

## COMMENTARIES

### Leptin Regulation of Bone Mass by the Sympathetic Nervous System and CART

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**Commentary on:** Eleftriou F, Ahn J, Takeda S, Starbuck M, Yang X, Liu X, Kondo H, Richards W, Bannon T, Noda M, Clement K, Vaisse C, Karsenty G. Leptin regulation of bone resorption by the sympathetic nervous system and CART. 2005; *Nature* 434: 514-520.

One of the most exciting developments in the area of bone metabolism research has been the discovery that neuronal signals play a significant role in the regulation of bone mass. Gerard Karsenty and colleagues have been at the forefront of this research program, since Ducy *et al.* (1) described a high bone mass phenotype in leptin-deficient *ob/ob* mice, in a paper that has now been cited over 300 times. In this earlier paper, it was suggested that leptin, a cytokine-like hormone produced by fat cells, is a powerful inhibitor of bone formation. The authors proposed that leptin exerts its antiosteogenic effects by binding to receptors in the hypothalamus, stimulating the release of noradrenaline from sympathetic nerve fibers in bone. Noradrenaline is then thought to inhibit bone formation by binding to  $\beta$ 2-adrenergic receptors on osteoblasts. Leptin-deficient *ob/ob* mice were observed by Ducy *et al.* (1) to have a 60% to 70% increase in trabecular bone formation, compared with normal mice, apparently because of a dramatic increase in bone matrix deposition by resident osteoblasts. Central intracerebroventricular infusion of leptin was also found to decrease trabecular bone mass in such mice, consistent with a role for leptin as an antiosteogenic hormone that regulates bone mass through a central hypothalamic pathway. A role for the sympathetic nervous system in regulating

bone turnover has been further indicated by additional data showing that pharmacological inhibition of  $\beta$ -adrenergic signaling using " $\beta$ -blockers" increases osteoblast number, bone formation rate, and bone mass in normal and ovariectomized mice (2).

The most recent paper published by this group in the journal *Nature* (3) sheds new light on leptin's regulation of bone mass via the sympathetic nervous system and points to a new role for leptin in the regulation of bone resorption by osteoclasts. Specifically, the authors have found that mice lacking the  $\beta$ 2-adrenergic receptor gene (*Adrb2*) show increased trabecular bone mass and are resistant to ovariectomy-induced bone loss. This result is surprising, as leptin treatment has previously been observed to decrease bone loss in ovariectomized rats (4). Loss of  $\beta$ -adrenergic signaling in osteoblasts seems not only to increase bone formation, as expected given the authors' previous work, but also to decrease bone resorption. A significant finding is that decreased bone resorption in *Adrb2*-deficient mice is the direct result of decreased expression of receptor activator of NF- $\kappa$ B ligand (RANKL) by osteoblasts. Previous studies have attributed increases in bone mass associated with leptin deficiency solely to increases in bone formation rate (1), whereas the new study (3) indicates that  $\beta$ -adrenergic signaling plays a major role in regulating osteoclast differentiation. Another novel result from the new paper by Eleftriou *et al.* (3) is that cocaine- and amphetamine-related transcript (CART), a neuropeptide regulated by leptin, is a powerful inhibitor of bone resorption. CART-deficient mice were

found to have a low bone mass phenotype and increased RANKL expression, and mice lacking CART showed greater bone resorption with leptin treatment than did normal mice receiving leptin. It is as yet not known exactly how CART increases RANKL expression in osteoblasts, but the new study (3) indicates that leptin can moderate osteoclast activity through two distinct and antagonistic pathways: first, by increasing RANKL expression by osteoblasts via *Adrb2* signaling, and second, by increasing CART expression, which in turn, decreases RANKL expression by osteoblasts.

The paper by Eleftriou *et al.* (3) raises several interesting questions for future research. The most obvious question concerns the signaling pathway(s) involved in the regulation of RANKL expression by CART. CART is present in blood, and CART receptors have thus far been detected in the posterior pituitary, adrenal medulla, and endocrine cells in the gut (5). The effects of central (intracerebroventricular) and peripheral infusions of CART peptide on bone turnover might provide some insights into the basic mechanisms involved. Second, some studies indicate that leptin can inhibit osteoclast differentiation directly (6;7), and *in vitro* experiments utilizing human bone marrow stromal cells show that leptin treatment inhibits RANKL expression while increasing osteoprotegerin expression (8). A goal of future research will be to determine the importance of leptin's direct effects on osteoclast differentiation relative to the centrally-mediated effects of leptin on bone resorption described by Eleftriou *et al.* (3). Another question deals with the effects of neuronal signals on cortical bone. Periosteum, supplied by a dense network of sensory and sympathetic fibers, is the most richly innervated surface of bone (9); however, it is not known how altered leptin signaling affects periosteal apposition during growth and aging. This is an important consideration, because periosteal expansion and femoral neck geometry are key determinants of hip fracture risk (10). The fact that obese Zucker rats, which lack the long form of the leptin receptor, have decreased femoral diameters and lower failure loads than normal rats suggests that

leptin deficiency may ultimately have a negative impact on limb bone strength (11).

The finding of Eleftriou *et al.* (3) that ovariectomy-induced bone loss is attenuated in *Adrb2* deficient mice merits further study. The ovariectomized mice included in the analysis were very young, only four weeks of age at the time of ovariectomy, and the effects of the procedure on bone histomorphometry were examined at two and four months of age. Mice experience rapid bone growth and mineral acquisition between four and 16 weeks of age, and it is likely that the lower bone mass observed in ovariectomized wild-type mice is more a result of decreased bone formation than increased bone resorption. Similar experiments in older animals (*e.g.*, those aged six months) would provide more definitive evidence that loss of  $\beta$ -adrenergic signaling inhibits the bone loss frequently observed with aging and estrogen depletion. Furthermore, previous studies (4) have shown that treatment of such older ovariectomized rats with leptin and estrogen preserves bone mass more effectively than does treatment with either estrogen or leptin alone, suggesting a synergistic effect. This idea is supported by the fact that ovariectomy decreases leptin receptor expression in the hypothalamus, but treatment of ovariectomized animals with either estrogen or raloxifene restores leptin receptor expression (12). The positive effects of hormone replacement therapy or raloxifene treatment on bone mass in postmenopausal women may therefore be explained in part by their positive effects on leptin sensitivity.

A final question concerns the use of  $\beta$  - blockers as potential pharmacological agents for the treatment and prevention of osteoporosis. It has been shown in animal models that  $\beta$ -blockers can inhibit bone loss in settings normally associated with increased bone resorption, such as hindlimb unloading (13) and ovariectomy (2), but leptin itself can have a similar preventive effect (4;14). A common theme underlying these seemingly contradictory findings is that conditions associated with bone loss, such as estrogen depletion, hindlimb unloading, and food restriction, are often

accompanied by noradrenaline release and decreased serum leptin (Fig. 1). The catabolic effects of these conditions may then be attenuated with leptin treatment,  $\beta$ -blockers, and/or CART (Fig. 1), but excess leptin beyond normal physiological levels may actually lead to increased bone resorption (1;15). Together, these studies reveal that leptin's effects on bone metabolism are, in many cases, condition- and dose-dependent and can also vary between axial and appendicular regions (16). These findings clearly pose a number

of challenges for the identification of new and effective molecular therapies for bone loss that target sympathetic signaling pathways. Indeed, a recent study (17) suggests that  $\beta$ -blocker use is not associated with a decreased risk of hip fracture, and bone mineral density at the hip is not strongly correlated with the use of  $\beta$ -blockers. Nevertheless, the neural control of bone metabolism, a field of research that is still in its infancy, has already demonstrated great potential to contribute novel insights into osteoporosis treatment and prevention.

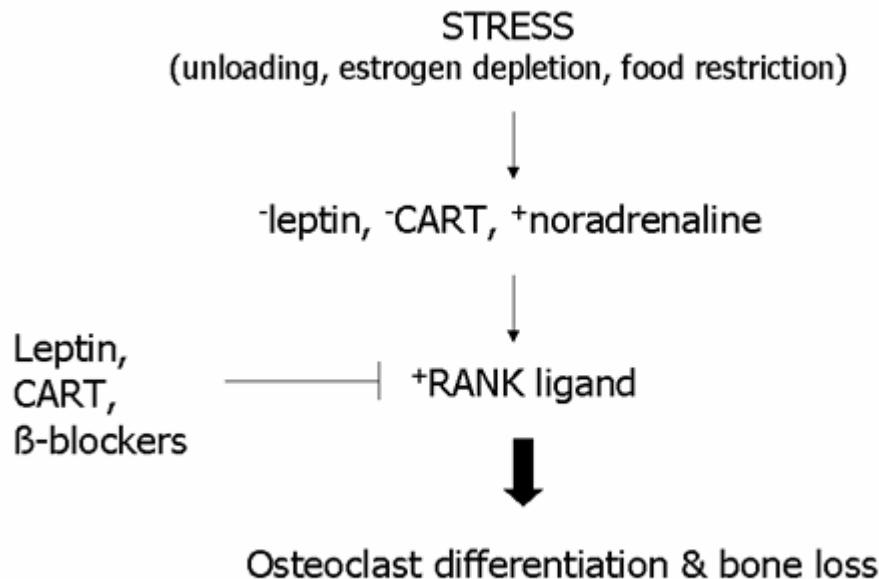


Figure 1. Noradrenaline release is increased with ovariectomy (18) and food restriction (19), and both hindlimb unloading and food restriction produce a drop in serum leptin (20;21). These conditions increase receptor activator of NF- $\kappa$ B ligand (RANKL) expression by osteoblasts, which can be inhibited by leptin treatment (14), beta-blockers (13), and/or cocaine- and amphetamine-related transcript (CART) (3) that suppress osteoclast differentiation and bone resorption. The inhibition of RANKL expression by leptin can occur both centrally, via upregulation of CART (3), and peripherally, by suppressing RANKL expression directly in osteoprogenitor cells (8).

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