

## **PERSPECTIVES**

### **Leptin and Bone: A Consensus Emerging?**

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#### **Abstract**

The cytokine-like hormone leptin has emerged as a major factor linking food intake with bone metabolism. Leptin can regulate bone formation through both central (hypothalamic) and peripheral (direct) pathways, and leptin deficiency, in the form of either caloric restriction or a congenital absence of leptin, is associated with low bone mass. Leptin resistance does, however, increase with age in both humans and laboratory animals. The problem of leptin resistance suggests that the potential utility of leptin as a treatment for bone loss is limited to states of energy deprivation and leptin deficiency, such as exercise-induced hypothalamic amenorrhea, anorexia nervosa, and weight loss. *BoneKEy-Osteovision*. 2007 March;4(3):99-107.

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*Keywords:* Bone mass; Leptin receptor; NPY; Stromal cell; Leptin resistance

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#### **Introduction**

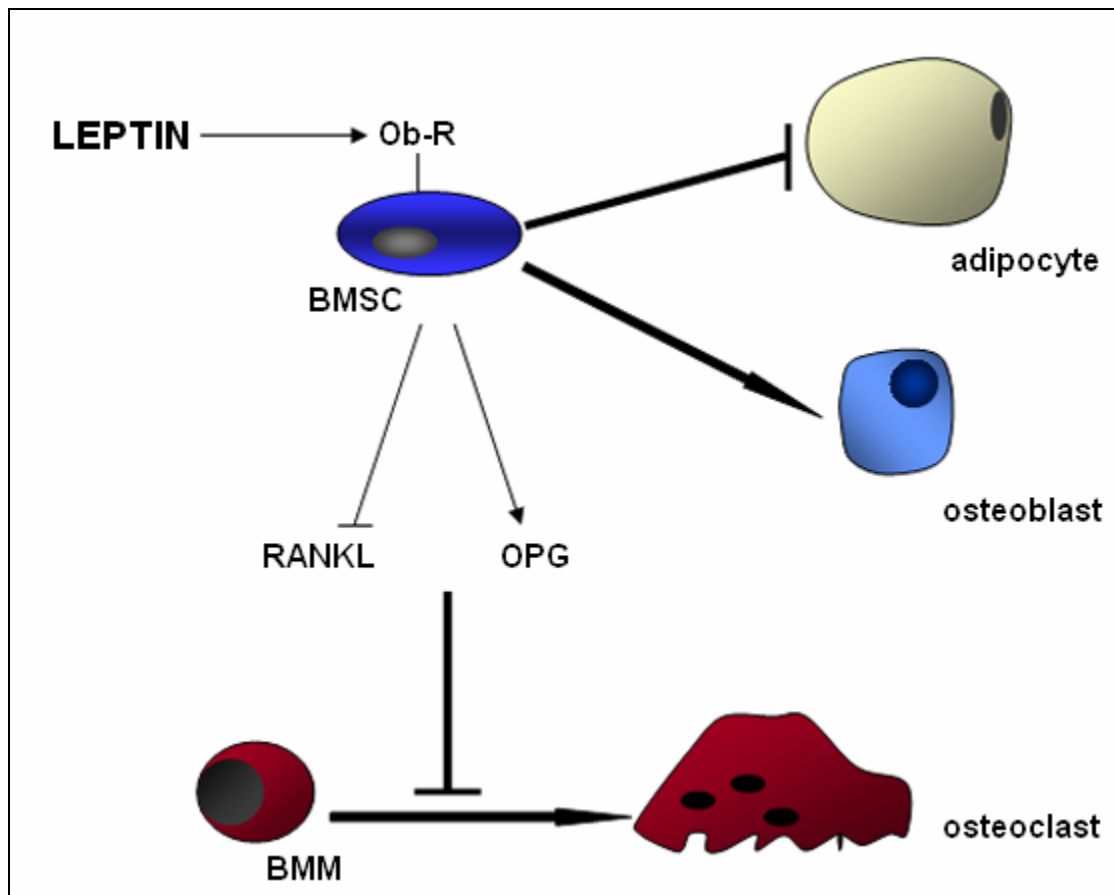
The cytokine-like hormone leptin was first described by Jeffrey Friedman's group at Rockefeller University in 1994 (1) and is now known to be a powerful regulator of appetite and energy balance. Leptin was initially observed to reduce food intake and induce weight loss in obese mice (1), and since its original discovery the biology of leptin has received considerable attention. In just over 10 years there have been literally thousands of papers published on the biology of leptin, and hundreds that have investigated the relationship between leptin and bone. The purpose of this brief *Perspective* is to highlight some of the general themes to emerge from these studies, with particular attention to a) the molecular mechanisms of leptin action in bone and cartilage, b) the skeletal phenotype of animal models in which leptin signaling is altered, and c) the evidence from human studies demonstrating a role (or lack thereof) for leptin in mediating age-related bone loss.

#### **Leptin Signaling in Brain and Bone**

Fat cells, or adipocytes, are the primary source of leptin in the body and as such lep-

tin plays an important role as a measure or signal of energy status. Leptin produced by peripheral fat depots enters the circulation and crosses the blood-brain barrier to reach its primary target, leptin receptors located in the hypothalamus. There are several isoforms of the leptin receptor, but the long form (Ob-Rb) is most abundant in the hypothalamus, where leptin binding activates the Jak/Stat signaling pathway (2). Leptin binding in the hypothalamus induces the expression of neuropeptides such as cocaine-amphetamine related transcript (CART) and alpha-melanocyte-stimulating hormone ( $\alpha$ -MSH), and suppresses the activity of genes such as those encoding neuropeptide Y (NPY) and agouti-related peptide (AgRP) that are involved in regulating food intake and energy expenditure (3). Leptin also regulates sympathetic outflows and functions as a  $\beta$ -adrenergic agonist (4). As discussed in more detail below, the central effects of leptin on  $\beta$ -adrenergic and NPY pathways have significant implications for the regulation of bone mass, since NPY is a powerful inhibitor of cortical bone formation and mice lacking

$\beta$ 1- and  $\beta$ 2-adrenergic receptors have low bone mass (5;6).



**Figure 1.** Leptin can affect bone metabolism not only via central, hypothalamic pathways (e.g., by regulating NPY expression) but also by directly binding to the leptin receptor (Ob-R) in bone marrow stromal (stem) cells (BMSCs) and inducing the expression of osteogenic genes (7;8;10). Leptin inhibits the differentiation of adipocytes from BMSCs *in vivo* and *in vitro* (8;10). Leptin also stimulates the production of osteoprotegerin (OPG) and inhibits the secretion of RANK ligand (RANKL) in BMSCs, inhibiting osteoclast differentiation from bone marrow monocytes (BMM) (14; 15).

Circulating leptin also regulates bone mass directly by binding to leptin receptors on bone marrow stromal (stem) cells (BMSCs), osteoblasts, and osteoclasts (Fig. 1; 7;8). Leptin binding to its receptor on stromal cells can activate the MAP-kinase signaling pathway in BMSCs (9), increasing the expression of osteogenic genes and directing BMSCs to the osteogenic rather than the adipogenic pathway (Fig. 1; 7;8;10). Interestingly, BMSCs isolated from osteoporotic donors show lower leptin binding capacity than BMSCs from donors with normal bone mass, and leptin is able to

effectively inhibit adipogenic differentiation in BMSCs from healthy donors but not in BMSCs from osteoporotic donors (8). Leptin treatment also directly increases osteoblast proliferation and mineralization *in vitro* and *in vivo* (11-13), and osteoblasts and bone marrow adipocytes themselves secrete leptin (7), raising the possibility that leptin may play a role in autocrine or paracrine signaling within the bone marrow microenvironment. It has been shown that leptin can also regulate osteoclast differentiation by increasing osteoprotegerin expression and decreasing RANK ligand

secretion by BMSCs (Fig. 1; 14). Leptin treatment decreases osteoclastogenesis and bone resorption *in vitro* and *in vivo* (14), which is consistent with studies demonstrating that leptin treatment can prevent bone loss associated with ovariectomy (15) and disuse (hindlimb unloading) (16). Finally, leptin receptors are detected in chondrocytes (17), and leptin treatment increases chondrocyte proliferation and expression of the IGF-1 receptor (IGF-1R) in cartilage of the growth plate and mandibular condyle (17-19).

### The Skeletal Phenotype of Leptin-Deficient Rodents

The mechanistic studies described above reveal that leptin can affect multiple cell types in the skeleton through both indirect (hypothalamic) and direct pathways. Animal models with altered leptin signaling have therefore proven invaluable for revealing the phenotypic effects of leptin on skeletal growth, development and aging. Two of the most well-known animal models with altered leptin signaling are mice lacking a functional leptin peptide, the leptin-deficient *ob/ob* mouse, and mice lacking a functional long form of the leptin-receptor, the *db/db* mouse (20). These mouse mutants show a 20-25% decrease in total bone mass measured as total body bone mineral content (BMC), a 20-25% decrease in cortical area of the femur, and a 30-40% decrease in mineralizing surface of the femur compared to normal mice (13;21-23). These data indicate that the primary effect of leptin deficiency on the skeleton is a reduction in cortical bone formation. Since approximately 80% of skeletal mass is accounted for by cortical bone, leptin deficiency results in a net loss of total bone mass compared to the wild-type condition. Leptin treatment increases bone formation in the appendicular skeleton and also decreases the population of bone marrow adipocytes in *ob/ob* mice, consistent with a role for leptin in regulating the differentiation of bone marrow stem cells (13;24-25).

The effects of leptin on cortical bone appear to be regulated by central, hypothalamic signals. Neuropeptide Y is an inhibitor of cortical bone formation, and leptin binding to

leptin receptors in the hypothalamus suppresses NPY expression. Mice lacking the Y2-receptor show a 15-20% increase in femur mass and cortical thickness compared to normal mice (5), suggesting that suppression of NPY expression by leptin is required for the normal acquisition of cortical bone in the limb skeleton. As noted earlier, leptin binding in the ventromedial hypothalamus can increase sympathetic tone, and leptin functions as a  $\beta$ -adrenergic agonist. Mice lacking the  $\beta$ 1- and  $\beta$ 2-adrenergic receptors show a 20-25% decrease in femur mass and cortical thickness compared to normal mice, demonstrating that normal  $\beta$ -adrenergic signaling is necessary for cortical bone formation (6). One of the more exciting findings in this regard is the recent observation that serum IGF-1 levels are significantly reduced in mice lacking  $\beta$ -adrenergic signaling, indicating that some of leptin's effects on bone involving the sympathetic nervous system may be mediated by IGF-1 signaling (26). This is also indicated by the fact, noted above, that leptin treatment can increase IGF-1R expression in chondrocytes.

Leptin does, however, appear to affect trabecular bone differently than cortical bone. Ducy and colleagues (27;28) originally described the leptin-deficient *ob/ob* mouse as having a "high bone mass" phenotype, based on their analysis of trabecular bone density in the spine of *ob/ob* animals. It was subsequently shown (5;13;22;24) that total bone mass is actually lower in *ob/ob* mice than normal mice, but that trabecular bone volume fraction (BV/TV) is increased with leptin deficiency in rodents. Why would leptin produce contrasting phenotypes in trabecular versus cortical bone? Sundeep Khosla (29) has suggested that a sparing of trabecular bone in the spine with leptin deficiency may be an adaptive mechanism to preserve mineral stores for calcium homeostasis during periods of food restriction. This adaptive explanation is supported by a later study demonstrating that caloric restriction in mice reduces total bone mass and femur mass but actually increases bone mass in the spine (30). It is likely, though, that the contrasting effects of leptin deficiency observed in the spine

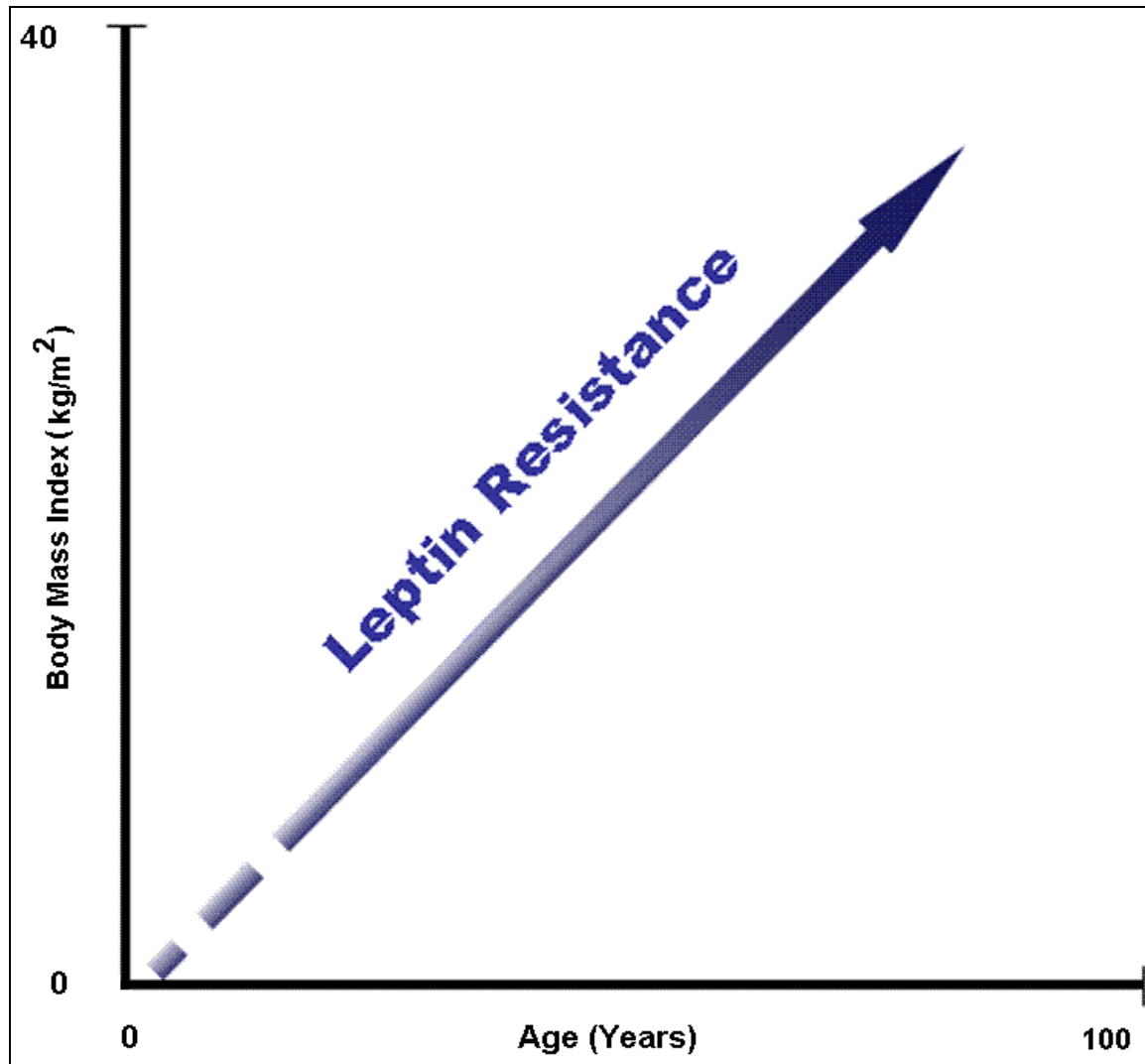
versus the femur may be specific to rodents, since humans with very low leptin levels (e.g., patients with anorexia) show low bone mass and density throughout the skeleton (see below). The case of leptin's varying effects throughout the mouse skeleton also provides a powerful example of why it is critical to examine all aspects of the skeletal phenotype in mouse mutant, knockout, and transgenic models. Technologies such as  $\mu$ CT provide such an accurate and rapid assessment of trabecular structure that other parameters, such as bone strength measurements and cortical bone dimensions, are frequently overlooked (31). Given that 80% of the mass of the skeleton is represented by cortical bone, measurements of trabecular bone alone may provide little insight into changes in "bone mass" resulting from a particular treatment or genetic manipulation.

### **Role of Leptin in Age-Related Bone Loss**

Evidence from the animal models described above suggests that in human studies we might expect serum leptin levels to be positively correlated with bone mass in the limb skeleton but inversely correlated with bone mass in the spine. However, a large meta-analysis examining the effects of leptin on BMD in 5803 postmenopausal women found that leptin levels explained less than 1% of the variation in BMD (32). Serum leptin showed no significant correlation with either femoral neck BMD ( $r = -0.04$ ) or lumbar spine BMD ( $r = -0.03$ ), and the correlation between serum leptin and whole body BMD was weak ( $r = 0.13$ ). These findings are reminiscent of the data showing that leptin has no significant effect on weight loss in humans, despite the fact that leptin induces significant weight loss in lab animals (33). The human bone and weight loss findings are both explained by the same phenomenon, leptin resistance. That is, with aging and with increases in endogenous, circulating leptin arising from greater food intake, leptin receptors are downregulated and leptin sensitivity is decreased (Fig. 2; 34-36). Moreover, leptin resistance in postmenopausal women may be further exacerbated by estrogen deficiency (37). The average serum leptin concentration for humans on a normal feeding schedule is

approximately 5-15 ng/ml (38), whereas in obese humans serum levels reach 40 ng/ml (39). Compared with an average serum leptin concentration of 1 ng/ml for primates in the wild (38), the average leptin concentration of most human populations is extremely high, suggesting that the overwhelming majority of people are relatively insensitive to leptin (Fig. 2). Because leptin resistance increases with age and estrogen deficiency, it is not surprising that meta-analyses do not detect significant correlations between leptin and BMD in postmenopausal women.

Leptin insensitivity due to aging or high levels of endogenous leptin can, however, be reversed with food restriction (40). In spite of the numerous benefits of caloric restriction (CR), it has been shown to reduce skeletal growth in young animals and cause bone loss in older animals (41-43). Severe CR, such as that which occurs in anorexia nervosa, is associated with markedly reduced leptin levels and osteoporosis (44-46). Even less severe, voluntary, weight loss is associated with increased rates of bone loss in adults (47;48). Systemic administration of leptin in rodents has been shown to reduce bone fragility (11), increase bone formation (49), and reverse the negative effects of CR on longitudinal bone growth (50;51). In food-restricted rats and mice, leptin treatment increases serum osteocalcin, serum testosterone, and serum growth hormone (49;52). Consistent with these animal studies, leptin treatment appears to have significant potential for reversing bone loss associated with hypothalamic amenorrhea in women (53). Leptin treatment (0.08  $\mu$ g/kg body weight) for three months increased serum IGF-1 and serum osteocalcin in women with exercise-induced hypothalamic amenorrhea (53). These women had serum leptin concentrations of approximately 3 ng/ml prior to treatment, which increased to approximately 6 ng/ml after three months of treatment. These findings underscore the point that, in leptin-sensitive individuals, leptin therapy increases markers of bone formation and bone growth; however, this also means that the utility of leptin as a potential treatment for bone loss may be limited to cases of energy deprivation, such



**Figure 2.** Leptin resistance increases with age and with body mass index (BMI). Serum leptin concentrations are closely correlated with BMI, so that a BMI of 40 is estimated to have a serum leptin concentration approaching 30-40 ng/ml, whereas a BMI of 20 is predicted to have a serum leptin concentration closer to 3-4 ng/ml (39;53). Leptin resistance also increases significantly with age. The problem of leptin resistance means that although leptin may stimulate bone formation at younger ages and in individuals with very low BMI (and low circulating leptin, <5 ng/ml), leptin levels will be poorly correlated with bone mass in older individuals or people with higher BMI and higher leptin levels (>5 ng/ml).

as food restriction, hypothalamic amenorrhea, and weight loss.

### Summary

Although reports of leptin's effects on bone appear in some cases to be inconsistent or even contradictory, the bulk of the literature on leptin and bone points to some basic conclusions that provide important insights into the relationship between nutrition and

bone metabolism (54). First, leptin deficiency, in the form of caloric restriction or a congenital absence of leptin, is associated with low total bone mass due primarily to decreased cortical bone formation. Trabecular bone mass may be increased with leptin deficiency in mice, but because trabecular bone comprises a relatively small portion (approximately 20%) of the total mass of the skeleton, this increase does not offset the decline in whole-body bone mass

resulting from the decrease in cortical bone. Second, leptin resistance increases with age, estrogen deficiency, and with increases in endogenous leptin. Hence, the majority of postmenopausal women with normal food intake are relatively insensitive to leptin treatment or to variations in circulating leptin levels. Finally, the problem of leptin resistance indicates that the potential utility of leptin as a therapy for bone loss is greatest in conditions of leptin deficiency and energy deprivation, such as food restriction, exercise-induced hypothalamic amenorrhea, anorexia nervosa, and perhaps weight loss.

### Acknowledgements

I am grateful to Dr. Gordon Strewler for the opportunity to prepare this *Perspective* for *BoneKey*, and to Dr. Carlos M. Isales, Dr. Clifton A. Baile, Dr. Mary Anne Della Fera, and Dr. Richard Lewis for helpful insights and discussion. This review is derived from a 2006 presentation on leptin and bone at the joint American Society for Bone and Mineral Research-International Bone and Mineral Society Symposium on Fat and Bone in Philadelphia, PA organized by Dr. Ian Reid and Dr. Clifford Rosen. Dr. Edith Gardiner, Dr. Sundeep Khosla, and Dr. Mary Anne Della Fera provided helpful comments that improved the manuscript.

**Conflict of Interest:** The author reports that no conflict of interest exists.

### References

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature*. 1994 Dec 1;372(6505):425-32.
2. Hegyi K, Fulop K, Kovacs K, Toth S, Falus A. Leptin-induced signal transduction pathways. *Cell Biol Int*. 2004;28(3):159-69.
3. Spiegelman BM, Flier JS. Obesity and the regulation of energy balance. *Cell*. 2001 Feb 23;104(4):531-43.
4. Collins S, Kuhn CM, Petro AE, Swick AG, Chrnyk BA, Surwit RS. Role of leptin in fat regulation. *Nature*. 1996 Apr 25;380(6576):677.
5. Baldock PA, Allison S, McDonald MM, Sainsbury A, Enriquez RF, Little DG, Eisman JA, Gardiner EM, Herzog H. Hypothalamic regulation of cortical bone mass: opposing activity of Y2 receptor and leptin pathways. *J Bone Miner Res*. 2006 Oct;21(10):1600-7.
6. Pierroz DD, Muzzin P, Glatt V, Boussein ML, Rizzoli R, Ferrari SL.  $\beta$ 1 $\beta$ 2-adrenergic receptor KO mice have decreased total body and cortical bone mass despite increased trabecular number. *J Bone Miner Res*. 2004 Oct;19(Suppl 1):S32.
7. Thomas T. The complex effects of leptin on bone metabolism through multiple pathways. *Curr Opin Pharmacol*. 2004 Jun;4(3):295-300.
8. Hess R, Pino AM, Rios S, Fernandez M, Rodriguez JP. High affinity leptin receptors are present in human mesenchymal stem cells (MSCs) derived from control and osteoporotic donors. *J Cell Biochem*. 2005 Jan 1;94(1):50-7.
9. Banks AS, Davis SM, Bates SH, Myers MG Jr. Activation of downstream signals by the long form of the leptin receptor. *J Biol Chem*. 2000 May 12;275(19):14563-72.
10. Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL. Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. *Endocrinology*. 1999 Apr;140(4):1630-8.
11. Gordeladze JO, Drevon CA, Syversen U, Reseland JE. 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.
12. Cornish J, Callon KE, Bava U, Lin C, Naot D, Hill BL, Grey AB, Broom N, Myers DE, Nicholson GC, Reid IR. Leptin directly regulates bone cell function in vitro and reduces bone fragility in vivo. *J Endocrinol.* 2002 Nov;175(2):405-15.
  13. Hamrick MW, Della-Fera MA, Choi YH, Pennington C, Hartzell D, Baile CA. Leptin treatment induces loss of bone marrow adipocytes and increases bone formation in leptin-deficient ob/ob mice. *J Bone Miner Res.* 2005 Jun;20(6):994-1001.
  14. Holloway WR, Collier FM, Aitken CJ, Myers DE, Hodge JM, Malakellis M, Gough TJ, Collier GR, Nicholson GC. Leptin inhibits osteoclast generation. *J Bone Miner Res.* 2002 Feb;17(2):200-9.
  15. Burguera B, Hofbauer LC, Thomas T, Gori F, Evans GL, Khosla S, Riggs BL, Turner RT. Leptin reduces ovariectomy-induced bone loss in rats. *Endocrinology.* 2001 Aug;142(8):3546-53.
  16. Martin A, de Vittoris R, David V, Moraes R, Begeot M, Lafage-Proust MH, Alexandre C, Vico L, Thomas T. Leptin modulates both resorption and formation while preventing disuse-induced bone loss in tail-suspended female rats. *Endocrinology.* 2005 Aug;146(8):3652-9.
  17. Maor G, Rochwerger M, Segev Y, Phillip M. Leptin acts as a growth factor on the chondrocytes of skeletal growth centers. *J Bone Miner Res.* 2002 Jun;17(6):1034-43.
  18. Kishida Y, Hirao M, Tamai N, Nampei A, Fujimoto T, Nakase T, Shimizu N, Yoshikawa H, Myoui A. Leptin regulates chondrocyte differentiation and matrix maturation during endochondral ossification. *Bone.* 2005 Nov;37(5):607-21.
  19. Figenschau Y, Knutsen G, Shahazeydi S, Johansen O, Sveinbjornsson B. Human articular chondrocytes express functional leptin receptors. *Biochem Biophys Res Commun.* 2001 Sep 14;287(1):190-7.
  20. Flier JS. What's in a name? In search of leptin's physiologic role. *J Clin Endocrinol Metab.* 1998 May;83(5):1407-13.
  21. Lorentzon R, Alehagen U, Boquist L. Osteopenia in mice with genetic diabetes. *Diabetes Res Clin Pract.* 1986 Jun;2(3):157-63.
  22. Steppan CM, Crawford DT, Chidsey-Frink KL, Ke H, Swick AG. Leptin is a potent stimulator of bone growth in ob/ob mice. *Regul Pept.* 2000 Aug 25;92(1-3):73-8.
  23. Takeshita N, Mutoh S, Yamaguchi I. Osteopenia in genetically diabetic db/db mice and effects of 1alpha-hydroxyvitamin D3 on the osteopenia. *Life Sci.* 1995 Feb 17;56(13):1095-101.
  24. Hamrick MW, Pennington C, Newton D, Xie D, Isales C. Leptin deficiency produces contrasting phenotypes in bones of the limb and spine. *Bone.* 2004 Mar;34(3):376-83.
  25. Hamrick MW, Della Fera MA, Choi YH, Hartzell D, Pennington C, Baile CA. Injections of leptin into rat ventromedial hypothalamus increase adipocyte apoptosis in peripheral fat and in bone marrow. *Cell Tissue Res.* 2007 Jan;327(1):133-41.
  26. Pierroz D, Baldock P, Bouxsein M, Ferrari S. Low cortical bone mass in mice lacking beta 1 and beta 2 adrenergic receptors is associated with low bone formation and circulating IGF-1. *J Bone Miner Res.* 2006 Sep;21(Suppl 1):S26.
  27. Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, Shen J, Vinson C, Rueger JM, Karsenty G. Leptin inhibits bone formation through a

- hypothalamic relay: a central control of bone mass. *Cell*. 2000 Jan 21;100(2):197-207.
28. Takeda S, Elefteriou F, Lévassieur R, Liu X, Zhao L, Parker KL, Armstrong D, Ducy P, Karsenty G. Leptin regulates bone formation via the sympathetic nervous system. *Cell*. 2002 Nov 1;111(3):305-17.
29. Khosla S. Leptin-central or peripheral to the regulation of bone metabolism? *Endocrinology*. 2002 Nov;143(11):4161-4.
30. Brochmann EJ, Duarte ME, Zaidi HA, Murray SS. Effects of dietary restriction on total body, femoral, and vertebral bone in SENCAR, C57BL/6, and DBA/2 mice. *Metabolism*. 2003 Oct;52(10):1265-73.
31. Jarvinen TL, Sievanen H, Jokihaara J, Einhorn TA. Revival of bone strength: the bottom line. *J Bone Miner Res*. 2005 May;20(5):717-20.
32. Pongchaiyakul C, Nguyen ND, Pisprasert V, Nguyen TV. A meta-analysis of relationships among leptin, fat mass, lean mass, and bone mineral density. *J Bone Miner Res*. 2005 Sep; 20(Suppl 1):S261.
33. Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T, Lubina JA, Patane J, Self B, Hunt P, McCamish M. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA*. 1999 Oct 27;282(16):1568-75.
34. Lin S, Thomas TC, Storlien LH, Huang XF. Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice. *Int J Obes Relat Metab Disord*. 2000 May;24(5):639-46.
35. Martin RL, Perez E, He YJ, Dawson R Jr, Millard WJ. Leptin resistance is associated with hypothalamic leptin receptor mRNA and protein downregulation. *Metabolism*. 2000 Nov;49(11):1479-84.
36. Qian H, Azain MJ, Hartzell DL, Baile CA. Increased leptin resistance as rats grow to maturity. *Proc Soc Exp Biol Med*. 1998 Nov;219(2):160-5.
37. Ainslie DA, Morris MJ, Wittert G, Turnbull H, Proietto J, Thorburn AW. Estrogen deficiency causes central leptin insensitivity and increased hypothalamic neuropeptide Y. *Int J Obes Relat Metab Disord*. 2001 Nov;25(11):1680-8.
38. Banks WA, Phillips-Conroy JE, Jolly CJ, Morley JE. Serum leptin levels in wild and captive populations of baboons (Papio): implications for the ancestral role of leptin. *J Clin Endocrinol Metab*. 2001 Sep;86(9):4315-20.
39. Benini ZL, Camilloni MA, Scordato C, Lezzi G, Savia G, Oriani G, Bertoli S, Balzola F, Liuzzi A, Petroni ML. Contribution of weight cycling to serum leptin in human obesity. *Int J Obes Relat Metab Disord*. 2001 May;25(5):721-6.
40. Fernandez-Galaz C, Fernandez-Agullo T, Perez C, Peralta S, Arribas C, Andres A, Carrascosa JM, Ros M. Long-term food restriction prevents ageing-associated central leptin resistance in Wistar rats. *Diabetologia*. 2002 Jul;45(7):997-1003.
41. Shires R, Avioli LV, Bergfeld MA, Fallon MD, Slatopolsky E, Teitelbaum SL. Effects of semistarvation on skeletal homeostasis. *Endocrinology*. 1980 Nov;107(5):1530-5.
42. Grinspoon SK, Baum HB, Kim V, Coggins C, Klibanski A. Decreased bone formation and increased mineral dissolution during acute fasting in young women. *J Clin Endocrinol Metab*. 1995 Dec;80(12):3628-33.
43. LaMothe JM, Hepple RT, Zernicke RF. Bone adaptation with aging and long-term caloric restriction in Fischer 344 x



- Brown-Norway F1-hybrid rats. *J Appl Physiol*. 2003 Oct;95(4):1739-45.
44. Lear SA, Pauly RP, Birmingham CL. Body fat, caloric intake, and plasma leptin levels in women with anorexia nervosa. *Int J Eat Disord*. 1999 Nov;26(3):283-8.
45. Kaufman BA, Warren MP, Dominguez JE, Wang J, Heymsfield SB, Pierson RN. Bone density and amenorrhea in ballet dancers are related to a decreased resting metabolic rate and lower leptin levels. *J Clin Endocrinol Metab*. 2002 Jun;87(6):2777-83.
46. Turner JM, Bulsara MK, McDermott BM, Byrne GC, Prince RL, Forbes DA. Predictors of low bone density in young adolescent females with anorexia nervosa and other dieting disorders. *Int J Eat Disord*. 2001 Nov;30(3):245-51.
47. Ensrud KE, Fullman RL, Barrett-Connor E, Cauley JA, Stefanick ML, Fink HA, Lewis CE, Orwoll E; Osteoporotic Fractures in Men Study Research Group. Voluntary weight reduction in older men increases hip bone loss: the osteoporotic fractures in men study. *J Clin Endocrinol Metab*. 2005 Apr;90(4):1998-2004.
48. Johnson JM, Maher JW, Samuel I, Heitshusen D, Doherty C, Downs RW. Effects of gastric bypass procedures on bone mineral density, calcium, parathyroid hormone, and vitamin D. *J Gastrointest Surg*. 2005 Nov;9(8):1106-10.
49. Goldstone AP, Howard JK, Lord GM, Ghatei MA, Gardiner JV, Wang ZL, Wang RM, Girgis SI, Bailey CJ, Bloom SR. Leptin prevents the fall in plasma osteocalcin during starvation in male mice. *Biochem Biophys Res Commun*. 2002 Jul 12;295(2):475-81.
50. Gat-Yablonski G, Ben-Ari T, Shtaf B, Potievsky O, Moran O, Eshet R, Maor G, Segev Y, Phillip M. Leptin reverses the inhibitory effect of caloric restriction on longitudinal growth. *Endocrinology*. 2004 Jan;145(1):343-50.
51. Hamrick MW. Leptin, bone mass, and the thrifty phenotype. *J Bone Miner Res*. 2004 Oct;19(10):1607-11.
52. Carro E, Senaris R, Considine RV, Casanueva FF, Dieguez C. Regulation of in vivo growth hormone secretion by leptin. *Endocrinology*. 1997 May;138(5):2203-6.
53. Welt CK, Chan JL, Bullen J, Murphy R, Smith P, DePaoli AM, Karalis A, Mantzoros CS. Recombinant human leptin in women with hypothalamic amenorrhea. *N Engl J Med*. 2004 Sep 2;351(10):987-97.
54. Reid IR, Cornish J, Baldock PA. Nutrition-related peptides and bone homeostasis. *J Bone Miner Res*. 2006 Apr;21(4):495-500.