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# COMMON ENDOCRINE CONTROL OF BODY WEIGHT, REPRODUCTION, AND BONE MASS

Shu Takeda[1], Florent Elefteriou[1], and Gerard Karsenty[1,2]
[1]*Department of Molecular and Human Genetics and Bone Disease Program of Texas, Baylor College of Medicine, Houston, Texas 77030; email: shu-tky@umin.ac.jp, florente@bcm.tmc.edu, karsenty@bcm.tmc.edu*
[2]*Author to whom correspondence should be addressed*



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■ **Abstract** Bone mass is maintained constant between puberty and menopause by the balance between osteoblast and osteoclast activity. The existence of a hormonal control of osteoblast activity has been speculated for years by analogy to osteoclast biology. Through the search for such humoral signal(s) regulating bone formation, leptin has been identified as a strong inhibitor of bone formation. Furthermore, intracerebroventricular infusion of leptin has shown that the effect of this adipocyte-derived hormone on bone is mediated via a brain relay. Subsequent studies have led to the identification of hypothalamic groups of neurons involved in leptin's antiosteogenic function. In addition, those neurons or neuronal pathways are distinct from neurons responsible for the regulation of energy metabolism. Finally, the peripheral mediator of leptin's antiosteogenic function has been identified as the sympathetic nervous system. Sympathomimetics administered to mice decreased bone formation and bone mass. Conversely, $\beta$-blockers increased bone formation and bone mass and blunted the bone loss induced by ovariectomy.

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## HORMONAL REGULATION OF BONE REMODELING

Because the integrity of the skeleton is a mandatory requisite to vertebrates for movement in a terrestrial environment, bones are the sites of a complex process constantly renewing their structure throughout life. This process, called bone remodeling, can be briefly described by the sequential action of osteoclasts resorbing preexisting mineralized bone and the subsequent action of osteoblasts laying down an extracellular matrix that will eventually become mineralized. This process occurs throughout the skeleton simultaneously to insure its complete renewal within 10 years and to maintain its structural properties and integrity when damaged. When the balance between bone resorption and bone formation is conserved, bone mass is kept constant. However, any unbalance between these two processes will inevitably lead to a pathologic state characterized either by a low bone mass or by a high bone mass that can potentially impair bone marrow functions. Osteoporosis, the most frequent bone remodeling disease, is characterized by a relative increased osteoclastic activity that is not compensated by an increased osteoblastic activity, leading to a low bone mass and a high risk of fracture (29, 30).

Bone remodeling is regulated by the paracrine or autocrine action of diverse cytokines, growth factors, and hormones, acting either on osteoblasts or osteoclasts. Several transcription factors (Pu.1, c-fos, NF$\kappa$B, Mi, etc.), cytokines (mcsf1, OPG, OPGL, etc.) and hormones (estrogen, PTH, etc.) are known to regulate osteoclast precursor differentiation or osteoclast function (5). Similarly, transcriptional factors (Cbfa1, Osterix) and cytokines (LRP5, PGE2, etc.) regulate osteoblast differentiation and function (13, 22). However, only a few hormones have been identified to regulate these processes. One experiment performed in our laboratory has suggested the existence of such humoral control of osteoblast function. In a mouse transgenic model of inducible osteoblast ablation (the osteocalcine-thymidine kinase transgenic mice), mice lost bone as a result of osteoblast deletion and continuous resorption by osteoclasts (9). However, cessation of osteoblast ablation led to a strikingly rapid and precise recovery of bone mass. The rapidity and the precision of this recovery suggested that osteoblasts had a way to sense when they had to produce a high amount of bone matrix and when they should reduce their activity at the time bone mass returns to normal. This observation suggested that a powerful homeostatic system could maintain bone mass and that this system might be of endocrine nature. Clinical observations have strengthened this hypothesis.

## LEPTIN IS AN INHIBITOR OF BONE FORMATION

Two observations taken from the literature drove us to the hormone we were seeking: Estrogen deprivation at menopause leads to bone loss and obesity to some extent protects from osteoporosis (19, 31, 40). These observations suggested that three distinct homeostatic functions, i.e., bone mass, reproduction, and body weight, could be regulated by the same hormone. Leptin arose as a plausible candidate because this adipocyte-secreted hormone coregulates two of these

functions, i.e., reproduction and body weight, through hypothalamic neurons expressing leptin receptors (1, 16, 18, 43). This hypothesis implicated that the regulation of bone formation, like the regulation of reproduction and body weight, would be under hypothalamic control. Leptin-deficient and leptin receptor–deficient mice are morbidly obese and sterile (20). According to the above observations, these animals should have a low bone mass because of their hypogonadism, and could be protected to some extent from this bone loss by their obesity. Surprisingly, in spite of their hypogonadism, leptin signaling–deficient mice have an increased bone formation rate and consequently 40% more bone than their wild-type littermates (12). This result is extraordinary because this mouse model is the only known example of a coexistence of a high bone mass and hypogonadism.

Leptin signaling deficiency, and not body weight, is responsible for this increased bone formation. Indeed the "fat-free" A-ZIP/F1 transgenic mice have a low level of leptin due to their lipodystrophy and also display a high bone mass phenotype in spite of their low body weight (12, 14). In contrast, melanocortin receptor 4–deficient mice are strongly obese yet do not display any bone mass abnormality (21, 34).



## LEPTIN EFFECT ON BONE MASS IS MEDIATED CENTRALLY

The hypothalamus constitutes the main brain center orchestrating the regulation of many—if not all—homeostatic functions. This part of the brain highly expresses the signaling form of leptin receptor (6, 18, 35). Our hypothesis that leptin would regulate bone formation through a central relay implied that leptin delivery by hypothalamic intracerebroventricular (ICV) infusion would affect bone mass and rescue the bone phenotype of leptin-deficient mice. It is indeed what we observed. Minimal dose of leptin ICV infusion led to marked bone loss in both leptin-deficient and wild-type mice. The same dose of leptin given peripherally was ineffective. This first result was a strong indication that leptin controls bone formation predominantly through the hypothalamus. A thorough testing of this hypothesis, however, was required to assess the consequences of leptin's direct effect on osteoblasts and bone mass, especially since several in vitro studies reported a direct effect of supraphysiologic doses of leptin on human or rat osteoblasts or osteoblastic cell lines (8, 25, 28, 39). In vitro studies led to negative results. Oncostatin M treatment of mouse primary osteoblasts induced the phosphorylation of Stat3, a downstream signaling molecule of both oncostatin M receptor (23) and leptin receptor (41). Treatment with physiologic and supraphysiologic doses of leptin did not induce Stat3 phosphorylation. Since *db/db* mice have a high bone mass phenotype, osteoblasts would be expected to produce more extracellular matrix than wild-type osteoblasts. However, among other parameters analyzed, in vitro nodule formation and collagen synthesis by leptin receptor–deficient osteoblasts were undistinguishable from wild-type osteoblasts. Finally, and more relevant because of the in vivo nature of these experiments, transgenic mice overexpressing leptin in osteoblasts

had a normal bone mass regardless of their age. This sharply contrasts with the ease with which leptin infusion decreases bone mass when delivered centrally. These results therefore indicate that leptin does not affect bone mass in vivo by a direct action on bone (34).

## TOWARD THE IDENTIFICATION OF DISTINCT HYPOTHALAMIC NEURONS OR NEURONAL PATHWAYS CONTROLLING BONE FORMATION AND BODY WEIGHT

One way to confirm the hypothalamic nature of bone mass regulation and to target neuronal structures involved in this function is the destruction of discrete regions of the hypothalamus. Such an approach was used with success five decades ago to identify hunger and satiety centers located in the basal and lateral hypothalamus (15). We chose to use the same strategy, in conjunction with genetic studies, to identify hypothalamic structure(s) and neuropeptides regulating bone formation. Two distinct neurotoxins—monosodium glutamate (MSG) and goldthioglucose (GTG)—can be used to lesion with a certain degree of specificity groups of neurons in the arcuate nuclei (ARC) and ventro-medial hypothalamic (VMH) nuclei, respectively (11, 27). These two hypothalamic nuclei highly express leptin receptors. MSG treatment did induce a gain of body weight because of the destruction of ARC neurons but did not affect bone mass, indicating that MSG-sensitive neurons are dispensable for leptin antiosteogenic function. By contrast, destruction of VMH-GTG-sensitive neurons led to a marked increase in bone mass, reaching a bone mass similar to the one observed in *ob/ob* mice (34). This result confirmed the involvement of the hypothalamus in the regulation of bone mass and defined a specific group of neurons characterized by their anatomical location and their sensitivity to GTG.

The next question was whether these GTG-sensitive neurons were the neurons by which leptin was affecting bone mass. To address this question, GTG-treated mice or control mice were administered leptin ICV. Unlesioned mice did lose bone, as expected, but GTG-treated mice did not, which indicated that GTG-sensitive neurons are the target of leptin for its antiosteogenic function. In contrast, MSG-treated mice infused with leptin ICV lost bone following leptin ICV infusion, which confirmed that MSG-sensitive neurons are not involved in the control of bone mass by leptin. This last result, however, does not exclude the existence of additional neuronal pathways involved in the control of bone mass. In line with this hypothesis, Baldock and colleagues (2) have shown that arcuate brain-specific deletion of neuropeptide Y (NPY)-receptor 2 leads to a high bone mass phenotype. Taken together, these results suggest the existence of distinct hypothalamic centers or neuronal pathways regulating body weight and bone mass.

Additional observations strengthen this notion. The sum of knowledge accumulated so far points to a main central pathway regulating body weight downstream of leptin: the melanocortin pathway (10, 26). A legitimate question is whether this pathway is also involved in leptin central control of bone formation. A negative

answer would suggest that there is a central pathway downstream of leptin controlling bone mass independently of body weight. If such a pathway exists, we could hope to manipulate it to increase bone mass without affecting body weight. To address this question, we made use of available genetic mutant mice. Agouti yellow mice (*Ay*) and melanocortin receptor 4 (*MC4R*)-deficient mice are two mouse models of genetic blockade of melanocortin signaling (4, 17, 21, 24). Histomorphometric analyses revealed that neither Ay nor MC4R-deficient mice had bone mass abnormalities. Moreover, treatment of ob/ob mice with the melanocortin agonist MTII did not reveal any bone mass abnormality either. Therefore blocking or stimulating melanocortin signaling does not affect bone mass, which indicates that the melanocortin pathway is not involved in the central control of bone mass (34).



## THE SYMPATHETIC NERVOUS SYSTEM: A LINK BETWEEN BRAIN AND BONE

The next question was what is the downstream effector of leptin's antiosteogenic function. This is important because the identification of this pathway may lead to a novel bone-forming therapy. A classic experiment, established decades ago and called parabiosis, was performed to address the question. This technique has been used to show the existence of a soluble molecule controlling body weight. The molecule is absent in *ob/ob* mice (7). Parabiosis between *ob/ob* and wild-type mice resulted in a dramatic loss of body weight in the *ob/ob* mice, which indicated that a molecule originating from the wild-type mouse and transported through the blood could rescue the phenotype of the *ob/ob* mouse. In the present study, two *ob/ob* mice were used and therefore there was no leptin in the system. Following parabiosis, a minimal dose of leptin ICV was administered to a single mouse. There was no measurable blood circulating leptin indicating that there was no leakage from the central nervous system to the general circulation. If the effector of leptin antiosteogenic action is a humoral nature, its effect would be seen not only in the ICV recipient mouse but also in the controlateral mouse. On the other hand, if the effector were of neuronal nature, the effect of leptin would be observed only in the mouse with leptin ICV. The latter possibility was the right one. A significant bone loss was observed only in the mouse receiving leptin ICV, but not in the controlateral mouse. This can be explained by the existence of either a neuronal mediator or a short live humoral mediator. We decided to focus first on the neuronal mediator based on what was already known about leptin signaling and human observations.

## MODULATION OF THE SYMPATHETIC NERVOUS SYSTEM—THERAPEUTIC IMPLICATIONS

It has been shown that one of the characteristics of *ob/ob* mice is a low sympathetic tone (42). In addition, leptin has been shown to increase sympathetic nervous activity through the VMH nuclei (32). Therefore, we hypothesized that leptin's

antiosteogenic action could be mediated through the sympathetic nervous system (SNS). To address this question, mice deficient in dopamine $\beta$-hydroxylase (DBH), an essential enzyme to produce epinephrine and norepinephrine from dopamine, were analyzed (36, 38). These mice are not obese despite the accumulating evidence that SNS is a negative regulator of body weight. Apart from that, these mice have been shown to have high concentrations of corticosterone and dopamine, both conditions known to induce osteopenia (3, 37). Surprisingly, these mice had high bone mass phenotype (34). More importantly, DBH-deficient mice lost all of their fat by leptin ICV treatment; however, they did not respond to leptin's antiosteogenic effect. These results emphasized two important aspects of leptin biology: (*a*) The SNS is not essential for the action of leptin on body weight, and (*b*) the antiosteogenic action of leptin is mediated by the SNS, at least partially.

If the SNS is a direct regulator of bone mass, functional adrenergic receptors must be located on bone cells. If so, which adrenergic receptor is responsible for the action of leptin on bone? Only the $\beta 2$ receptor was present on osteoblasts and none of other post-synaptic beta or alpha receptors were detected. Moreover, primary osteoblasts produced cAMP in response to a beta agonist treatment, suggesting that these receptors are functional (34).

Then, modulating sympathetic nervous pathway should affect bone mass, as modulating leptin signaling does. Isoproterenol was used to mimic an increase in SNS activity in *ob/ob* mice and completely rescued the high bone mass of *ob/ob* mice, although it did not affect their body weight. The same results were obtained using wild-type mice. These results suggest that stimulation of the SNS leads to

**TABLE 1** Body weight and bone mass effects following manipulation of leptin, hypothalamic, melanocortic, and sympathetic signaling

| | **Body weight** | **Bone mass** |
|---|---|---|
| Leptin signaling deficiency | ↗ | ↗ |
| Leptin ICV | ↘ | ↘ |
| MSG treatment | ↗ | = |
| MSG + leptin ICV | ↘ | ↘ |
| GTG treatment | ↗ | ↗ |
| GTG + leptin ICV | ↘ | = |
| Melanocortin signaling deficiency | ↗ | = |
| Melanocortin signaling deficiency + ICV | ↘ | ↘ |
| DBH deficiency | = | ↗ |
| Isoproterenol treatment | = | ↘ |
| Propranolol treatment | = | ↗ |
| DBH deficiency + leptin ICV | ↘ | = |

a decrease in bone mass. Furthermore, as there was no effect on body weight, the fact that leptin's actions on body weight regulation and bone metabolism use different pathways was further strengthened (Table 1).

The last but most important question is the following one: Could antagonists of the SNS be used to oppose the antiosteogenic action of leptin and increase bone mass? Wild-type mice treated with propranolol, one of the most commonly used $\beta$-blockers, had increased bone mass. Again, no effect on body weight was observed. Moreover, mice treated with propranolol were protected from bone loss induced by ovariectomy (34). These results suggest that $\beta$-blockers could be used to treat osteopenic diseases such as osteoporosis.

Are these findings are clinically relevant? For now, there is no evidence that treatment with $\beta$-blockers is effective against osteoporosis in humans. Prospective clinical trials are awaited, but it will take several years to reach a clear answer. However, there is one indication that modulating SNS leads to dysregulation of bone metabolism in humans. Reflex sympathetic dystrophy is a disease characterized by the upregulation of sympathetic nervous system in a localized lesion (33). It is accompanied by a focal osteopenia and one of the most effective treatments for this disease is . . . $\beta$-blockers.

How do these findings relate to the higher bone mass observed in obese people? It is known that obesity is marked by a state of leptin resistance. Although the molecular mechanisms of leptin resistance in obesity are not well understood, obese people can be seen as being functionally deficient in leptin signaling and therefore one would expect that they would have a high bone mass phenotype. Thus the high bone mass phenotype of obese people is consistent with these findings.

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