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Serum leptin as a determinant of bone resorption in healthy postmenopausal women

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Abstract

To examine the relationships between serum leptin and bone metabolism, we measured bone mineral density (BMD) at the spine and the hip, fasting serum leptin, and osteocalcin and urinary excretion of C-terminal crosslinking telopeptide of type I collagen (CTX), as markers of bone formation and resorption, respectively, in 121 postmenopausal women aged 54 ± 5 years. These parameters were also assessed at 6 months and 2 years of treatment with either 2.5 mg tibolone ($n = 34$), 1.25 mg tibolone ($n = 45$), or 2 mg estradiol plus 1 mg norethindrone acetate ($n = 42$). At baseline, serum leptin correlated positively with spine ($r = 0.21$, $P = 0.02$) and total hip ($r = 0.26$, $P = 0.0044$) BMD and negatively with CTX ($r = -0.38$, $P < 0.0001$) and osteocalcin ($r = 0.21$, $P = 0.025$). After adjustment for BMI and for fat mass, the association between serum leptin and CTX persisted with a partial correlation coefficient of -0.18 ($P = 0.046$) and of -0.22 ($P = 0.03$), respectively. Women in the highest quartile of leptin levels had 11% higher total hip ($P = 0.0039$) and lumbar spine BMD ($P = 0.016$), 21% lower osteocalcin ($P = 0.01$), and 38% lower CTX ($P = 0.0005$) than women in the lowest quartile ($P < 0.05$). During treatment, serum leptin levels increased (+14.7 ± 47.3%, $P = 0.019$), without significant difference between the groups. This increase correlated with the increase in body weight ($r = 0.46$, $P < 10^{-4}$). No correlation was found between the changes in leptin and the changes in bone parameters. In conclusion, leptin may play a role as a determinant of bone resorption in healthy, untreated postmenopausal women, but the effect of estradiol or tibolone on bone are not mediated by leptin.

Keywords: Bone mineral density; Bone turnover; Hormone replacement therapy; Leptin; Tibolone

Introduction

Leptin is an adipocyte-derived, cytokine like protein, produced by the ob gene. Beyond its initial function in regulating appetite and energy expenditure, this hormone appears to play important roles in various parameters in human health. Serum leptin levels increase in obesity, and a reduction in body weight results in a significant decrease in leptin concentration [1]. It is well established that obesity has a protective effect against osteoporosis. Because both bone density and leptin levels depend on body weight, it has been suggested that leptin may play a mediating role in maintaining bone mass in obese subjects. Moreover, a sexual dismorphism in serum levels has been observed, with women having two to threefold higher level [2,3], independent of adiposity, leading to the hypothesis that estrogen might play a stimulating role in leptin secretion. Thus, leptin has emerged as a candidate for bone effects of estrogens and hormonal deficiencies.

It has been previously suggested that leptin may be of importance for osteoblastic cell growth and bone mineralization. In vitro studies showed the expression of leptin in primary cultures of normal human osteoblasts in the mineralization and/or the osteocyte transition period [4], and others showed that leptin inhibits osteoclasts generation and

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may contribute to linkage of formation and resorption in bone [5]. In animals, some studies showed that leptin stimulates bone growth in leptin deficient ob/ob mice [6], whereas Ducy et al, [7] showed that these ob/ob mice have high bone mass despite hypogonadism and hypercortisolism; intracerebroventricular infusion of leptin causes bone loss in these mice, and leptin may regulate bone metabolim via the sympathetic nervous system [8]. In ovariectomized rats, changes in bone metabolism are very similar to those observed in human menopause, and administration of leptin prevents the increase in bone turnover [9].

In humans, data are conflicting about the relationship between bone mineral density and serum leptin [3,10,11] and the relationship between leptin and bone metabolism has not been clarified. The main objective of the present study was to examine which effects the leptin exerts on bone metabolism in humans; therefore we assessed the relationships between serum leptin, bone mineral density, and bone turnover markers, in healthy postmenopausal women before any hormone replacement therapy (HRT). To find out whether the effects of HRT on bone are mediated by leptin, we studied the relationships between the changes in bone parameters and the changes in leptin levels during 2-year treatment with HRT or tibolone.

Patients and methods

Subjects

This study was part of a prospective, randomised, double-blind study, comparing the effects on bone of tibolone to those of estradiol (E_2) and norethindrone acetate (NETA) [12]. This study was conducted in two centers, using two different randomization lists. Data presented in this study come from one center in which blood and urine samples were available for biological assessments. One hundred twenty-one postmenopausal women are the basis of this study. All participants were at least 45-years-old with intact uterus. Criteria for entry in the study included menopause confirmed by serum levels of estrogen $\leq$ 50 pg/ml and FSH $\geq$ 20 Ul/l. Exclusion criteria were a body mass index (BMI) below 19 or over 27 kg/m^2, bilateral oophorectomy, undiagnosed vaginal bleeding, endometrial hyperplasia, inflammatory disease, or any condition likely to require corticosteroid treatment, bone diseases, liver enzyme abnormalities, a history of cancer, or cigarette consumption $>$ 10 cigarettes daily. Subjects were also excluded if they had used estrogens within the last 6 months, or had calcitonin or vitamin D treatment within the last 2 months, and drugs known to interfere with calcium metabolism. All subjects gave written informed consent to participate in the study which was carried out in accordance with the Helsinki declaration.

Treatments

Subjects received either tibolone 2.5 mg (n = 34), tibolone 1.25 mg (n = 45), or E_2 2 mg plus NETA 1 mg (n = 42), daily at bedtime. All women received a daily supplementation of 500 mg of calcium with a meal. Follow-up duration was 2 years.

Assessments

Height, weight, and BMI (calculated as the weight in kg per height in m^2) were determined at baseline for each subject. BMD (g/cm^2) was measured at the left hip (the femoral neck and the total hip), and the lumbar spine L2–L4 in the antero–posterior scan, by dual energy-X-ray absorptiometry (DEXA), using a QDR 2000 W (Hologic, MA, USA), at baseline, 6 months, and 2 years of follow-up. For 96 patients, fat mass was measured at baseline and at 2 years using the same device. In the whole population, at baseline, 6 months, and 2 years of follow-up, serum concentrations of osteocalcin (using two-side radioimmunoassay, Elsa Osteo, CisBio International, France) and the urinary excretion of C-terminal crosslinking telopeptide of type I collagen (CTX) corrected for creatinine, on the morning second-void urine sample (using enzyme linked immunosorbent assay, Crosslaps ELISA, Nordic Biosciences, Denmark) were measured after an overnight fast. Fasting serum leptin levels were measured using a radioimmunoassay for human leptin (Linco Research Inc., St. Charles, MO, USA). All blood samples were drawn between 7 and 9 AM. All assays have an intra-and intervariability lower than 10% and were all performed in a central laboratory with extensive experience in biochemical marker measurements (Synarc, Lyon, France).

Statistical analysis

Quantitative variables are expressed as median (range). As the distributions of several -variables, and notably leptin, CTX, or osteocalcin levels, were skewed, nonparametric statistical methods were used for analysis. The relationships between leptin and other variables were evaluated using Spearman rank correlation coefficients. Because leptin is influenced by body weight, partial rank correlation coefficients adjusted for BMI or fat mass were also computed. Changes in leptin levels and in other parameters at 6 and 24 months were expressed as relative percent changes as compared to baseline value. Changes for the whole cohort and within each treatment arm were tested using (one sample) Wilcoxon signed rank sum tests. Between groups comparisons were performed using Kruskal-Wallis nonparametric test, and no post hoc two-by-two comparisons was done as the comparison of the three treatment was not the purpose of this study. The associations between relative changes in leptin and changes in other parameters were also evaluated using Spearman rank correlation coefficients. All tests were

Table 1
Baseline characteristics of the study population

	All women (n = 121)	Tibolone 2.5 mg (n = 34)	Tibolone 1.25 mg (n = 45)	E2 + NETA (n = 42)
Age (years)	53 (45–68)	54 (47–66)	54 (45–63)	53 (47–68)
YSM (years)	2 (1–14)	3 (1–13)	2 (1–14)	2 (1–13)
Height (cm)	160 (147–178)	157 (148–175)	160 (147–172)	162 (148–178)
Weight (kg)	59 (43–86)	58 (46–75)	59 (46–86)	61 (43–84)
BMI (kg/m^2)	23.0 (16.6–33.2)	23.3 (18.9–28.4)	23.1 (16.6–29.9)	22.1 (17.1–33.2)
Total hip BMD (g/cm^2)	0.835 (0.599–1.168)	0.846 (0.607–1.003)	0.839 (0.599–1.168)	0.826 (0.6–1.048)
Femoral neck BMD (g/cm^2)	0.708 (0.467–1.000)	0.710 (0.502–0.848)	0.703 (0.471–1.000)	0.708 (0.467–0.934)
Spine BMD (g/c m^2)	0.865 (0.586–1.223)	0.870 (0.602–1.065)	0.900 (0.586–1.129)	0.847 (0.672–1.223)
Serum leptin (ng/ml)	10.7 (2.0–33.2)	10.7 (3.6–33.2)	11.1 (3.7–25.2)	10.1 (2.0–30.3)
Serum osteocalcin (ng/ml)	25.2 (9.3–51.5)	25.4 (14.1–46.1)	25.8 (9.3–41.9)	24.8 (9.3–51.5)
Urinary CTX (μg/mmol cr)	330 (52–892)	365 (203–863)	308 (155–892)	326 (52–702)

Note. Values are median (range). YSM, time since menopause in years: BMI, body mass index: E2 + NETA, estradiol 2 mg and norethindrone acetate 1 mg.

two-sided, and at a 0.05 significance level. All analyses were carried out using S-Plus 2000 statistical software package (MathSoft, Inc., Seattle, WA).

Results

Baseline characteristics of the patients are shown in Table 1. There was no statistically significant difference between groups.

Cross-sectional analyses

At baseline, leptin showed a strong positive correlation with weight ($r = 0.61$, $P < 10^{-4}$) and BMI ($r = 0.73$, $P < 10^{-4}$) and a weaker correlation with BMD at the spine ($r = 0.21$, $P = 0.022$), total hip ($r = 0.26$, $P = 0.0044$), and femoral neck ($r = 0.18$, $P = 0.06$). Serum leptin correlated negatively with urinary CTX ($r = -0.38$, $P < 10^{-4}$), and to a lesser degree with serum osteocalcin ($r = -0.21$, $P = 0.025$). However, the only association that remained significant after adjustment for BMI was between serum leptin and urinary CTX with a partial rank correlation coefficient of -0.18 ($P = 0.046$). After adjustment for fat mass, the association between serum leptin and urinary CTX was maintained ($r = -0.22$, $P = 0.03$), whereas no significant relationship was found with BMD. No significant correlation was found between serum leptin and age ($r = -0.075$, $P = 0.4$) or time since menopause ($r = 0.025$, $P = 0.79$). We then categorized women into quartiles of serum leptin distribution at baseline. There was no significant difference between quartiles of leptin in age, years since menopause, and BMI. There was a significant, although modest association between levels of serum leptin and BMD at the spine and total hip (Table 2), with women in the highest quartile of leptin having on average 11% higher total hip ($P = 0.0039$) (Fig. 1), and spine BMD ($P = 0.016$) than women in the lowest quartile. On the other hand, there was an inverse significant association between serum leptin and bone turnover, with women in the highest quartile having on average a 21% ($P = 0.010$) and 38% ($P = 0.0005$) lower levels of serum osteocalcin and urinary CTX, respectively, than women in the lowest quartile of leptin (Fig. 1). After adjustment for BMI, these percentages were 11% ($P = 0.19$) for serum osteocalcin and 23% ($P = 0.036$) for urinary CTX.

Table 2
Baseline characteristics according to quartiles of serum leptin levels

	Serum leptin				
	First quartile (n = 31)	Second quartile (n = 31)	Third quartile (n = 29)	Fourth quartile (n = 30)	P[a]
Age (years)	54 (45–64)	54 (47–63)	54 (48–66)	53 (45–68)	0.89
YSM (years)	1.5 (1–14)	3 (1–10)	3 (1–13)	2 (1–13)	0.59
BMI (kg/m^2)	19.8 (16.6–25.5)	22.2 (17.4–27.5)	24.0 (21.1–26.8)	25.7 (20.4–33.2)	0.077
Total hip BMD (g/cm^2)	0.799 (0.599–0.977)	0.863 (0.600–1.048)	0.841 (0.607–1.093)	0.868 (0.710–1.168)	0.012
Femoral neck BMD(g/cm^2)	0.673 (0.471–0.839)	0.748 (0.467–0.934)	0.708 (0.502–0.993)	0.722 (0.592–1.000)	0.10
Spine BMD (g/cm^2)	0.823 (0.586–1.017)	0.896 (0.602–1.129)	0.874 (0.626–1.129)	0.870 (0.743–1.223)	0.033
Serum osteocalcin (ng/ml)	26.8 (16.1–47.2)	26.2 (11.3–41.0)	25.7 (15.8–42.2)	22.9 (9.3–51.5)	0.0011
Urinary CTX (μg/mmol cr)	404 (106–892)	345 (97–737)	323 (140–674)	246 (52–566)	$<10^{-4}$

Note. Values are median (range).
[a] Between groups comparison by Kruskal-Wallis test.

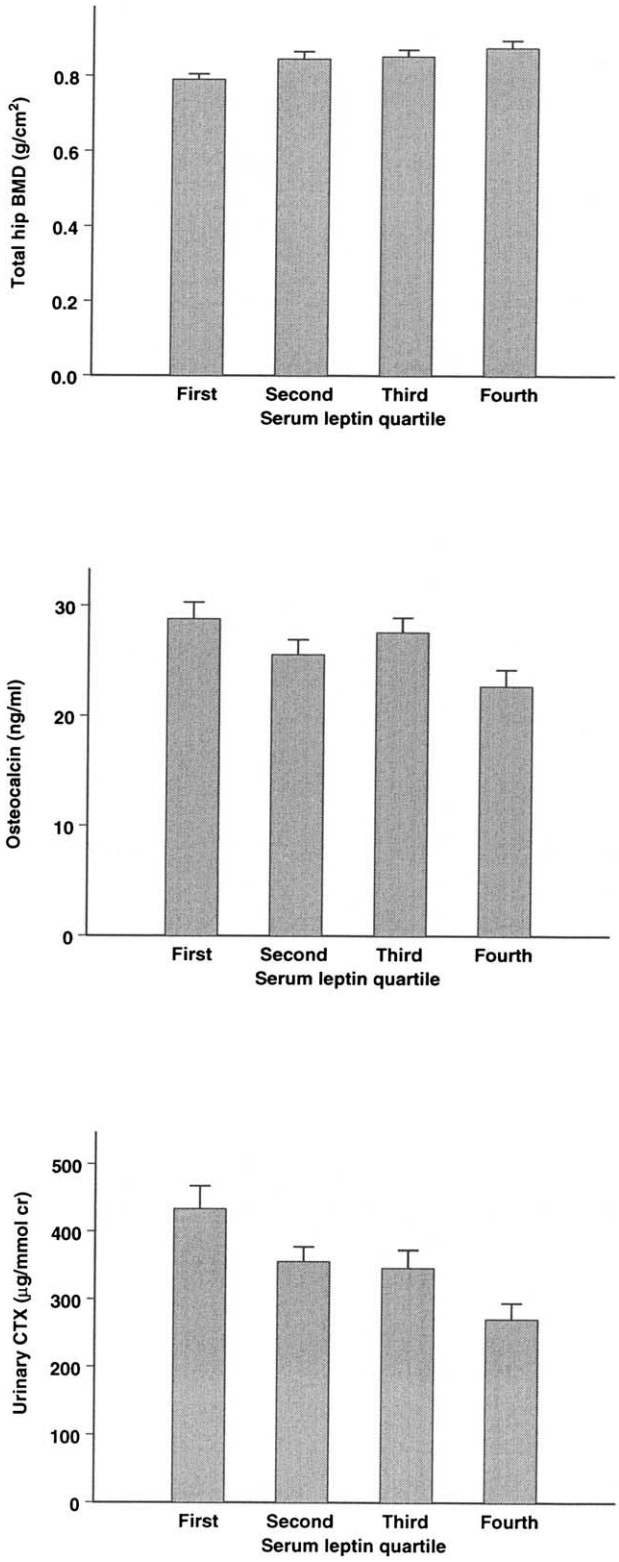

Fig. 1. Mean (± SEM) values of total hip BMD, serum osteocalcin, and urinary CTX in a population of healthy postmenopausal women, according to quartiles of serum leptin levels at baseline (from first (lowest) to fourth (highest)). For comparison between first and fourth quartiles, $P = 0.0039$ for total hip BMD, $P = 0.01$ for serum osteocalcin, and $P = 0.0005$ for urinary CTX.

During treatment

The percentage of changes in serum leptin levels and other variables during follow-up are shown in Table 3. The changes in leptin at 6 months significantly differed among treatment groups ($P = 0.0018$). At 2 years, leptin increased significantly in the whole population (mean ± SD: 14.7 ± 47.3%, $P = 0.019$), without difference between treatment groups. There was an increase in body weight (median 3.12%), which was statistically significant in each of the three groups ($P < 10^{-4}$), without difference among them ($P = 0.71$). There was a correlation between the increase in leptin level, and the increase in body weight ($r = 0.46$, $P < 10^{-4}$). Women with changes in body weight above the median had a significantly higher increase in serum leptin than others: 30.3 ± 50.5% vs −0.42 ± 38.7%; $P = 0.0003$. The decrease in biochemical markers of remodeling and the increase in BMD were statistically significant in all groups, with a significant treatment effect for the majority of these parameters (Table 3). No significant correlation was found between changes in leptin and changes in bone parameters, either at 6 months or at 2 years.

Discussion

In healthy nonobese, early postmenopausal women, we found that serum leptin was a significant independent predictor of bone resorption. There was a positive correlation between serum leptin levels and bone mineral density, which was no longer significant after adjustment for BMI. The effect of leptin on bone resorption was observed only at baseline, before starting any hormonal therapy. Although serum leptin increased during HRT and tibolone treatment, changes in bone parameters were not related to changes in leptin levels.

In our study, leptin levels correlated positively with BMD at both spine and hip, and correlated strongly with body weight and BMI. The protective effect of obesity on bone mass is well established, and has been attributed to several factors including the production of estrogens by adipose tissue, weight bearing effect, and anabolic effect of insulin on osteoblasts. However, this bone sparing effect of obesity has not been fully explained, and because both bone mass and leptin are related to body weight, it has been suggested that this hormone may be a mediator regulating this effect. The association between serum leptin levels and bone density in women was reported in some studies [11,13–15] and in one study in nonobese women, the positive correlation between leptin and bone mass was independent of body weight [14]. However, in agreement with the findings of a large cross-sectional study including 1148 postmenopausal women [3], this association was not significant after adjustment for BMI or fat mass. We found a strong inverse relationship between urinary CTX, a specific and sensitive marker of bone resorption, and serum leptin,

Table 3
Percent changes of serum leptin, bone markers, and bone mineral density after 6 months and 2 years of treatment

	Tibolone (2.5 mg)	Tibolone (1.25 mg)	E2 + NETA	All women	P^a
6 months					
Serum leptin	−12.0 (−53.3;67.5)¶	−4.4 (−87.5;274.1)	12.9 (−80.6;218.1)†	−2.0 (−87.5;274.1)	0.0018
Serum osteocalcin	−32.2 (−63.2;34)‡	−28.1 (−64.4;47.8)‡	−34.6 (−58.8;−6.4)‡	−31.8 (−64.4;47.8)‡	0.22
Urinary CTX	−72 (−91.7;−3.1)‡	−49.5 (−92;6.6)‡	−73.3 (−95.8;9.4)‡	−65.4 (−95.8;9.4)‡	0.0001
BMD spine	1.7 (−2.7;15.5)†	1.1 (−5.1;6.2)†	3.7 (−2.5;9.8)‡	2.2 (−5.1;15.5)‡	10^{-4}
BMD total hip	0.3 (−3.5;5.1)	0.6 (−2.7;5.4)	2 (−2.4;10.2)¶	1.1 (−3.5;10.2)¶	0.32
2 years					
Serum leptin	0 (−56.3;102.5)‡	12 (−57.9;175.5)‡	11.1 (−58.9;144.3)‡	7.1 (−58.9;175.5)‡	0.13
Serum osteocalcin	−42.7 (−66.8;64)‡	−32 (−61.9;19)‡	−43.6 (−72.9;58.7)‡	−41.6 (−72.9;64)‡	0.022
Urinary CTX	−68.3 (−88.5;−5.2)‡	−47.9 (−91.9;66.9)†	−65.5 (−94.8;28)‡	−62.4 (−94.8;66.9)‡	0.0054
BMD spine	3.9 (−2.6;9.1)‡	1.2 (−7.1;13.7)¶	6.3 (−4.5;17.4)‡	3.9 (−7.1;17.4)‡	10^{-4}
BMD total hip	3.1 (−2.4;8.9)‡	1.4 (−4.0;6.3)¶	3.4 (−4.6;12.3)‡	2.5 (−4.6;12.3)‡	0.0014

Note. Values are median (range). Symbols indicate significant changes within group: ¶ $P < 0.05$; † $P < 0.01$; ‡ $P < 10^{-4}$.
[a] Between groups comparison by Kruskal-Wallis test.

even after adjustment for BMI or fat mass. In addition, women with serum leptin levels in the highest quartile had about a 38% lower levels of urinary CTX, compared with women in the lowest quartile. Data on the relationship between leptin and bone markers are conflicting. Some studies did not find correlation with bone markers in women [16,17]. Conversely, Blain et al. [15] found a borderline relationship with bone formation markers and, in accordance with our data, a significant negative correlation with CTX after adjustment for fat mass. The disappearance of correlation between leptin and bone density, and the persistence of the correlation between leptin and CTX, after adjustment for BMI and fat mass in our study, suggest that leptin is not directly related to bone mass in women, but may play an important role in determining bone turnover, in healthy postmenopausal women by limiting the excessive bone resorption related to hormonal deficiency, independently of body weight. Yamauchi et al. found that low plasma leptin levels and not percentage fat mass was associated with the presence of vertebral fractures in postmenopausal women, suggesting that circulating leptin plays a physiological role in maintaining better bone quality [13].

Inhibition of bone resorption by leptin has been studied both in vivo and in vitro. Burguera et al. [9] showed that subcutaneous administration of leptin prevents ovariectomy-induced bone loss in rats, and that combination of estrogen and leptin further decreases bone turnover compared with that in estrogen-treated ovariectomized rats. Holloway et al. [5] showed that leptin inhibits osteoclast generation in culture of human peripheral blood mononuclear cells (hPBMC) and murine spleen cells incubated in bone. These studies have suggested that leptin modulates bone remodeling by stimulating the expression of osteoprotegerin (OPG), the potent inhibitor of osteoclastogenesis, by stromal cells or hPBMC, and by inhibiting the expression of RANKL, the major cytokin controlling osteoclastogenesis. Furthermore, leptin directly induces the secretion of interleukin 1 receptor antagonist in human monocytes [18], interleukin 1 being a key cytokin involved in bone loss during estrogen deficiency.

Besides its effects on bone resorption, it has also been reported that peripheral leptin administration may stimulate bone formation in ob/ob mice [6] and that leptin acts on human marrow stromal cells, enhancing osteoblast differentiation and inhibiting adipocyte differentiation [19]. This inhibition of adipogenesis by leptin, the adipocytes-secreted hormone, could be the result of a negative feedback phenomenon. However, although medullary adipocytes share several histological and functional characteristics with white adipocytes, it has been shown that in contrast to extramedullary adipocytes, marrow adipocytes are unresponsive to insulin [20]. Thus, the process of adipogenesis and the sensibility to different factors including leptin may differ in the marrow and in the extramedullary adipose tissues.

At baseline, leptin did not correlate with age or time since menopause. Although in one cross-sectional study, serum estradiol correlated negatively with leptin level in untreated postmenopausal women, suggesting that ovarian senescence may lead to increase in leptin secretion [21] several previous reports showed no influence of menopause status on serum leptin [2,22–24]. The bone loss occurring with menopause despite the protective effect of leptin, which does not decline, may be partly due to a resistance to the action of leptin resulting from impaired transport to the brain, as it has been reported in ovariectomized mice [25].

During follow-up, leptin levels increased, and these changes correlated with changes in body weight. Although there were higher changes in the E2+NETA group than in the tibolone ones, we were not able to show a difference in the effect of treatments on leptin ($P = 0.13$). Others have shown that during long-term administration, neither HRT [2,26–29], nor tibolone [30] affects serum leptin concentrations. However, bone parameters changes were not related to changes in leptin, precluding the hypothesis that leptin may act as a mediator between HRT or tibolone and bone.

Larger randomized placebo-controlled studies would be required to investigate adequately the effects of HRT and tibolone on serum leptin.

In conclusion, the results of our study suggest that, in healthy nonobese untreated postmenopausal women, leptin may play an important role as a determinant of bone resorption. Effects of HRT and tibolone on bone turnover and BMD are not explained by changes in leptin levels.

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