ± 5.5	86.9 ± 13.9	0.2 ± 2.7[2]
BMI (kg/m^2)	32.7 ± 4.6	−10.2 ± 5.5	33.1 ± 3.8	0.2 ± 2.7[2]
Waist-to-hip ratio	0.87 ± 0.07	−4.4 ± 9.1	0.87 ± 0.05	0.1 ± 2.0
TBBMD (g/cm^2)	1.16 ± 0.08	−1.15 ± 1.2	1.16 ± 0.87	0.62 ± 2.1[3]
TBBMC (g)	2441.8 ± 287.6	−0.5 ± 3.2	2389.9 ± 318.5	0.2 ± 3.4
Fat mass (g)	40.3 ± 7.2	−18.7 ± 11.3	38.5 ± 10.3	7.0 ± 14.4[3]
Osteocalcin (nmol/L)	0.7 ± 0.2	12.8 ± 30.0	0.8 ± 0.3	−4.3 ± 22.5
Pyd (nmol/mmol creat)	20.3 ± 6.5	36.4 ± 44.9	27.2 ± 7.6	9.5 ± 28.6[2]
Dpd (nmol/mmol creat)	5.1 ± 2.6	52.5 ± 66.5	7.2 ± 2.2	4.9 ± 30.2[2]

[1] $\bar{x}$ ± SD. Dpd, deoxypyridinoline; Pyd, pyridinoline; TBBMC, total-body bone mineral content; TBBMD, total-body bone mineral density; creat, creatinine.
[2,3] Significantly different from weight-loss group (ANOVA and Scheffe's test): [2] $P < 0.01$, [3] $P < 0.05$.

Bone and other body-composition changes

Women in the WL group lost 10.2 ± 5.5% ($P < 0.001$) of their body weight, whereas there was no significant change in the WM group (Table 1). Total-body BMD decreased by 1.15 ± 1.2% (n = 14) in the WL group and by 0.6 ± 2.1% in the WM group (n = 7; some individuals in this group declined to have a final DXA measurement). Total-body bone mineral content did not change significantly in either group. Fat mass decreased significantly in the WL group but not in the WM group. Lean tissue mass was not significantly different between the WL (43.9 ± 4.1 kg) and WM (43.2 ± 4.7 kg) groups at baseline, and there was no significant change in the WL (−2 ± 3%) or WM (−1± 2%) group after the 6-mo study. The WHR did not change significantly after weight loss.

Bone turnover and hormones during weight loss

Urinary pyridinoline and deoxypyridinoline increased significantly with energy restriction after 1 wk and remained elevated throughout the study (**Figure 1**); both compounds increased significantly more in the WL than in the WM group from baseline to after weight loss (Table 1). Serum osteocalcin did not increase significantly after weight loss; however, ANOVA showed that values measured over time were significantly greater in the WM than in the WL group (**Figure 2**). Final PTH concentrations tended to increase after weight loss (**Table 3**, $P < 0.06$); however, serial measurements of PTH were not significantly greater in the WL than in the WM group (**Figure 3**).

Serum SHBG increased and leptin decreased significantly in the WL group, whereas serum 25(OH)D and estrone concentrations did not change significantly after weight loss (Table 3). Loss of fat mass (%) was positively associated with the percentage change in estrone (**Figure 4**), leptin (R^2 = 0.30, $P < 0.05$), and SHBG (R^2 = 0.34, $P < 0.05$) in the WL group. None of these indexes changed significantly in the WM group during or at the end of the study period.

The most significant predictor of the rise in pyridinium cross-links, as determined by stepwise multiple regression analysis, was SHBG (R^2 = 0.57, $P < 0.05$). Osteocalcin values were predicted by dietary protein and sodium (R^2 = 0.44, $P < 0.05$); the strongest predictors of osteocalcin included dietary protein, sodium, and energy and serum PTH (R^2 = 0.96, $P < 0.01$).

Complete 24-h urine collections for determination of urinary calcium were not available for all subjects. Baseline 24-h urinary calcium concentrations (57.3 ± 26.3 mg/d; n = 10) did not differ significantly between groups or change significantly after ≈6 mo of weight loss. In addition, 24-h urinary creatinine excretion in both groups was 12.0 ± 3.9 mmol/d at baseline and did not differ significantly between groups or decrease significantly in either group. There was a 14% increase in 24-h urinary pyridinoline and deoxypyridinoline excretion from baseline to the end of the study that was significant for pyridinoline ($P < 0.05$) but not for deoxypyridinoline in the WL group.

DISCUSSION

This study showed that markers of bone resorption increase during energy restriction. Although the rate of bone formation

TABLE 2
Daily nutrient intakes at baseline and during weight loss or maintenance[1]

	Weight-loss group (n = 14)		Weight-maintenance group (n = 13)	
Variable	Baseline	% Change	Baseline	% Change
Energy (MJ)	7.9 ± 2.5	5.2 ± 1.0	7.4 ± 2.8	7.0 ± 1.3[2]
Protein (g)	89.9 ± 30.7	57.4 ± 14.3	100.7 ± 55.1	84.1 ± 28.3[3]
Calcium (mg)	665.9 ± 267.1	503.9 ± 130.0	698.8 ± 147.8	635.7 ± 119.2[3]
Vitamin D (μg)	2.5 ± 1.5	1.3 ± 2.7	1.3 ± 1.3	2.3 ± 0.5
Phosphorus (mg)	1772.7 ± 1947.6	882.6 ± 193.2	1346.0 ± 791.4	1077.4 ± 580.5
Magnesium (mg)	380.3 ± 428.0	204.1 ± 57.2	276.1 ± 216.5	268.0 ± 206.8
Sodium (mg)	2206.7 ± 809.9	1511.5 ± 665.5	2004.0 ± 528.4	2268.4 ± 437.6[3]

[1] $\bar{x}$ ± SD.
[2,3] Significantly different from the weight-loss group (two-way ANOVA and Scheffe's test): [2] $P < 0.05$, [3] $P < 0.01$.

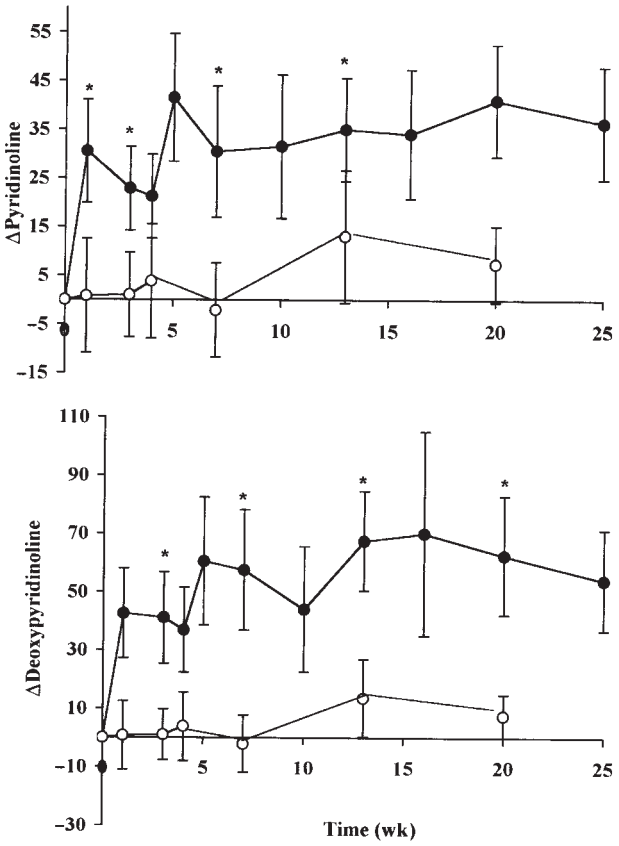

FIGURE 1. Mean (±SEM) change (Δ) in pyridinoline and deoxypyridinoline (normalized for creatinine) from baseline over 6 mo in the weight-loss (●; n = 14) and weight-maintenance (○; n = 13) groups. There was a significant interaction of treatment and time between groups, $P < 0.05$ (ANOVA). *Significantly different from the weight-maintenance group, $P < 0.05$.

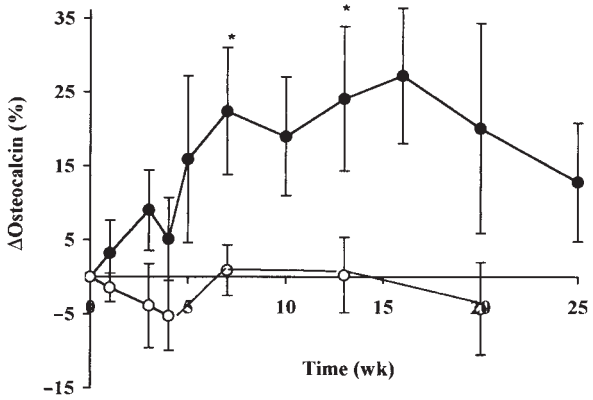

FIGURE 2. Mean (±SEM) change (Δ) in osteocalcin from baseline over 6 mo in the weight-loss (●; n = 14) and weight-maintenance (○; n = 13) groups. There was a significant effect of treatment between groups, $P < 0.05$ (ANOVA).

also increased, values did not increase significantly until week 5 of weight loss. These serial measurements of bone turnover help to elucidate previous inconsistent findings of both increases and decreases in bone turnover during energy restriction (7, 8) by showing the magnitude, timing, and duration of the change. This study also clarified the hormonal regulation of bone mass and turnover due to weight loss.

The mechanisms by which moderate energy restriction increases bone resorption are unclear. In the present study, serum PTH tended to increase after 6 mo of weight loss. Although the rise in PTH was small, with values within the reference range, it may have contributed to the rise in bone resorption, as was observed in a previous study (24). A rise in PTH may have occurred because of a reduction in calcium intake (Table 3) during energy restriction, which would explain the increased rate of bone resorption (Figure 1). However, there was a significant acute rise in bone resorption (Figure 1), but not in serum PTH (Figure 3). It is possible that the circadian rhythm of PTH (25) may have resulted in missed critical blood samples, limiting our ability to observe a significant acute rise in serum PTH during energy restriction. The mechanisms that limit the availability of calcium to enter the circulation (resulting in a rise in PTH) were not clarified in this study. It is possible that the low dietary calcium intake in these women (Table 2), which was compounded by the stress of energy restriction and a reduced intake of other nutrients, caused the rise in PTH. Additionally, limited calcium availability may have been due to a decrease in gut calcium absorption. Studies that address these issues could provide further insight into the underlying mechanisms of bone loss.

Hormonal factors other than PTH could increase bone turnover. Estrogens (estradiol and estrone) were shown to be positively related to BMD (26, 27) and negatively related to bone loss (28, 29) in postmenopausal women. Estrone is the most abundant circulating estrogen in postmenopausal women. Concentrations of estrone in adipose tissue and serum are greater in obese than in lean postmenopausal women (30). In the present study, serum estrone remained unchanged during weight loss. However, the women who lost the most fat mass showed the greatest reduction in serum concentrations of estrone (Figure 4).

TABLE 3
Serum hormone concentrations at baseline and the percentage changes after weight loss or maintenance[1]

	Weight-loss group		Weight-maintenance group	
Variable	Baseline	% Change	Baseline	% Change
25(OD)D (nmol/L)	43.7 ± 17.5	−0.8 ± 20.8	60.4 ± 32.7	3.5 ± 30.7
PTH (pmol/L)	2.4 ± 0.69	25.4 ± 17.6	3.2 ± 0.99	0.5 ± 37.2
SHBG (nmol/L)	73.8 ± 35.2	37.0 ± 21.4	72.7 ± 43.6	−4.1 ± 27.8[2]
Estrone (pmol /L)	94.4 ± 52.5	5.9 ± 32.5	76.2 ± 27.0	8.6 ± 20.2
Leptin (μg/L)	44.1 ± 20.9	−40.7 ± 29.1	37.1 ± 16.6	3.9 ± 25.0[2]

[1] $\bar{x}$ ± SD. 25(OD)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; SHBG, sex hormone binding globulin.
[2] Significantly different from the weight-loss group, $P < 0.05$ (ANOVA and Scheffe's test).

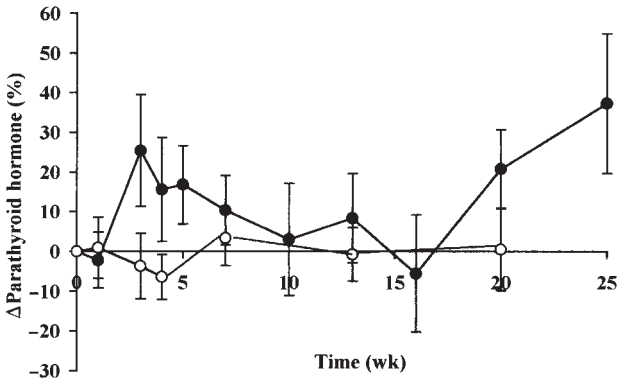

FIGURE 3. Mean (±SEM) change (Δ) in parathyroid hormone from baseline over 6 mo in the weight-loss (●; $n = 11$) and weight-maintenance (○; $n = 10$) groups.

Nevertheless, we observed no direct relation between serum estrone and markers of bone turnover. We recognize that a limitation of this study was the small number of subjects and the potential lack of power to show significant relations. Hence, it is possible that serum estrone concentrations would have decreased as body weight decreased (rather than only with fat mass) had more subjects (as well as heavier subjects) been included.

Both serum SHBG and WHR were shown previously to be correlated with BMD (5, 12, 31–33). Women with more abdominal fat (ie, a higher WHR) than peripheral fat (ie, fat in the gluteal-femoral region) tend to have an androgenic profile (high free testosterone and low SHBG) (34) and higher BMDs (31, 32). We hypothesized that serum SHBG and WHR may predict changes in bone turnover and bone mass because of their relations with free circulating sex steroids (both estrogens and androgens) (13, 34). Serum SHBG increased after weight loss (Table 3) and partially predicted changes in bone resorption but not in BMD. Researchers showed conflicting results concerning the relation of WHR with weight loss (35–39). In the present study, weight loss did not significantly alter WHR (Table 1) and therefore cannot explain alterations in bone metabolism.

Serum leptin decreased with weight loss, as expected (17). A 40% decrease in leptin was accompanied by a 19% loss of fat mass and was not associated with any bone indexes. Our results in the WL group agree with and extend the findings of others—during weight maintenance there is no relation between serum leptin and markers of bone turnover in adults (15, 16). Nevertheless, leptin resistance in obese individuals may minimize the response of bone markers to a change in serum leptin concentrations (19).

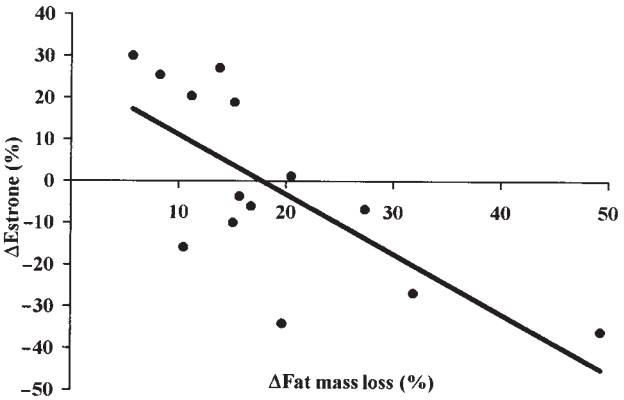

FIGURE 4. Relation between the change (Δ) in serum estrone and the loss of fat mass after weight loss in obese postmenopausal women. $P < 0.01$, $R^2 = 0.50$.

Much research has focused on calcium and vitamin D intakes as important regulators of BMD; however, there are many other nutrients that influence bone and that should be considered in studies of energy restriction. This study examined self-reported dietary records for changes in various nutrients that may have influenced bone turnover (Table 2) and showed that reductions in protein and sodium are associated with changes in bone formation, but not bone resorption, as determined by multiple regression analysis. Additionally, Svendsen et al (8) found that bone turnover increased in postmenopausal women who consumed a low-energy liquid diet supplemented with nutrients (including 800 mg Ca/d). The rise in bone turnover, therefore, may be more a result of the weight loss rather than a chronic reduction in nutrient intake. Nevertheless, our data are limited because the endpoint of our study design was weight loss, and specific nutrient intake was dependent on self-reported intake. Consequently, studies designed to specifically address the role of micronutrients (other than calcium and vitamin D) on bone indexes during energy-restricted diets are indicated.

Bone loss could also result from a decrease in mechanical loading associated with a reduction in body weight. It is hypothesized that an elevation in BMD concomitant with an increase in body weight is due to skeletal adaptations resulting from an increased mechanical load (40). Osteocytes are believed to be important mechanosensors for bone (41) that detect mechanical loading and may be affected by weight loss. Bone loss due to weight loss may be due to a reduction of these stress-related events. However, in weight-loss studies that address the level of physical activity, there have not been consistent positive effects of exercise on bone mass (6, 8).

In summary, the present study showed a decline in BMD after moderate weight loss due to negative bone balance, secondary to an increase in bone resorption relative to formation. Energy restriction appears to result in an elevation in bone resorption that may be regulated, in part, by a decrease in sex steroid concentrations and an increase in PTH. These observations clarify earlier ambiguities about the response of bone turnover to energy restriction and identify potential mechanisms for future research areas.

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