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Leptin Reduces Ovariectomy-Induced Bone Loss in Rats

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Bone mineral density increases with fat body mass, and obesity has a protective effect against osteoporosis. However, the relationship between fat body mass and bone mineral density is only partially explained by a combination of hormonal and mechanical factors. Serum leptin levels are strongly and directly related to fat body mass. We report here the effects of leptin administration compared with estrogen therapy on ovariectomy-induced bone loss in rats. Leptin was effective at reducing trabecular bone loss, trabecular architectural changes, and periosteal bone formation. Interestingly, the combination of estrogen and leptin further decreased bone turnover compared with that in estrogen-treated ovariectomized rats. Leptin also significantly increased osteoprotegerin mRNA steady state levels and protein secretion and decreased RANK ligand mRNA levels in human marrow stromal cells *in vitro*. Our findings suggest that leptin could modulate bone remodeling in favor of a better bone balance in rats. This study is the first evidence that leptin therapy has a significant effect in preventing ovariectomy-induced bone loss, and this effect may at least in part be mediated by the osteoprotegerin/RANK ligand pathway. (*Endocrinology* 142: 3546–3553, 2001)

OSTEOPOROSIS AND OBESITY are two major health problems in Western societies (1). However, these two diseases are inversely correlated, as bone mineral density (BMD) increases with weight, body mass index, and fat body mass (FBM) (2). In fact, numerous studies have shown that obesity is associated with increased BMD (3, 4) and has a protective effect against osteoporosis, leading to a lower risk of bone fractures compared with normal weight controls (5). To date, this protective effect of obesity has been partially explained by a combination of hormonal (peripheral aromatization of adrenal androgens to estrone in adipose tissue) and mechanical factors (weight-bearing effect) (6). Indeed, peripheral aromatization of adrenal androgens to estrone in adipose tissue may lead to a relative increase in serum estrogen levels, which is one of the most important determinants of bone remodeling. In addition, mechanical strains exerted by obesity have osteogenic effects on weight-bearing bone sites. However, these two mechanisms may not fully explain the increased BMD associated with obesity, as FBM and BMD are still directly and strongly correlated, even after adjusting for differences in serum estrogen levels (7), and this relationship has been observed regardless of the weight-bearing status of the bone site (8). Moreover, fat mass (9) and body weight (10) are correlated with BMD in women independently of their menopausal status. Despite normal serum 17β-estradiol levels and ample calcium intake, premenopausal women lose 1% of spinal trabecular BMD yearly during the fifth decade, suggesting that the association between obesity and bone density is at least partially independent of sex steroids in premenopausal women (11).

Because leptin is almost entirely produced by white adipose tissue, serum leptin levels are increased in obesity and are strongly and directly related to fat mass (11). Leptin circulates in plasma and, after crossing the blood-brain barrier, acts in the hypothalamic nuclei to regulate food intake, energy expenditure, growth, and sexual maturation (12). In addition to its effects in the central nervous system (13, 14), recent studies have shown that leptin also acts in the periphery, where leptin receptors have been described (12). Leptin has the capability of suppressing specific biochemical reactions that contribute to lipid accumulation and adipocyte differentiation (15), as well as modulating T cell immune responses and reversing the immunosuppressive effects of acute starvation (16). Using a conditionally immortalized human marrow stromal (hMS) cell line with the potential to differentiate to either osteoblastic or adipocytic lineages (17, 18), we have recently demonstrated that stromal cells produce functional leptin receptors. Furthermore, leptin exerted direct osteogenic effects *in vitro* by enhancing osteoblastic differentiation and inhibiting late adipocytic differentiation of the hMS cells (18). The hMS cell line also expresses the two key osteoclast determinants, RANK ligand (RANKL) and OPG (18, 19). RANKL is a novel member of the TNF ligand superfamily that is essential for osteoclast differentiation and activation (20). The stimulatory effects of RANKL in the bone microenvironment are neutralized by the secreted decoy receptor OPG (21). The balance between OPG and RANKL secretion by stromal cells is critical to the regulation of osteoclast formation. For example, factors known to induce osteoclast formation may increase both OPG and RANKL

Abbreviations: AN, Anorexia nervosa; BFR, bone formation rate; BMD, bone mineral density; BV/TV, cancellous bone volume; FBM, fat body mass; hMS, human marrow stromal; MAR, mineral apposition rate; MSC, marrow stromal cells; OVX, ovariectomy/ovariectomized.

levels, with the increase in RANKL exceeding that in OPG (22).

The *in vivo* effects of leptin on bone metabolism are controversial. Ducy *et al.* (23) have recently shown that leptin-deficient (*ob/ob*) and leptin receptor-deficient (*db/db*) mice have increased bone mass despite hypogonadism and hypercortisolism. On the contrary, Steppan *et al.* (24) showed that leptin administration led to a significant increase in femoral length, total body bone area, bone mineral content, and bone density in *ob/ob* mice compared with those in vehicle-treated controls.

To further evaluate whether leptin could be a hormonal mediator relating fat and bone mass, we explored the effects of leptin on bone metabolism *in vivo*. The effect of leptin on bone mass was studied for 1 month in ovariectomized (OVX) Sprague Dawley rats and was compared with that of 17α-ethinyl estradiol (E2) and a combination of leptin and E2.

Materials and Methods

Animals and drugs

All procedures were approved by the institutional animal care and use committee at Mayo Clinic (Rochester, NY) before beginning the study.

Sprague Dawley rats were obtained from Charles River Laboratories, Inc. (Portage, MI), at 6 months of age and body weight of 283 ± 5.1 g (mean ± SEM). Bilateral ovariectomy (n = 56; OVX) or sham surgery (n = 8; intact) was performed at 5 months and 3 wk of age. At 4 d postsurgery, OVX rats were stratified by weight and randomized to a baseline (n = 8) or one of the five groups (n = 8 rats/group): 1) leptin; 2) E2; 3) leptin plus E2; 4) vehicle; and 5) and a group pair fed to the leptin-treated group. Treatment was not started until 7 d post-OVX to allow for the estrogen levels to become completely depleted. A continuous sc administration of leptin (100 μg/day) was performed using Alzet osmotic pumps (ALZET, Cupertino, CA). This dose was selected from the literature, based on its potential to effectively modulate food intake without impairing growth (25–27). We used recombinant human leptin provided by Eli Lilly & Co. (Indianapolis, IN), according to previous studies that showed the effectiveness of human leptin in rodents (28, 29). E2 was administered sc at a concentration of 200 μg/kg·d, using slow release tablets. Fluorochromes to label mineralizing bone surfaces were administered by juxta-tail vein injection 1 d before starting treatment (calcein-tetracycline-HCl, 20 mg/kg BW; Sigma, St. Louis, MO), 10 d before death (tetracycline, 20 mg/kg BW; Sigma), and 3 d before death (calcein as described). The two fluorochrome labels are readily differentiated under UV illumination, with tetracycline fluorescing in pale yellow and calcein fluorescing in bright green.

Bone histomorphometry

Histomorphometric measurements were made with the osteomeasure semiautomatic analysis system (Osteomeasure Instruments, Inc., Atlanta, GA) as previously described (30). The regions of interest were traced, and the line lengths and defined areas were calculated by computer.

Cortical bone measurements. Ground transverse sections were used for histomorphometric analysis of cortical bone. Cross-sections of 150 μm were cut at a site just proximal to the tibia-fibula synostosis with a low speed saw (Isomet, Buehler, Lake Bluff, IL) equipped with a diamond wafer blade. The sections were ground to a thickness of 15–20 μm on a roughened glass plate and mounted in glycerol before microscopic examination under UV illumination to visualize fluorochrome labeling. The following parameters were determined as previously described (30): cross-sectional area, defined as the area of bone and marrow cavity bounded by the periosteal surface of the specimen; medullary area, defined as the area delineated by the endocortical surface of the specimen; cortical bone area, calculated as the difference between the cross-sectional and the medullary area; periosteal perimeter, defined as the total perimeter enclosing the cross-section (periosteal perimeter includes fluorochrome-labeled and nonlabeled perimeters); periosteal bone formation rate, calculated as the area bounded by tetracycline labels divided by the labeling period of 26 d; and periosteal mineral apposition rate (MAR), defined as the periosteal bone formation rate divided by the labeled perimeter. These measurements have been described previously (30) and follow the standard nomenclature recommended by Parfitt *et al.* (31).

Cancellous bone histomorphometry. After fixation for a minimum of 2 d in 70% ethanol, the proximal tibial metaphyses were dehydrated in a series of increasing concentrations of ethanol and embedded without demineralization in a mixture of methylmethacrylate-2-hydroxyethylmethacrylate. The embedded tibiae were sectioned at an indicated thickness of 5 μm on the Reichert-Jung Supercut 2050 microtome to obtain midcoronal sections. Bone measurements were performed in toluidine blue-stained sections. The sampling site used was 1 mm distal to the growth plate in the secondary spongiosa and extended bilaterally in each section excluding the endocortical surfaces.

Bone formation measurements and calculations. The bone formation rate was calculated as the product of the double labeled surfaces and the MAR. The MAR, expressed as microns per d, was the mean distance between the tetracycline and calcein labels divided by the labeling interval of 10 d. Double labeled surfaces were determined as the bone surfaces with both tetracycline and calcein labels.

Dynamic measurement related to bone resorption. Calcein label perimeters were measured in a growth-adjusted metaphyseal sampling site, and subsequent resorption was calculated as previously described by Westerlind *et al.* (30).

Northern analysis

Total RNA was isolated from conditionally immortalized hMS cells (17) cultured in the presence of 10% FCS using the QIAGEN RNeasy kit in combination with the QiaShredder (QIAGEN, Hilden, Germany). These cells are authentic osteoblastic precursors and are capable of differentiating into osteoblasts or adipocytes with a complete mature phenotype depending on the culture conditions as previously described (17). Ten micrograms of total RNA were separated on a 1.5% (wt/vol) agarose gel containing formaldehyde (2.2 M) using continuous buffer circulation and then transferred to a nylon membrane (Hybond N+, Amersham Pharmacia Biotech, Arlington Heights, IL) by capillary blotting (19). The human cDNA inserts, a β-actin cDNA that hybridized to a 2.0-kb mRNA, a full-length OPG cDNA (GenBank accession no. U94332) that hybridized to a 2.9-kb mRNA, and a RANKL cDNA (GenBank accession no. AB036798) that hybridized to a mRNA species of 2.4 kb were radiolabeled by random primer labeling. Band intensity was quantified by densitometry. Control hybridization with human β-actin verified that equal amounts of RNA were loaded. All experiments were carried out at least three times, and representative blots are shown.

OPG protein assay

The primary marrow stromal cells (MSC) were plated at a density of 5000 cells/cm^2, grown for 7 d in basal proliferation medium containing 10% charcoal-stripped FCS, and treated for up to 21 d in at 37 C in osteogenesis induction medium containing 10% charcoal-stripped FCS, 0.1 μM dexamethasone, 0.05 mM ascorbic acid-2-phosphate, and 10 mM β-glycerol phosphate as previously described (32). At the time of cell harvest, the MSC covered more than 90% of the petri dishes, and more than 90% of cells were viable. MSC were treated with freshly prepared leptin at 100 or 1000 ng/ml (resuspended in phosphate buffer disodium, 120 μM, pH 7.5) or vehicle for 0, 3, 7, or 21 d. The medium (PT-3001, BioWhittaker, Inc., Walkersville, MD) was changed twice per week, and the leptin treatment was repeated at the time of the medium change. The conditioned medium (n = 9 or n = 6) was centrifuged at 1000 rpm to remove cell debris and stored at −80 C until analysis. OPG protein was measured using the OPG ELISA system from Immundiagnostik (Bensheim, Germany) according to the manufacturer's instructions. The protein data were normalized to the total RNA content as a surrogate of the cell number.

Statistical analysis

ANOVA was performed with *post-hoc* Newman-Keuls multiple comparison testing. $P < 0.05$ was considered statistically significant.

Results

OVX induced weight gain

Figure 1 shows the effect of sc leptin therapy *vs.* E2, leptin plus E2, and sham treatment on body weight in rats over 28 d. The expected OVX-induced weight gain was prevented by the administration of estrogen. The leptin-treated, vehicle-treated, and pair-fed groups gained a statistically significant amount of body weight compared with the sham-operated, E2-treated, or E2- plus leptin-treated groups. On the other hand, the leptin-treated group had a significantly lower body weight at the end of the study compared with the vehicle-treated group ($P < 0.05$), but it was not significantly different from that of the pair-fed group. Thus, the dose and route of administration we used for leptin treatment were partially effective in modulating appetite and limiting weight gain. However, the rate of body weight gain in the leptin-treated group was intermediate between the vehicle and the E2-treated groups, therefore avoiding any bias due to starvation on bone metabolism. Food intake in the leptin-treated group was not statistically significantly different from that in the vehicle-treated group (Fig. 2). Food intake in the leptin plus E2 group was significantly lower compared with those in the vehicle and sham group during the first 10 d of the study; however, these differences resolved with time. The leptin and leptin plus E2 groups had significantly higher levels of plasma leptin than the control ($P < 0.05$), but levels remained within the physiological range (Table 1). Levels of leptin in the cerebrospinal fluid were not statistically significant different between the groups (Table 1).

Leptin partially prevented OVX-induced trabecular bone changes

Cancellous bone volume (BV/TV) was reduced by 60% 1 month after OVX in vehicle-treated rats compared with that in sham-operated rats, whereas this decrease was totally prevented by E2 treatment (Fig. 3) as well as the combination of leptin and E2. Leptin treatment alone was also effective in attenuating the decrease in BV/TV (69% higher than vehicle) and had a protective effect on trabecular bone architecture, with preservation of trabeculae number (Fig. 4). Trabecular thickness in the leptin-treated group was not significantly different from that in the vehicle-treated group (data not shown).

We did not observe any changes after leptin treatment in the assessed cellular parameters, the number of osteoclasts (osteoclast surface/bone surface), the number of osteoblasts (osteoblast surface/bone surface and labeled surface/bone surface), or the activity of osteoblasts [MAR and bone formation rate (BFR)/bone surface], compared with those in the vehicle-treated group (Table 2). These data suggest that leptin effects on OVX-induced trabecular bone loss could be mediated by impairing osteoclast activity, rather than by altering osteoclast number or bone formation.

Leptin prevented the OVX-induced increase in BFR

In cortical bone, leptin therapy prevented the OVX-induced increase in BFR, an effect similar to that observed

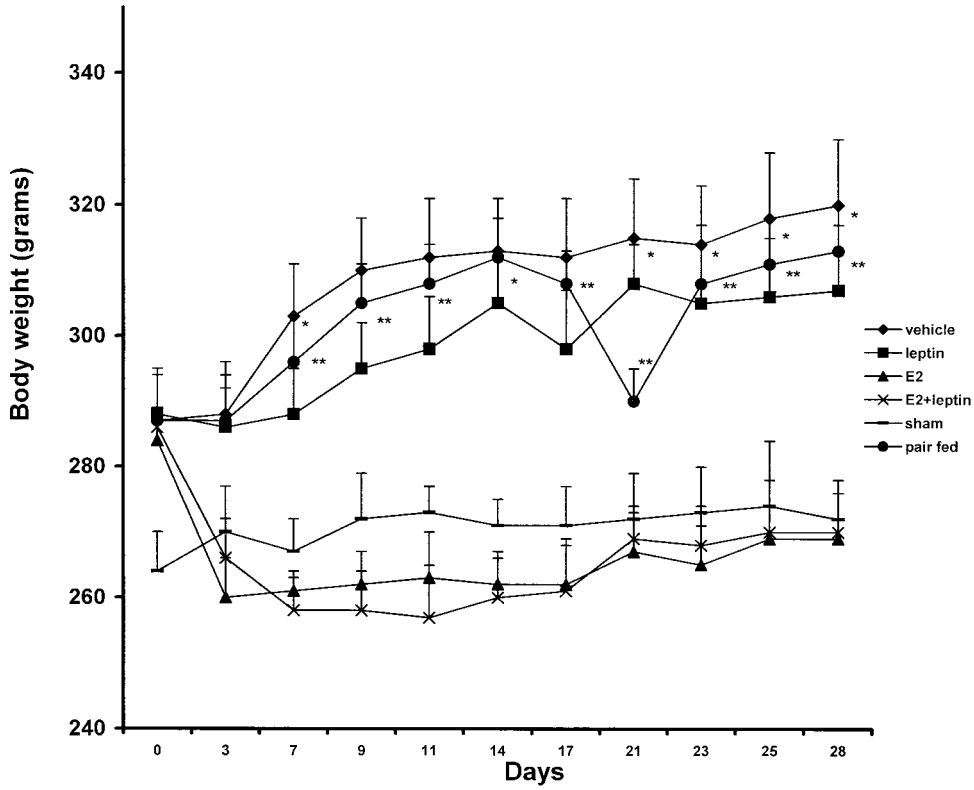

FIG. 1. Effect of sc leptin administration *vs.* estrogen, leptin plus estrogen, pair feeding, and sham operation on rat body weight over 28 d. *, $P < 0.05$; **, $P < 0.001$ (*vs.* vehicle).

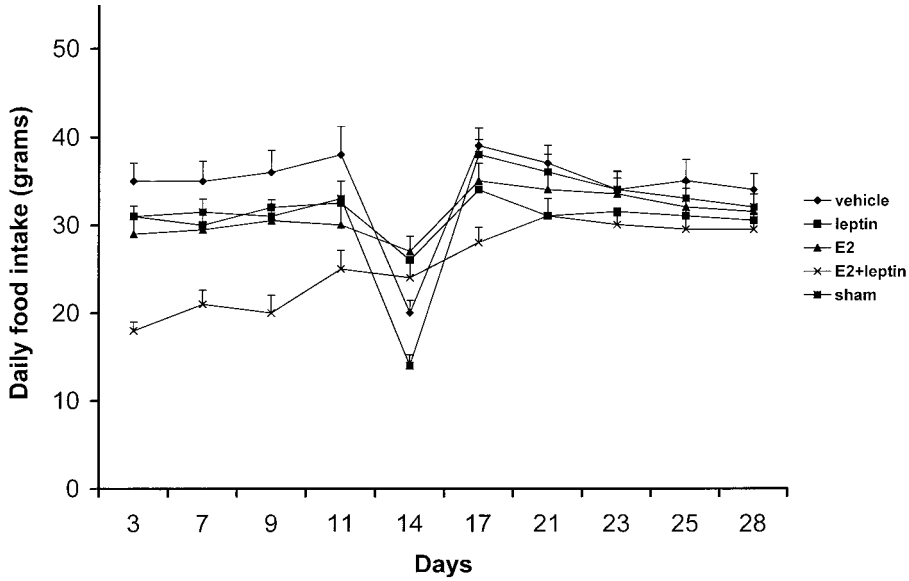

FIG. 2. Effect of sc leptin administration *vs.* estrogen, leptin plus estrogen, pair feeding, and sham operation on rat food intake over 28 d.

TABLE 1. Plasma and CSF leptin levels (nanograms per ml)

	Plasma			CSF	
	d 0	d 15	d 28	d 0	d 28
Control	1.5 ± 0.23			0.19 ± 0.23	
Vehicle		1.31 ± 0.17	1.14 ± 0.1		0.2 ± 0.02
Leptin		2.23 ± 0.23[a]	2.77 ± 0.39[a]		0.22 ± 0.05
E_2		1.52 ± 0.14	1.52 ± 0.2		0.23 ± 0.03
E_2 + leptin		1.91 ± 0.25	2.75 ± 0.66[a]		0.19 ± 0.02
Pair-fed		1.50 ± 0.21	1.74 ± 0.21		0.2 ± 0.03
Sham		1.42 ± 0.09	1.19 ± 0.14		0.18 ± 0.05

n = 8 rats/group.
[a] $P < 0.05$ *vs.* vehicle.

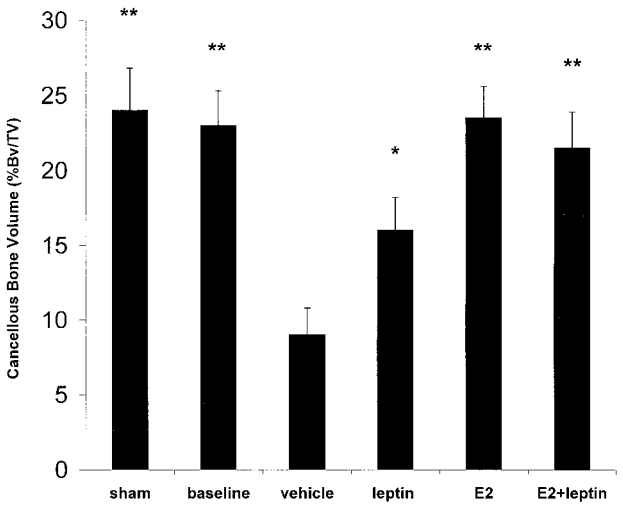

FIG. 3. Effect of sc leptin administration *vs.* estrogen, leptin plus estrogen, pair feeding, and sham operation on rat cancellous bone volume (%BV/TV) after 28 d of therapy. *, $P < 0.05$; **, $P < 0.001$ (*vs.* vehicle).

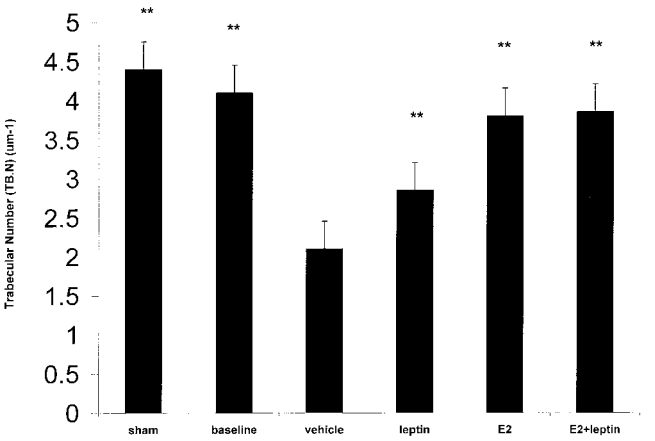

FIG. 4. Effect of sc leptin administration *vs.* estrogen, leptin plus estrogen, and sham operation on rat trabecular number (Tb.N; microns^{-1}) after 28 d of therapy. *, $P < 0.05$; **, $P < 0.001$ (*vs.* vehicle).

with E2 treatment (Fig. 5). As in cancellous bone, the combination of E2 and leptin further decreased cortical BFR compared with values in estrogen-treated OVX and sham rats. This effect was due to a decrease in MAR.

Leptin induced OPG gene expression and protein secretion in stromal cells in vitro

As leptin was able to partially prevent OVX-induced bone loss, which, in turn, is the result of accelerated bone turnover, leptin may alter the balance between the levels of RANKL and its decoy receptor OPG, two key effectors of osteoclast differentiation and activation as well as bone remodeling

TABLE 2. Trabecular and cortical bone parameters

	Trabecular			Cortical	
	Oc.s/BS (%)	Ob.s/BS (%)	MAR (μm/day)	BFR/BS ($\mu m^3/\mu m^2$·d)	MAR (μm/d)
Control	8.9 ± 1.2	3.5 ± 1.7			
Vehicle	9.8 ± 1.1	10.5 ± 1.6	0.9 ± 0.05	52.2 ± 9.4	1.34 ± 0.2
Leptin	10.4 ± 1.0	11.6 ± 3.1	0.89 ± 0.07	51.3 ± 9.7	1.25 ± 0.06
E_2	6.5 ± 1.1[a]	1.52 ± 0.7[a]	0.83 ± 0.06	13.8 ± 4.8[a]	1.25 ± 0.13
E_2 + leptin	5.8 ± 1.1[a]	0.4 ± 0.3[b]	0.93 ± 0.15	5.8 ± 1.6[b]	0.69 ± 0.11
Sham	7.1 ± 1.3	6.2 ± 1.9[a]	0.88 ± 0.03	13.6 ± 3.3[a]	0.59 ± 0.07

Oc.S/BS, Osteoclast surfaces; Ob.S/BS, osteoblast surfaces; MAR, mineral apposition rate; BFR/BS, bone formation rate, tissue level. n = 8 rats/group.
[a] $P < 0.05$ *vs.* vehicle.
[b] $P < 0.001$ *vs.* vehicle.

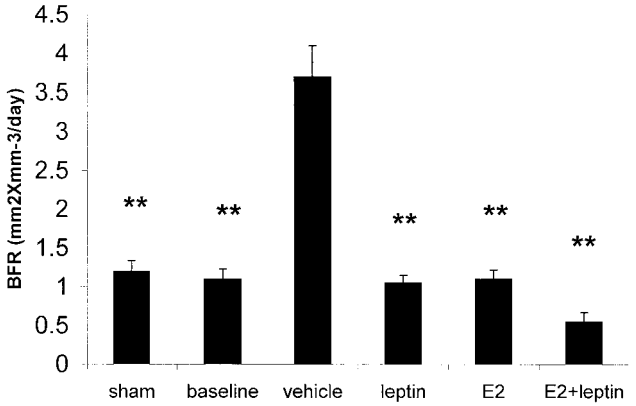

FIG. 5. Effect of sc leptin administration *vs.* estrogen, leptin plus estrogen, pair feeding, and sham operation on BFR (square millimeters × millimeters^{-3} per d) after 28 days of therapy. *, $P < 0.05$; **, $P < 0.001$ (*vs.* vehicle).

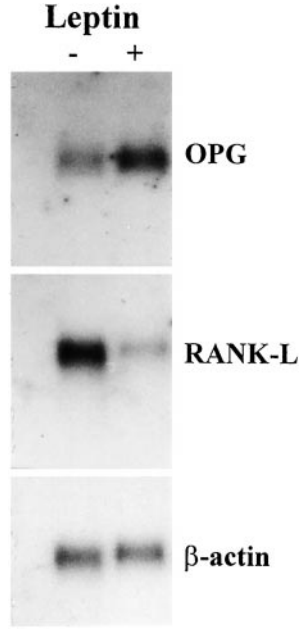

FIG. 6. Northern blot analysis of the regulation of OPG and RANKL mRNA steady state levels in conditionally immortalized hMS cells by leptin. A, Ten micrograms of total RNA were isolated from hMS cells treated with leptin (2.4 μg/ml) for 24 h. *Upper panel*, OPG mRNA (2.9 kb); *middle panel*, RANKL mRNA (2.4 kb); *lower panel*, control hybridization with the housekeeping gene β-actin (2.0 kb).

(20–22). In fact, we (19) and others (31) demonstrated that hMS cells expressed functional leptin receptors (19) as well as OPG and RANKL (32). Moreover, leptin stimulated osteoblastic differentiation of hMS cells (19), and the OPG/RANKL ratio increased with acquisition of the osteoblastic phenotype (33). Thus, we next investigated the hypothesis that leptin may mediate some of its effects on bone remodeling through the OPG/RANKL pathway. Leptin differentially regulated this pathway by stimulating OPG mRNA levels (by 2.5-fold) and decreasing RANKL mRNA levels (by 3.0-fold) in conditionally immortalized hMS cells (Fig. 6). To confirm OPG regulation by leptin in normal nontransformed primary MSC, we employed the MSC assay. OPG secretion by MSC increased 6-fold over the course of 21 d ($P < 0.001$, by ANOVA), and leptin dose-dependently enhanced OPG protein secretion (by 15–31% at a concentration of 1 mg/ml) at each of the three time points ($P < 0.005$ after 3 d, by ANOVA; $P < 0.01$ after 7 and 21 d, respectively, by ANOVA; Fig. 7).

Discussion

Our data show that administration of leptin to rats was effective in reducing trabecular bone loss and trabecular architectural changes associated with estrogen deficiency. The dose of leptin we selected based on shorter studies (25–27), was designed to exert physiological effects without causing severe anorexia and weight loss. Plasma leptin levels remained within physiological levels. Whether higher doses of leptin administered for a longer period of time may have an even more potent effect on preventing bone loss after OVX in rats remains unknown.

Obesity appears to have a protective effect on osteoporosis in humans and inversely correlates with the risk of bone fractures (33, 34). Even though the effects of estrogen and body weight-bearing are partially responsible for these actions, other factors closely related to body fat may have a crucial effect on the increased bone density characteristic of obese humans. Ozata *et al.* (35) have recently shown that four patients with human leptin deficiency caused by a missense mutation had alterations in PTH and calcium metabolism, and one of these patients had a marked decrease in BMD, which it is in contrast to nonleptin-deficient obese individuals, who are protected against osteoporosis. Farooqi *et al.* (36) also reported that 1 yr of leptin therapy in a child with congenital leptin deficiency induced a decrease in body fat by 15.6 kg and a decrease in lean mass by 0.82 kg, but

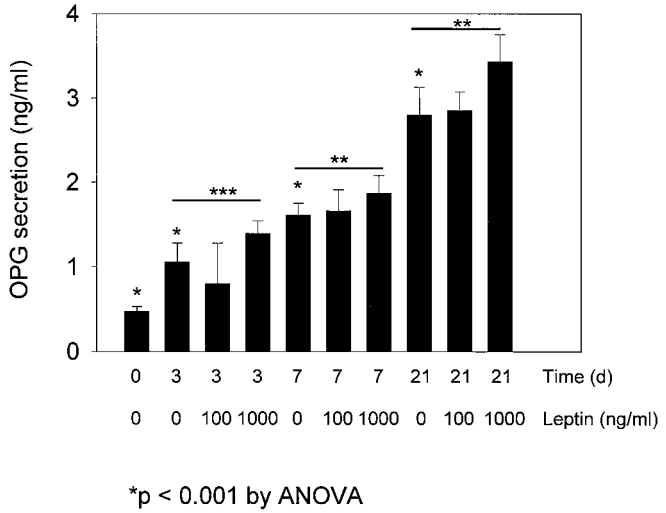

FIG. 7. Effect of leptin on OPG protein secretion. Conditioned medium was harvested from MSC cultured at 37 C in osteogenesis induction medium in the presence of 10% charcoal-stripped FCS and treated with freshly prepared leptin at 100 or 1000 ng/ml or vehicle for 0, 3, 7, and 21 d. OPG protein was measured using an OPG ELISA system. Data are the mean ± SD and are normalized to the total RNA content as a surrogate of the cell number. *, $P < 0.001$ for OPG secretion over time in untreated MSC (n = 9 on d 0, 3, and 7; n = 6 on d 21); **, $P < 0.01$ for leptin dose responses at 7 and 21 d; ***, $P < 0.005$ for leptin dose response on d 3 (all by ANOVA).

increased bone mineral mass by 0.15 kg. Thomas *et al.* (37) recently reported that serum leptin levels were directly related to BMD in women in both weight-bearing and non-weight-bearing sites, suggesting that leptin may mediate at least part of the protective effect of fat mass on the skeleton.

This hypothesis is further supported by our *in vitro* data showing that leptin significantly increased OPG mRNA steady state levels and protein production, whereas it decreased RANKL mRNA expression by human marrow stromal cells. Transgenic mice overexpressing OPG (21) have generalized increased bone mass (osteopetrosis) due to decreased osteoclastogenesis and bone resorption, whereas OPG-deficient mice have severe osteoporosis due to increased osteoclastogenesis and bone resorption (38). *Takahashi et al.* (39) showed that leptin induced MAPK-dependent cell proliferation in the mouse embryonic cell line C3H10T1/2. Interestingly, activation of MAPK enhanced osteoblastic differentiation, whereas it induced phosphorylation of PPARγ, which prevented adipogenesis. Indeed, we showed that leptin inhibited adipocyte differentiation of hMS cells, whereas it promoted osteoblastic differentiation (18). On the other hand, we demonstrated that the progression along this pathway resulted in increased OPG mRNA levels and decreased RANKL mRNA in MSC (40). Thus, regulation of the OPG/RANKL pathway by leptin may be related to its differentiating effect on osteoblastic precursor cells. Indeed, Takahashi *et al.* (40) showed that leptin induced mitogen-activated protein kinase-dependent cell proliferation in the mouse embryonic cell line C3H10T1/2. Interestingly, activation of MAPK enhanced osteoblastic differentiation, whereas it induced phosphorylation of PPARγ, which prevented adipogenesis. In accordance with our results, Holloway *et al.* (41) recently reported that leptin was able to inhibit osteoclastogenesis *in vitro* through an increase in OPG mRNA and protein expression in human peripheral blood mononuclear cells and a decrease in RANKL mRNA expression. A plausible teleological explanation for the observed bone changes seen in rats may be that high levels of circulating leptin are sensed by early bone marrow precursor cells as a signal that extra fat is being accumulated, and there will be a need for bone formation to bear this extra fat. Interestingly, in humans, physiological situations such as puberty and pregnancy, characterized by body weight gain, are associated with increased leptin levels (13, 42, 43).

Recently, Ducy *et al.* (23) showed that *ob/ob* and *db/db* mice have an increased bone mass despite hypogonadism and hypercortisolism. Furthermore, the intracerebroventricular infusion of leptin caused bone loss in leptin-deficient mice, suggesting that leptin is a potent inhibitor of bone formation acting through the central nervous system. Contrary to these studies, Steppan *et al.* (24) showed that leptin administration led to a significant increase in femoral length, total body bone area, bone mineral content, and bone density in *ob/ob* mice compared with those in vehicle-treated controls. This increase in total body bone mass was the result of an increase in both trabecular and cortical bone mass. Several factors may explain these discrepancies. The deficiency of leptin during development (24) may somehow impair postnatal development and response to leptin administration. In fact, there is growing evidence that leptin largely produced by the placenta (44) could play a significant role during fetal growth (45).

Supporting the hypothesis that leptin may have positive effects on bone metabolism, a recent study by Ogueh *et al.* has shown that there is a negative correlation between fetal leptin levels and levels of cross-linked carboxyl-terminal telopeptide of type I collagen, a marker of bone resorption (46). They postulated that leptin decreased bone resorption with the overall effect of increasing bone mass in humans. They concluded that leptin might play a role in fetal bone metabolism as part of its effect on fetal growth and development.

The route of leptin administration is an obvious difference between Ducy's studies and others. Thus, to reconciliate these different results, we postulated that leptin effects on bone may result from a balance between negative central effects and positive peripheral effects. In fact, the concept of leptin resistance (47) that could occur with the development of obesity despite increasing levels of leptin is at least partly related to the observed decrease in blood-brain barrier permeability (48–50). The serum levels of leptin may also modulate this balance, as we observed that serum leptin levels were correlated with BMD in women, but not in men, with leptin levels 2- to 3-fold higher in the former.

Ducy *et al.* (23) have recently shown that *ob/ob* and *db/db* mice have increased bone mass despite hypogonadism and hypercortisolism. Moreover, the intracerebroventricular infusion of leptin caused bone loss in leptin-deficient mice. The researches postulated that leptin is a potent inhibitor of bone formation acting through the central nervous system. The findings of the present study would suggest that leptin has a protective peripheral skeletal effect, and it may be that overall leptin effects on bone result from a balance between negative central effects and positive direct peripheral effects

depending on serum leptin levels or blood-brain barrier permeability. There may be also important species differences in rats compared with mice with regard leptin action on bone.

Anorexia nervosa (AN) is characterized by a very similar hormonal profile to that of *ob/ob* mice with hypercorticism, hypogonadism, and a dramatic decrease in serum leptin levels. However, the features of AN include osteoporosis, which is more severe than the bone loss secondary to hypothalamic amenorrhea and is critically dependent upon nutritional factors in addition to the degree or duration of estrogen deficiency itself (51). Interestingly, Karlsson *et al.* (52) recently reported that AN occurring during adolescence impaired both mineral accrual, as measured by volumetric BMD (vBMD), and bone size. More importantly, whereas reduced volumetric BMD may be related to estrogen deficiency, reduced bone size no longer existed after adjusting for fat and lean masses. Weight, but not estrogen use, is a significant predictor of BMD in anorexic women at all skeletal sites (53). Overall, these data suggest that the dramatic decline in leptin levels observed during AN may be one of the major hormonal factors in the pathogenesis of the associated bone fragility through diminishing cortical BFR and skeletal growth.

Based on our data, we believe that leptin may play an important protective role in bone metabolism by inhibiting bone resorption. Consequently, it could be a major contributing factor to the protective effect of obesity against osteoporosis. The results obtained from these studies may have significant implications in understanding the mechanisms involved in the osteogenic effects exerted by obesity as well as for the therapy of osteoporosis. Studies are needed to evaluate whether leptin therapy of postmenopausal women can prevent bone loss-associated estrogen deficiency.

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