Leptin as an Endocrine Signal in Bone

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Leptin and its actions in bone came to prominence in 2000, with the publication of two landmark articles identifying a novel interaction between energy and bone homeostasis, as well as a novel hypothalamic circuit to the skeleton. However, they also revealed the dichotomous nature of leptin's effect on the skeleton. Subsequent research has increased understanding of the factors critical to interpretation of the leptin–bone signaling. These include opposing effects in cortical and cancellous bone, central and peripheral effects, involvement of other neural and endocrine factors, and leptin receptor polymorphisms in human populations. It is clear that leptin can markedly influence the regulation of bone mass, and that study of this pathway continues to increase our knowledge of the biology of skeletal tissue and its interactions with other tissues. However, this relationship is complex and requires careful interpretation.

Introduction

A balance between bone formation and resorption is essential for bone development and homeostasis. Disruption of this balance can diminish bone mass and microarchitectural integrity of bone tissue, resulting in an increase in bone fragility and susceptibility to fractures, as so commonly evident in osteoporosis [1]. The etiology of osteoporosis is strongly tied to endocrine signals initiated in the hypothalamus (most notably, sex hormones) [1,2]. In 2000, however, a publication identified an entirely novel skeletal interaction between endocrine signals and the hypothalamus, resulting from a signal produced by adipose tissue, leptin [3]. Obese individuals, with high serum leptin, are protected from osteoporosis, and thus it was a surprise when leptin deficiency was found to stimulate bone formation. Considerable effort has since been made to further characterize this relationship. The skeletal effects of leptin, however, are complex and regulated across multiple pathways, resulting in a range of effects. As a result, understanding the actions of this hormone must take several issues into account.

The use of rodent models, in particular leptin-deficient *ob/ob* mice and leptin receptor–deficient *db/db* mice, has been critical to the elucidation of the effects of leptin on the skeletal system. However, a degree of controversy exists regarding the skeletal phenotype of these mice, and therefore the true effect of leptin in bone. In 2000, the Karsenty laboratory published an examination of the cancellous phenotype of *ob/ob* mice and elucidated the hypothalamic nature of the effect [3]. They postulated an anti-osteogenic effect of leptin based on the greater cancellous bone volume of *ob/ob* mice, demonstrating an increase in bone formation sufficient to overcome the elevated bone resorption [3]. In contrast, 6 months later, Steppan et al. [4] reported the reduced bone mass of *ob/ob* long bones and the increase in bone size and mass after leptin treatment, even in the face of reduced body weight. They concluded that leptin had a powerful, positive effect on bone mass and growth. Thus, the early picture of leptin's action in bone, derived as it was from murine models, was somewhat controversial. Nonetheless, these initial studies generated significant interest and conjecture surrounding this pathway, with subsequent investigation in both animal and human systems. This review aims to examine recent literature in order to seek a consensus view on the skeletal response to leptin, as well as highlight potential confounders.

Murine Modeling of Leptin Action

A number of studies have examined the apparently dichotomous skeletal response in leptin-deficient mice. It is now established that leptin deficiency does indeed have differing effects on cortical and cancellous bone. The original "high bone mass" phenotype of *ob/ob* mice could only be confirmed for cancellous bone. In contrast, other parameters were found to be reduced, including total body bone mineral density (BMD), long-bone bone mineral content (BMC), cortical area, and mineralizing surface, clearly defining this mouse model as "low bone mass" phenotype. Although leptin deficiency also reduced longitudinal bone growth [4], the reductions in cortical bone were also

evident in *ob/ob* mice on a mixed background in which bone length was unaffected [5•], reinforcing the decrease in bone mass as a postdevelopmental occurrence. A number of theories have been put forward concerning the cortical–cancellous dichotomy, including mineral sparing during the predominant downturn on anabolism during severe caloric restriction [6]. A recent article suggested that increased leptin during obesity may represent a mechanism for increasing bone size, cortical bone density, and thus bone resistance to cope with increased body weight, with cancellous bone reduced in response [7]. Consistent with the focus on calorie-induced changes in bone mass, Tatsumi et al. [8••] recently demonstrated that caloric restriction in control mice and rats suppresses bone formation, resulting in reduced bone mass. This effect was absent in leptin receptor–defective *db/db* mice and inhibited by treatment with a β-adrenergic blocker. However, additional studies are needed to determine whether this is a direct effect of high-dose leptin or, as is more likely, due to reduced energy intake and hormonal changes such as decreased insulin-like growth factor-1 levels.

Central Effects of Leptin on Bone

It is now well established that bone metabolism is under the direct control of centrally mediated pathways. Both sensory and autonomic nerve fibers innervate bone tissue [9]. The presence of several neuropeptides and neurotransmitters in bone, including adrenaline, noradrenaline, serotonin, calcitonin gene–related peptide, vasoactive intestinal peptide, and neuropeptide Y (NPY), supports a direct signaling system between the brain and bone, with accumulating evidence suggesting that these neuropeptides are directly involved in the regulation of bone remodeling [9,10].

Leptin crosses the blood–brain barrier and binds to its receptors in the hypothalamus after being secreted into the circulation by adipocytes. The long form of the leptin receptor, Ob-Rb, is the only form that has been shown to mediate signal transduction in vivo [11] and is highly expressed in the hypothalamus, especially in the arcuate nucleus and ventromedial hypothalamic nucleus [12]. A central anti-osteogenic role for leptin was conclusively demonstrated by decreasing cancellous bone volume after intracerebroventricular infusion of leptin into ovariectomized *ob/ob* and wild-type mice [3]. This was further supported by parabiosis, whereby cancellous bone mass was significantly reduced in the mice that received intracerebroventricular leptin, without change in bone mass in the contralateral mouse [12]. The central leptin pathway was recently investigated using the more targeted approach of a single hypothalamic intracerebroventricular injection of leptin, producing adeno-associated virus in *ob/ob* mice. The resultant normalization of body weight and correction of skeletal abnormalities indicated that hypothalamic leptin functions as a permissive factor for normal bone growth [13••]. Consistent with initial studies, femur and vertebral cancellous bone volume decreased to wild-type levels, reinforcing the anti-osteogenic action in this skeletal envelope. In contrast, however, femur length and total femur bone volume were increased to wild-type levels, highlighting the pro-anabolic actions of leptin, and moreover, emanating from the same region of the brain. Importantly, these effects occurred despite no detection of leptin in the circulation. Thus, the central hypothalamic regulation of bone by leptin is sufficient without peripheral or local effects. Moreover, this study clearly identifies the dichotomous skeletal effect of this hormone's actions in the hypothalamus.

Involvement of the Sympathetic Nervous System

The sympathetic nervous system (SNS) has been identified as an important downstream mediator of the central effects of leptin on bone. It has long been established that *ob/ob* mice have decreased sympathetic tone [14] and that leptin infusion to the ventromedial hypothalamus (VMH) causes catecholamine secretion [15]. Functional β2-adrenergic receptors have been demonstrated in mouse primary osteoblast cultures [12], a requirement for central sympathetic signaling to be able to directly control bone metabolism. Dopamine β-hydroxylase–deficient mice that are unable to produce the catecholamine ligands adrenaline and noradrenaline have greater cancellous bone mass, which is unchanged by intracerebroventricular infusion of leptin [12]. Normalizing the cancellous bone mass of *ob/ob* mice without altering body weight or bone resorption parameters by the β-adrenergic receptor agonist isoproterenol further supports the involvement of the SNS [12]. This was complemented with blockade of the SNS by the β-adrenergic receptor antagonist propranolol, resulting in increased cancellous bone mass in wild-type mice and again protecting *ob/ob* mice against cancellous bone loss by intracerebroventricular leptin [12]. Thus, it is clear that modulation of SNS activity can regulate bone metabolism and that SNS signaling is essential for the cancellous anti-osteogenic actions of leptin.

Interaction with NPY

Although adrenergic signaling is consistent with the cancellous effects of leptin, the mechanism responsible for the opposing cortical effect is less well defined. One possibility is the change in NPY levels in hypothalamic neurons subsequent to leptin signaling. Leptin-deficient mice show an increase in hypothalamic NPY expression [16], whereas leptin binding in the hypothalamus results in a suppression of NPY expression [17]. Intracerebroventricular infusion of NPY into wild-type mice led to a significant reduction in cancellous bone volume [3]. Similarly, increased NPY expression confined to the hypothalamus by viral vector–mediated delivery reduced bone mass and bone formation [18]. Leptin and Y-receptors are coexpressed on NPY neurons in the arcuate nucleus and have been shown to interact in mediating leptin's effects on energy homeostasis [19] and

thus may also be linked to some extent in regulating bone homeostasis. Blocking central NPY signaling increases bone mass. Deletion of the Y2 receptor solely from the hypothalamus of adult mice produced a significant increase in cancellous bone volume [20] as well as a 40% increase in cortical bone mass and density along the entire femur [5•]. A recent series of papers suggests that Y1 receptors on bone cells may represent the link between the hypothalamus and bone [21,22]. The NPY-based models induce unidirectional changes in bone anabolism throughout the skeleton [20,22,23]. Thus, increased NPY signaling may account for the decrease in cortical bone mass of leptin deficiency; however, this also suggests that adrenergic signaling is capable of overriding this effect in cancellous bone. Consistent with this hierarchy, NPY effects are more pronounced in cortical than cancellous bone, and when *ob/ob* and $Y2^{-/-}$ mice were crossed, bone formation was greater in cortical bone in $Y2^{-/-}$*ob/ob* double mutant mice compared with *ob/ob* but was not elevated in cancellous bone [5•,18]. Thus, the skeletal response to leptin may represent the coordination of multiple centrally mediated pathways.

Peripheral Effects of Leptin on Bone

Similar to experiments advocating leptin as a centrally acting bone regulator, ample evidence has accumulated to identify a local and direct effect of leptin on bone metabolism. Despite early evidence to the contrary [3], it has been clearly demonstrated in vitro and in vivo that leptin and its signal-transducing receptor Ob-Rb [24] are expressed in primary cultures and bone cells of human [25,26] and murine [27,28] origin. Moreover, further evidence showed presence of the signal-transducing Ob-Rb leptin receptor on ossifying fetal cartilage [28], immortalized marrow stromal cells [29], chondrocytes [4,30–32], and primary osteoblasts [4]. Thus, it is possible that a local circuit exists in bone tissue, with both ligand and receptor present in osteoblasts.

Bone marrow stromal cells (BMSCs) can differentiate into osteoblasts, adipocytes, chondroblasts, and myoblasts [33]. Direct leptin signaling may stimulate osteoblastic differentiation and suppresses adipogenesis, directing BMSCs to the osteogenic rather than the adipogenic pathway. Consistent with this, a dose- and time-dependent increase in osteoblast markers and decrease in adipocyte markers was evident in a human stromal cell line after leptin administration [29]. Besides the differentiation effect of leptin on BMSCs, leptin treatment has been demonstrated to stimulate human and rat osteoblast proliferation and mineralization in vitro, leading to an increased number of mineralized nodules [25,31,34,35]. Leptin has also been shown to inhibit apoptosis, hence enhancing osteoblastic activity, as shown in primary human osteoblast cultures [35]. Such direct responses are consistent with findings of elevated bone mass and protection from osteoporosis in obesity and its concurrent elevation in serum leptin levels. Moreover, obesity is associated with central leptin insensitivity and reduced transmission of leptin across the blood–brain barrier [36]. Thus, obesity may preferentially promote peripheral leptin signaling over central, thereby stimulating bone formation.

Interestingly, leptin exerted a similar stimulatory osteogenic effect on BMSCs isolated from both control and osteoporotic donors, whereas it significantly inhibited adipogenic differentiation only in control cells but not in osteoporotic cells [37•]. Thus, in addition to a direct stimulation of osteoblast production, the leptin response in bone has further complexity as a result of the changes in adipocytes within the bone microenvironment. The anti-adipogenic response of leptin can be seen clearly in the marked increase in marrow adipocyte numbers in *ob/ob* mice, and changes in adipocyte number would alter leptin concentration in a local manner. However, marrow adipocytes are also regulated by central leptin signaling; injection of leptin into the VMH of rats reduced bone marrow adipocytes and increased adipocyte apoptosis [38]. Thus, the marrow adipocyte and its ability to locally produce leptin as well as respond to central signaling has the potential to act as an integrator of serum and hypothalamic leptin tone. Indeed, marrow fat in adults is now being considered a risk factor for low bone mass and fractures [39].

Association of Leptin and Bone in Humans

Given the previous discussion, it is not surprising that studies concerning the role of leptin in the regulation of bone metabolism in humans have yielded conflicting results. Some studies report both positive and negative associations of leptin with BMD, whereas other studies reveal no associations between leptin and skeletal status.

Both bone mineral and serum leptin concentration increase at the initiation of puberty [40]. However, other studies showed no association between serum leptin concentration and total-body BMD [41,42]. In healthy premenopausal women, it appears that plasma leptin concentration is positively associated with BMD [43]; however, other studies found no direct effect on BMC and BMD values after adjustment for body mass index (BMI) [44]. On the other hand, studies in perimenopausal women showed that serum leptin levels were negatively correlated with lumbar spine and total-body BMD [45].

Studies in postmenopausal women showed either a lack of association (after adjustment for BMI) [46] or positive association [43,47] with BMD. Di Carlo and colleagues [48] could not detect any correlation between leptin status and bone in a cross-sectional study of healthy postmenopausal women. These authors did find a significant correlation between leptin levels, BMI, and total-body BMD in a longitudinal evaluation of early postmenopausal women [49]. However, the correlation with BMD is lost over time during the progression of the postmenopausal period. Findings in men are similarly mixed. Studies have reported either no association [43,46] or a negative association [50–52] between leptin and BMD.

Association of Leptin Receptor Gene (*LEPR*) Polymorphism and Bone in Humans

One factor that may contribute to this variation in skeletal responses to leptin is the existence of a number of *LEPR* polymorphisms. Limited information is available on the relationship between *LEPR* polymorphisms and bone. However, a more widely investigated polymorphism is a Gln223Arg polymorphism of the *LEPR* gene. The substitution of an arginine for a glutamine occurs in the extracellular domain common to all isoforms of the leptin receptor, with potential functional consequences on leptin binding activity [53].

Significant associations between *LEPR* genotypes and BMD and vertebral fracture risk have been shown in postmenopausal white women, linking lower BMD and increased risk of vertebral fracture with heterozygotes at the Gln223Arg [54]. However, in elderly men, *LEPR* polymorphisms were shown to have no important role in either baseline BMD or longitudinal change of BMD at the hip or the forearm [55]. A study by Koh and colleagues [56] indicates that the association between Gln223Arg polymorphism of *LEPR* and BMD in young men is influenced by estrogen receptor α gene polymorphisms. In agreement with this, *LEPR* Gln223Arg polymorphism was shown to be associated with bone mass through indirect mechanisms in growing boys [57].

Conclusions

The biology of leptin is complex. The pleiotropic nature of this hormone makes isolation of individual effects (eg, on bone) extremely challenging. Leptin may signal to bone on multiple levels, with local and systemic checks and balances impacting the final outcome. As a result, we still must define the clinical utility of this pathway. The central regulation of bone remodeling is clearly established and remains a fertile area of research, which may provide much needed therapies for stimulating bone formation as well as critical insight into skeletal responses to a number of conditions, such as anorexia nervosa and depression.

Disclosures

No potential conflicts of interest relevant to this article were reported.

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