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Leptin administration does not prevent the bone mineral metabolism changes induced by weight loss

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ABSTRACT

The objective was to examine the effects of weight loss and leptin administration following weight loss on calciotropic hormones and bone turnover. This was a prospective, single-blinded study of 12 subjects (8 women, 4 men; 2 nonobese, 10 obese; age range, 19-46 years) who were studied on an inpatient basis while maintaining their usual weight [$Wt_{initial}$] and during maintenance of 10% weight loss while receiving twice-daily injections of either a placebo [$Wt_{-10\%P}$] or replacement doses of leptin [$Wt_{-10\%L}$]. The main outcome measures were markers of bone formation (bone alkaline phosphatase and procollagen type 1 amino terminal propeptide) and resorption (N-telopeptide) as well as parathyroid hormone, calcium, and 25-hydroxy vitamin D measured from fasting morning serum. As expected, serum leptin declined with weight loss. Bone alkaline phosphatase decreased by 12.3% ± 3.9% between $Wt_{initial}$ and $Wt_{-10\%P}$ and remained suppressed after leptin administration (both $P < .01$ compared with baseline). N-telopeptides increased by 37.2% ± 11.3% from $Wt_{initial}$ to $Wt_{-10\%L}$ ($P < .01$). Procollagen type 1 amino terminal propeptide, parathyroid hormone, calcium, and 25-hydroxy vitamin D did not change. These results suggest that both decreased bone formation and increased bone resorption underlie bone loss associated with weight loss. Leptin administration did not prevent the uncoupling of bone remodeling that accompanies weight loss.

1. Introduction

Dietary weight loss in both lean and obese subjects is associated with reductions in total body and regional bone mass [1,2]. Studies examining bone turnover have linked weight loss to an increase in bone-resorption markers and to variable changes in markers of bone formation [1,2]. The signals that mediate these changes are not fully understood but likely include changes in levels of circulating hormones that occur with a reduction in body weight.

Recently, it has been recognized that the adipocyte-derived hormone leptin modulates bone metabolism [3,4]; however, conflicting data exist as to how and to what extent leptin influences bone formation and resorption. In vitro, leptin has been shown to stimulate osteoblast and chondrocyte formation and inhibit osteoclastogenesis [5,6]. In vivo, peripheral leptin administration to leptin-deficient animals resulted in reduced bone fragility [6]. Intracerebroventricular leptin infusion to leptin-deficient and wild-type mice led to restoration of cortical and cancellous bone volume in the femur and vertebrae in one study [7], but led to decreased bone mass

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following a similar procedure in others [8,9]. Conflicting data also exist for human studies of leptin administration in low leptin states (ie, congenital leptin deficiency, lipodystrophy, and hypothalamic amenorrhea), where varying effects on bone have been reported, ranging from net bone formation to no change at all [10-14].

To further understand the role of leptin in bone metabolism, we prospectively studied bone metabolic markers under 3 conditions: maintenance of initial weight, maintenance of 10% weight loss while receiving injections of placebo, and maintenance of 10% weight loss while receiving injections of leptin. Serum markers of bone formation (bone alkaline phosphatase [BSAP] and amino-terminal propeptide of type I procollagen [P1NP]) and bone resorption (N-telopeptide [NTX]) were quantified, in addition to parathyroid hormone (PTH), calcium (Ca), and 25-hydroxy vitamin D (25D). We hypothesized that a net increase in bone resorption, reflected by increased NTX as well as decreased BSAP and P1NP, would occur following weight loss. Furthermore, we believed that leptin administration to subjects maintaining a reduced body weight would lead to increases in P1NP and BSAP, as well as decreases in NTX, reversing the weight-loss–associated changes in bone turnover.

2. Subjects and methods

Studies were approved by the Institutional Review Board of The New York Presbyterian Medical Center and are consistent with guiding principles for research involving humans [15]. Twelve inpatient subjects were studied: 4 men, 8 women; 2 nonobese, 10 obese; age range, 19 to 46 years (mean, 30.3 ± 8.7 years) (Table 1). All women were premenopausal and had regular menses. As described previously [16], subjects were fed a liquid formula diet (40% of calories as fat [corn oil], 45% as carbohydrate [glucose polymer], and 15% as protein [casein hydrolysate]; caloric density = 1.25 digestible kcal of energy per gram]) plus vitamin and mineral supplements (800 mg Ca and 400 IU vitamin D per day) in quantities sufficient to maintain a stable weight (defined as the absolute value of the slope of the line relating weight [kilograms] to time [days] less than 0.01 kg over a 2-week period, measured on an electronic scale [Model 6002; Scaletronix, White Plains, NY] to the nearest 0.1 kg). This weight plateau is designated as $Wt_{initial}$. Each subject's aerobic fitness was measured by bicycle ergometry upon admission. Supervised exercise (treadmill walking or stationary bicycling) was performed 3 times per week throughout the duration of the study at specified intensities and durations that were adjusted to maintain each subject's anaerobic threshold at his or her initial level throughout the study [16,17].

Following completion of studies (described below) at $Wt_{initial}$, subjects were provided with 800 kcal/d of the same liquid formula diet until they had lost approximately 10% of $Wt_{initial}$. Once 10% weight loss had been achieved, intake was adjusted upward until subjects were again weight stable as described above. Subjects then received 5 weeks of twice-daily (8:00 AM and 6:00 PM) subcutaneous injections of saline, designated as $Wt_{-10\%P}$, or recombinant human leptin (A-100, provided by Amgen, Thousand Oaks, CA; patent was taken over by Amylin Pharmaceuticals, San Diego, CA, and the same formulation was relabeled *Metreleptin*), designated as $Wt_{-10\%L}$. Studies were again completed, and subjects underwent a 2-week washout period during which they received no intervention. They were then crossed over to receive the other intervention for 5 weeks, after which studies were performed a final time. Ten subjects received placebo followed by leptin, and 2 subjects received leptin followed by placebo. The imbalance in the order of treatment occurred because the first 4 subjects were not randomized in the pilot phase of the leptin studies [16]; consequently, there was a predominance of subjects receiving placebo first. Initial total daily leptin doses were 0.08 mg/kg fat mass per dose in male subjects and 0.14 mg/kg fat mass per dose in female subjects [16]. Circulating leptin concentrations at 8:00 AM were measured weekly in subjects receiving leptin, and dosages were adjusted until circulating leptin concentrations were similar to those measured at 8:00 AM at $Wt_{initial}$ [16,17]. Subjects remained on a diet isocaloric to that initially demonstrated necessary to maintain a 10% reduced body weight throughout the leptin and placebo arms of the study. Fasting (8:00 AM) morning blood samples were drawn at $Wt_{initial}$, $Wt_{-10\%P}$, and $Wt_{-10\%L}$; and serum levels of BSAP, P1NP, NTX, PTH, Ca, and 25D were measured.

Table 1 – Levels of bone mineral metabolic markers before ($Wt_{initial}$) and after ($Wt_{-10\%P}$) weight loss and following leptin administration ($Wt_{-10\%L}$)

	$Wt_{initial}$	$Wt_{-10\%P}$	$Wt_{-10\%L}$
Weight (kg)	115.9 ± 11.2	101.1 ± 9.8†	100.8 ± 10.3†
Leptin (ng/mL)	32.1 ± 4.5	21.4 ± 3.4†	38.8 ± 5.7*‡
P1NP (μg/L)	49.3 ± 5.1	46.4 ± 4.0	49.0 ± 5.3
BSAP (U/L)	26.2 ± 1.2	22.7 ± 1.2†	22.3 ± 1.5†
NTX (nmol/L BCE)	11.9 ± 0.8	13.8 ± 1.0	15.7 ± 1.3*
PTH (pg/mL)	31.6 ± 5.5	32.4 ± 3.7	31.9 ± 6.6
Ca (mg/dL)	9.3 ± 0.1	9.3 ± 0.2	9.1 ± 0.1
25D (ng/mL)	15.8 ± 1.3	15.8 ± 1.7	15.1 ± 1.8

P1NP = procollagen type I amino terminal propeptide; BSAP = bone-specific alkaline phosphatase; NTX = N-telopeptides; PTH = parathyroid hormone; Ca = calcium; 25D = 25 hydroxy vitamin D.
* $P < .05$ compared with $Wt_{initial}$.
† $P < .01$ compared with $Wt_{initial}$.
‡ $P < .01$ compared with $Wt_{-10\%P}$.

2.1. Analytical methods

Serum was drawn and stored at –70°C until assayed in a central laboratory (Clinical Research Center at Columbia University Medical Center). An enzyme-linked immunosorbent assay was used to determine serum NTX (Inverness Medical, Princeton, NJ). Intra- and interassay precision was 4.6% and 6.9%, respectively. Serum P1NP was measured using a radioimmunoassay (Orion Diagnostica, Espoo, Finland) with intra- and interassay precision of 6.5% and 8.3%, respectively. An enzyme immunoassay (Quidel, San Diego, CA,) with intra- and interassay coefficients of variation (CVs) of 3.9% and 7.6%, respectively, was used to determine serum BSAP. Leptin levels were measured using an enzyme-linked immunosorbent assay (Diagnostic Systems Laboratories, Webster, TX) with intra- and interassay CVs of less than 5%. Serum PTH (1-84) was measured using an immunoradiometric assay (Nichols

Institute, San Juan Capistrano, CA) with a detection limit of 0.5 pmol/L and intra- and interassay CVs of 4.8% and 6.8%, respectively. Serum Ca was measured by the standard autoanalyzer method (Hitachi 912, Roche Diagnostics, Indianapolis, IN). Serum 25D was measured using a radioimmunoassay kit (DiaSorin, Stillwater, MN) with intra- and interassay CVs of 8.6% and 9.1%, respectively.

2.2. *Statistical analysis*

Data were analyzed using a linear mixed model analysis of within-subject differences for the fixed effect of weight condition ($Wt_{initial}$, $Wt_{-10\%P}$, and $Wt_{-10\%L}$) with continuous covariates for sex and initial somatotype (obese or nonobese), random effects for subject and error, and a compound symmetry covariance structure. Between-condition comparisons were made from the mixed model estimated means and 95% confidence intervals when the fixed effect of weight condition tested with a *P* value less than .05. A compound symmetry covariance structure was empirically determined to best fit the autocorrelation of the within-subject repeated measures across the 6 dependent variables before testing. The relative number of obese vs never-obese subjects in this study reflects that of the subject population expressing an interest in these studies. Our previous studies of healthy never-obese and obese subjects have not demonstrated any significant somatotype-related differences in the metabolic response to attempts to sustain weight loss [18-20]. The preponderance of obese subjects in this population did not permit analyses of data using initial somatotype as a covariate. Results are reported as mean ± SEM.

3. Results

Subjects required an average of 8 weeks to reduce their weight by 10% (range, 5-10 weeks) and another 4 to 6 weeks to achieve weight stabilization. Leptin administration to subjects ingesting the same number of calories that were required to maintain their weight at $Wt_{-10\%P}$ resulted in no significant further reduction in body weight (Table 1). Leptin levels declined significantly with 10% weight loss and increased significantly with leptin administration. Leptin concentrations were significantly higher at $Wt_{-10\%L}$ as compared with $Wt_{initial}$ ($P < .05$), but were still within physiological range. Leptin levels were greater in the obese subjects (32.1 ± 4.5 ng/mL) than in the 2 nonobese subjects (7.5 and 8.9 ng/mL) ($P < .05$).

Amino-terminal propeptide of type I procollagen remained in the reference range and did not change throughout the study (Table 1). Although BSAP remained in the reference range during the study, there was a significant decrease of 12.3% ± 3.9% after weight loss (26.2 ± 1.3 to 22.7 ± 1.3 U/L, $P = .0063$). This decline was not affected by leptin administration. Conversely, there was a significant increase of 37.2% ± 11.3% in serum NTX from $Wt_{initial}$ to $Wt_{-10\%L}$ (11.9 ± 1.0 to 15.7 ± 1.1 nmol/L BCE, $P = .011$) (Fig. 1). The increases in serum N-telopeptides from $Wt_{initial}$ to $Wt_{-10\%P}$ and from $Wt_{-10\%P}$ to $Wt_{-10\%L}$, however, were not statistically significant ($P = .178$ and $P = .167$, respectively; Table 1). Controlling for sex and initial somatotype in the linear mixed models did not change the pattern of statistical significance among the treatment conditions; neither did reanalysis of data after exclusion of the 2 lean subjects. There was no difference in PTH, Ca, and 25D between the 3 study conditions (Table 1).

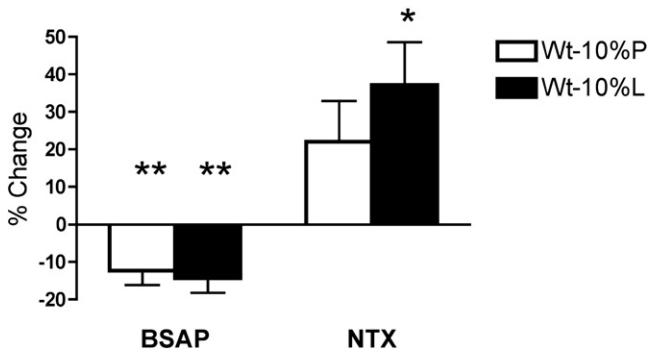

Fig. 1 – Percentage change of serum NTX and BSAP after weight loss and leptin administration. *P < .05 and **P < .01 compared with $Wt_{initial}$.

4. Discussion

Weight reduction is associated with bone loss in both lean and obese individuals. The role of leptin in the bone loss that accompanies weight reduction has continued to be unclear, favoring bone formation in some studies and bone resorption in others [7-9,21]. The major finding of this study is that, contrary to our initial hypothesis, leptin replacement in subjects maintained at approximately 10% less than their usual weight did not reverse weight-loss–associated changes in bone mineral markers (decreased circulating levels of BSAP and increased NTX levels).

Our findings are consistent with others that noted an increase in markers of bone resorption following weight loss [1,2,22,23]. Redman et al [1] found that body weight reductions of 10.4% and 13.9% after 6 months of low and very low calorie diets, respectively, were accompanied by decreases in BSAP of 16% in the former and increases in NTX of 31% in the latter. Fleischer et al [22], in patients post Roux-en-Y gastric bypass, observed a 57% increase in NTX after a 17% weight loss 6 months postoperatively and a 106% increase in NTX after a 34% decrease in weight 12 months postbypass. The larger degree of change in NTX after bypass is likely a reflection of the larger decrease in weight as opposed to the duration of weight loss, as our study and that of Redman et al found smaller degrees of change in NTX with less weight loss. After Roux-en-Y gastric bypass, Bruno et al [23] observed inverse correlations between serum levels of leptin and levels of NTX, suggesting that an increase in levels of leptin is associated with a decrease in levels of NTX and therefore may have a bone protective effect. In our study, however, NTX concentrations did not decrease with administration of leptin.

The effects of leptin on bone metabolism have been shown to be both centrally and peripherally mediated. Centrally, leptin increases the expression of cocaine amphetamine regulated transcript, which regulates the expression of receptor activator of nuclear factor–κB ligand) [4]. Receptor

activator of nuclear factor–κB ligand activates osteoclasts, thereby promoting bone resorption. In addition, hypothalamic activation of leptin leads to increased sympathetic nervous system activity, which acts on the osteoblast to regulate born formation [4]. In the periphery, leptin has been shown to act at the osteoblast, promoting cell proliferation, collagen synthesis, and mineralization [5]. These osteoblasts were also noted to increase interleukin 6 and osteoprotegerin expression in response to leptin, both of which stimulate osteoclast activity to facilitate total bone remodeling [5]. In vivo studies determined that central administration of leptin to leptin-deficient (*ob/ob*) mice resulted in a reduction in their high bone mass [9], whereas peripheral administration of leptin to these mice led to an increase in bone formation and growth [21]. Results from leptin administration in other mouse models indicate a bone-promoting effect as well [3].

Our findings are congruent with some studies of mouse models [9], but they are in contrast to some of the few studies that examined the effects of leptin administration on skeletal metabolism in humans. Welt et al [10] studied the effects of leptin administration to 8 nonobese women with hypothalamic amenorrhea and found an increase in BSAP and osteocalcin levels after 3 months of treatment. In contrast, Simha et al [11] examined the effects of leptin therapy on 2 patients with lipodystrophy and found no changes in levels of Ca, PTH, BSAP, osteocalcin, or NTX after 6 to 8 months of treatment, which were similar to our findings. These variations between studies may be a reflection of differences in diet composition, physical activity, or physiological states such as insulin sensitivity, as these variables were not controlled. Changes in body weight as a result of leptin administration or duration of leptin treatment could have contributed to the variations as well.

Although it is clear that obese rodents are less responsive to exogenously administered leptin than never-obese rodents, it is not clear whether there is actual leptin resistance in obese human subjects [24]. Studies of obese and never-obese human subjects at their usual weight have reported little, if any, effect of leptin administration, even at supraphysiological doses, on body weight [25]. In contrast, Westerterp-Plantegna et al [26] reported that administration of pegylated leptin to subjects who were maintaining a reduced weight, rather than losing it, increased satiation and energy expenditure. Although most of our subjects were still obese after 10% weight loss, in other studies performed with this protocol, subjects were responsive to leptin treatment in terms of reversal of decreased energy expenditure, decreased sympathetic nervous system activity, and changes in thyroid function [16,27]. It therefore seems unlikely that the lack of effect of leptin on bone turnover markers in this study was due to residual leptin resistance. However, we cannot rule out the possibility that different thresholds of leptin resistance may exist for various physiological functions of this hormone.

The lack of significant change in levels of Ca, 25D, and PTH was not unexpected. The dose of Ca provided was slightly less than the recommended daily allowance of 1000 mg. The dose of vitamin D administered, although still the recommended daily allowance for adults, has been shown to be less than what is actually required for maintenance of normal 25D levels [28]. The lack of significant changes in these values from baseline, along with the fact that bone resorption would be favored by higher serum levels of Ca and 25D, led us to infer that the likelihood of these parameters having any effect on our results is minimal.

The present study is unique in a number of respects. First, the same otherwise healthy subjects were compared before and after weight loss and before and after leptin administration, thus permitting separation of the effects of reduced body energy stores, which are evident both at $Wt_{-10\%P}$ and $Wt_{-10\%L}$, from those of reduced circulating concentrations of leptin, which are evident only at $Wt_{-10\%P}$. This is in contrast to the subjects of other studies, who were leptin insufficient or deficient upon time of treatment [10-13]. Our study subjects did not undergo significant weight loss following leptin administration. This finding is similar to prior studies involving healthy subjects but is in contrast to others that noted weight loss during treatment [10-13]. The effects of weight loss and leptin on energy intake were tested in this study by a design that, by virtue of using a liquid formula diet only, was free of the possible confounding effects of diet composition; and subjects were maintained at the same level of fitness throughout the study, thereby eliminating any effects of exercise on bone remodeling.

There are a number of potential weaknesses to this study. Our participants received only 5 weeks of leptin therapy, which may not have been sufficient time to overcome any changes in bone remodeling. Longer duration of treatment may have led to less bone-resorptive effects or perhaps heightened bone-promoting ones. Another weakness to the study is that only 2 subjects received leptin treatment followed by placebo. To truly understand the effects of leptin beyond the 5 weeks of therapy, more subjects would have been needed in the study group that received leptin before placebo. Although most of our subjects were randomized, 4 subjects were not, as they were enrolled in the study during its pilot phase. The significant increase in NTX observed in the last phase of the study may conceivably be attributable to the longer duration of a weight-reduced state and may not necessarily be a consequence of leptin therapy; further, it may reflect the negative energy balance during leptin administration that is not apparent in body weight measurements [29-31]. We did not measure bone mineral density or other markers of body composition in our study subjects, as significant changes in body composition would not have been likely to occur after 5 weeks of leptin treatment. Other markers that can provide useful information with respect to the effects of leptin on bone metabolism, such as C-telopeptides, tartrate-resistant acid phosphatase 5b, and osteocalcin, were not measured. It should also be noted that subcutaneous administration of leptin twice daily does not mimic the normal pulsatility of leptin secretion. Other changes during weight loss including, but not limited to, decreased mechanical loading and fluctuations in hormonal concentrations such as insulin, estrogen, growth hormone, insulin-like growth factor 1, and cortisol may also have affected some of the changes in bone turnover that were observed. Finally, we did not account for the effect of leptin on these hormones and their subsequent impact on bone metabolism.

In summary, serum markers of bone remodeling activity suggest that both decreased bone formation and increased

bone resorption could underlie the bone loss often seen with weight reduction. Leptin administration does not appear to reverse the uncoupling of bone remodeling that accompanies weight reduction.

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