Published in final edited form as:

Metabolism. 2011 September ; 60(9): 1222–1226. doi:10.1016/j.metabol.2011.02.010.

Leptin Administration Does Not Prevent the Bone Mineral Metabolism Changes Induced by Weight Loss

Rushika Conroy, MD, MS², Monica Girotra, MD¹, Elizabeth Shane, MD¹, Donald J. McMahon¹, Katherine H. Pavlovich², Rudolph L. Leibel, MD², Michael Rosenbaum, MD^{2,*}, and Judith Korner, MD, PhD^{1,*}

¹ Columbia University College of Physicians and Surgeons, Department of Medicine, New York, New York 10032

² Department of Pediatrics, New York, New York 10032

Abstract

Objective—To examine the effects of weight loss and leptin administration following weight loss on calciotropic hormones and bone turnover.

Materials/Methods—This was a prospective, single blinded study of twelve subjects (8 women, 4 men; 2 non-obese, 10 obese; age range 19–46 years) who were studied on an in-patient 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.

Results—As expected, serum leptin declined with weight loss. Bone alkaline phosphatase decreased by $12.3 \pm 3.9\%$ between Wt_{initial} and Wt_{-10%P}, and remained suppressed after leptin administration (both $P < 0.01$ compared to baseline). N-telopeptides increased by $37.2 \pm 11.3\%$ from Wt_{initial} to Wt_{-10%L} ($P < 0.01$). Procollagen type 1 amino terminal propeptide, parathyroid hormone, calcium and 25-hydroxy vitamin D did not change.

Conclusions—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.

Keywords

leptin; bone; weight loss

© 2011 Elsevier Inc. All rights reserved.

Address correspondence and reprint requests to: Judith Korner, M.D., Ph.D., Columbia University, 650 West 168th St, Black Building, Room 905, New York, NY 10032, Tel: 212-305-3725, Fax: 212-305-2283, jk181@columbia.edu.

*Contributed equally to this manuscript

Clinical Trial Registration Number: NCT00265980

Disclosures: Dr. Korner is a member of the Scientific Advisory Board of Nutrisystem and receives research support from Covidien and NGM Biopharmaceuticals. Dr. Shane receives support from Lilly, Novartis, and Merck Pharmaceuticals.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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 following a similar procedure in others [8, 9]. Conflicting data also exist for human studies of leptin administration in low leptin states (i.e., 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].

In order to further understand the role of leptin in bone metabolism, we prospectively studied bone metabolic markers under three 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, PINP) 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 PINP, would occur following weight loss. Furthermore, we believed that leptin administration to subjects maintaining a reduced body weight would lead to increases in PINP and BSAP, as well as decreases in NTX, reversing the weight loss-associated changes in bone turnover.

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 non-obese, 10 obese; age range 19–46 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/gram], plus vitamin and mineral supplements (800 mg calcium 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 (kg) to time (days) less than 0.01kg over a 2 week period, measured on an electronic scale (Scaletronix Inc, Model #6002 White Plains, NY) to the nearest 0.1kg). This weight plateau is designated as “W_{t initial}”. Each subject’s aerobic fitness was measured by bicycle ergometry upon admission. Supervised exercise (treadmill walking or stationary bicycling) was performed three 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, 18].

Following completion of studies (described below) at $W_{t_{\text{initial}}}$, subjects were provided with 800 kcal/day of the same liquid formula diet until they had lost approximately 10% of $W_{t_{\text{initial}}}$. Once 10% weight loss had been achieved, intake was adjusted upwards until subjects were again weight stable as described above. Subjects then received 5 weeks of twice daily (0800h and 1800h) subcutaneous injections of saline, designated as " $W_{t-10\%P}$," or recombinant human leptin (A-100, provided by Amgen, Inc., Thousand Oaks, CA; patent was taken over by Amylin Pharmaceuticals Inc., San Diego, CA and the same formulation was re-labeled Metreleptin) designated as " $W_{t-10\%L}$ ". 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 two subjects received leptin followed by placebo. The imbalance in the order of treatment occurred because the first four 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/dose in males, and 0.14 mg/kg fat mass/dose in females [16]. Circulating leptin concentrations at 0800h were measured weekly in subjects receiving leptin, and dosages were adjusted until circulating leptin concentrations were similar to those measured at 0800h at $W_{t_{\text{initial}}}$ [16, 18]. 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 (0800h) morning blood samples were drawn at $W_{t_{\text{initial}}}$, $W_{t-10\%P}$ and $W_{t-10\%L}$, and serum levels of BSAP, P1NP, NTX, PTH, Ca, and 25D were measured.

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 inter-assay precision were 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 Corporation, San Diego, CA,) with intra- and interassay 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, Texas) 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/liter 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). Serum 25D was measured using a radioimmunoassay kit (DiaSorin, Inc. Stillwater, MN) with intra- and interassay CVs of 8.6 and 9.1%, respectively.

Statistical Analysis

Data were analyzed using a linear mixed model analysis of within-subject differences for the fixed effect of weight condition ($W_{t_{\text{initial}}}$, $W_{t-10\%P}$ and $W_{t-10\%L}$) with continuous covariates for gender and initial somatotype (obese or non-obese), 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 0.05. A compound symmetry covariance structure was empirically determined to best fit the autocorrelation of the within-subject repeated measures across the six dependent variables prior to testing. The relative

number of obese versus 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 [17, 19, 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 $\pm$ SEM.

Results

Subjects required an average of 8 weeks to reduce their weight by 10% (range 5–10 weeks) and another 4–6 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 to $Wt_{initial}$ ($P<0.05$), but were still within physiological range. Leptin levels were greater in the obese subjects (32.1 ± 4.5 ng/ml) than in the two nonobese subjects (7.5 and 8.9 ng/ml) ($P<0.05$).

P1NP remained in the normal range and did not change throughout the study (Table 1). Although BSAP remained in the normal range during the study, there was a significant decrease of $12.3 \pm 3.9\%$ after weight loss (26.2 ± 1.3 to 22.7 ± 1.3 U/L, $P=0.0063$). This decline was not affected by leptin administration. Conversely, there was a significant increase of $37.2 \pm 11.3\%$ in serum NTX from $Wt_{initial}$ to $Wt_{-10\%L}$ (11.9 ± 1.0 to 15.7 ± 1.1 nM BCE, $P=0.011$) (Figure 1). The increases in serum NTX from $Wt_{initial}$ to $Wt_{-10\%P}$ and from $Wt_{-10\%P}$ to $Wt_{-10\%L}$, however, were not statistically significant ($P=0.178$ and $P=0.167$, respectively; Table 1). Controlling for gender and initial somatotype in the linear mixed models did not change the pattern of statistical significance among the treatment conditions; neither did re-analysis of data after exclusion of the two lean subjects. There was no difference in PTH, Ca, and 25D between the 3 study conditions (Table 1).

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% below 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. 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 [1]. Fleischer et al., in patients post Roux-en-Y gastric bypass, observed a 57% increase in NTX after a 17% weight loss 6 months post-operatively, and a 106% increase in NTX after a 34% decrease in weight 12 months post-bypass [22]. 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 RYGB Bruno et al 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 [23]. 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 CART (cocaine amphetamine regulated transcript), which regulates the expression of RANKL (Receptor activator of nuclear factor kappa-B ligand) [4]. RANKL 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 bone 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], while 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. studied the effects of leptin administration to eight non-obese women with hypothalamic amenorrhea and found an increase in BSAP and osteocalcin levels after three months of treatment [10]. In contrast, Simha et al. examined the effects of leptin therapy on two patients with lipodystrophy and found no changes in levels of Ca, PTH, BSAP, osteocalcin, or NTX after 6–8 months of treatment, which was similar to our findings [11]. 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.

While 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. reported that administration of pegylated leptin to subjects who were maintaining a reduced weight, rather than losing it, increased satiation and energy expenditure [27]. 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, 26]. 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 calcium provided was slightly below the RDA of 1000mg daily. The dose of vitamin D administered, while still the RDA for adults, has been shown to be below what is actually required for maintenance of normal 25D levels [29]. 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 re-modeling.

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 two subjects received leptin treatment followed by placebo. In order 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 prior to placebo. Although most of our subjects were randomized, four 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 [30, 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.

Acknowledgments

We thank the staff of the Irving Center for Clinical Research at The New York Presbyterian Hospital, Columbia University College of Physicians & Surgeons for their invaluable assistance. Leptin A-100 was generously provided by Alex De Paoli, Amgen Inc., Thousand Oaks, California, USA and Metreleptin by Amylin Pharmaceuticals Inc., San Diego, California, USA.

Research Support: This study was supported by NIH grants RR 00645, RR024156, DK64773, P30-DK286687, K24, and AR 052661.

Abbreviations

BSAP	bone alkaline phosphatase
P1NP	amino-terminal propeptide of type I procollagen
NTX	N-telopeptide
PTH	parathyroid hormone
Ca	calcium
D-25D	25-hydroxy vitamin
WL	weight loss
Wt	weight

References

1. Redman LM, Rood J, Anton SD, et al. Calorie Restriction and Bone Health in Young, Overweight Individuals. *Arch Intern Med.* 2008; 168(17):1859–1966. [PubMed: 18809812]
2. Unusi-Rasi K, Sievanen H, Kannus P, et al. Influence of weight reduction on muscle performance and bone mass, structure and metabolism in obese premenopausal women. *J Musculoskelet Neuronal Interact.* 2009; 9(2):72–80. [PubMed: 19516082]
3. Thomas T. The complex effects of leptin on bone metabolism through multiple pathways. *Current Opinion in Pharmacology.* 2004; 4:295–300. [PubMed: 15140423]
4. Karsenty G. Convergence between bone and energy homeostasis: Leptin regulation of bone mass. *Cell Metabolism.* 2006; 4:341–348. [PubMed: 17084709]
5. Gordeladze JO, Drevon CA, Syversen U, et al. Leptin stimulates human osteoblastic cell proliferation, de novo collagen synthesis, and mineralization: Impact on differentiation markers, apoptosis, and osteoclastic signaling. *J Cell Biochem.* 2002; 85(4):825–36. [PubMed: 11968022]
6. Cornish J, Callon KE, Bava U, et al. Leptin directly regulates bone cell function in vitro and reduces bone fragility in vivo. *Journal of Endocrinology.* 2002; 175:405–415. [PubMed: 12429038]
7. Iwaniec UT, Boghossian S, Lapke PD, et al. Central leptin gene therapy corrects skeletal abnormalities in leptin-deficient ob/ob mice. *Peptides.* 2007; 28:1012–1019. [PubMed: 17346852]
8. Elefteriou F, Takeda S, Ebihara K, et al. Serum leptin level is a regulator of bone mass. *Proc Natl Acad Sci U S A.* 2004; 101(9):3258–63. [PubMed: 14978271]
9. Ducy P, Amling M, Takeda S, et al. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell.* 2000; 100(2):197–207. [PubMed: 10660043]
10. Welt CK, Chan JL, Bullen J, et al. Recombinant human leptin in women with hypothalamic amenorrhea. *N Engl J Med.* 2004; 351(10):987–97. [PubMed: 15342807]
11. Simha V, Zerwekh JE, Sakhaee K, et al. Effect of subcutaneous leptin replacement therapy on bone metabolism in patients with generalized lipodystrophy. *J Clin Endocrinol Metab.* 2002; 87(11):4942–5. [PubMed: 12414854]
12. Farooqi IS, Matarese G, Lord GM, et al. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J Clin Invest.* 2002; 110(8):1093–2103. [PubMed: 12393845]
13. Farooqi IS, Jebb SA, Langmack G, et al. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Eng J Med.* 1999; 341:879–84.
14. Chan JL, Mantzoros CS. Role of leptin in energy-deprivation states: normal human physiology and clinical implications for hypothalamic amenorrhea and anorexia nervosa. *Lancet.* 2005; 366:74–85. [PubMed: 15993236]
15. Guiding principles for research involving animals and human beings. *Am J Physiol Regul Integr Comp Physiol.* 2002; 283:R281–R283. [PubMed: 12121837]

16. Rosenbaum M, Murphy EM, Heymsfield SB, et al. Low dose leptin administration reverses effects of sustained weight-reduction on energy expenditure and circulating concentrations of thyroid hormones. *J Clin Endocrinol Metab.* 2002; 87:2391–94. [PubMed: 11994393]
17. Leibel R, Rosenbaum M, Hirsch J. Changes in energy expenditure resulting from altered body weight. *N Eng J Med.* 1995; 332:621–28.
18. Rosenbaum M, Goldsmith R, Bloomfield D, et al. Low dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. *J Clin Invest.* 2005; 115:3579–3586. [PubMed: 16322796]
19. Rosenbaum M, Hirsch J, Gallagher DA, et al. Long-term persistence of adaptive thermogenesis in subjects who have successfully maintained a reduced body weight. *Amer J Clin Nutr.* 2008; 88:906–12. [PubMed: 18842775]
20. Rosenbaum M, Hirsch J, Murphy E, et al. The effects of changes in body weight on carbohydrate metabolism, catecholamine excretion, and thyroid function. *Amer J Clin Nutr.* 2000; 71:1421–32. [PubMed: 10837281]
21. Stepan CM, Crawford DT, Chidsey-Frink KL, et al. Leptin is a potent stimulator of bone growth in ob/ob mice. *Regul Pept.* 2000; 92 (1–3):73–8. [PubMed: 11024568]
22. Fleischer J, Stein EM, Bessler M, et al. The decline in hip bone density after gastric bypass surgery is associated with extent of weight loss. *J Clin Endocrinol Metab.* 2008; 93(10):3735–3740. [PubMed: 18647809]
23. Bruno C, Fulford AD, Potts JR, et al. Serum markers of bone turnover are increased at six and 18 months after Roux-en-Y bariatric surgery: correlation with the reduction in leptin. *J Clin Endocrinol Metab.* 2010; 95(1):159–166. [PubMed: 19858320]
24. Myers MG, Leibel R, Seeley R, et al. Obesity and Leptin Resistance: Distinguishing Cause from Effect. *Trends Endocrinol Metab.* 2010; 21:643–651. [PubMed: 20846876]
25. Heymsfield SB, Greenberg AS, Fujioka K, et al. Recombinant Leptin for Weight Loss in Obese and Lean Adults; A Randomized, Controlled, Dose-Escalation Trial. *JAMA.* 1999; 282(16):1568–1575. [PubMed: 10546697]
26. Rosenbaum M, Goldsmith R, Bloomfield D, et al. Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. *J Clin Invest.* 2005; 115 (12):3579–86. [PubMed: 16322796]
27. Westerterp-Plantenga M, Saris W, Hukshorn C, et al. Effects of Weekly Administration of Pegylated Recombinant Human OB Protein on Appetite Profile and Energy Metabolism in Obese Men. *Am J Clin Nutr.* 2001; 74:426–434. [PubMed: 11566639]
28. Leibel, RL.; Rosenbaum, M. Metabolic Responses to Weight Perturbation. In: Clement, K., et al., editors. *Novel Insights into Adipose Cell Functions, Research and Perspectives in Endocrine Interactions.* Berlin, Heidelberg: Springer-Verlag; 2010. p. 121-339.
29. Hanley DA, Davison KS. Symposium: Vitamin D insufficiency: A significant risk factor in chronic diseases and potential disease-specific biomarkers of vitamin D sufficiency. *J Nutr.* 2005; 135:332–337. [PubMed: 15671237]
30. Weyer C, Walford R, Harper I, et al. Energy metabolism after 2 y of energy restriction: the biosphere 2 experiment. *Am J Clin Nutr.* 2000; 72:946–53. [PubMed: 11010936]
31. Leibel R, Hirsch J. Diminished energy requirements in reduced-obese patients. *Metabolism.* 1984; 33:164–170. [PubMed: 6694559]

Figure 1.
Percent change of serum NTX and BSAP after weight loss and leptin administration.
* $P < 0.05$, ** $P < 0.01$ compared to $W_{t_{initial}}$

Table 1

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

	$Wt_{initial}$	$Wt_{-10\%P}$	$Wt_{-10\%L}$
Wt (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 (ug/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 (nM 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
25 D (ng/ml)	15.8 ± 1.3	15.8 ± 1.7	15.1 ± 1.8

* $P < 0.05$,

** $P < 0.01$ compared to $Wt_{initial}$;

[†] $P < 0.01$ compared to $Wt_{-10\%P}$