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Editorial

Leptin and the skeleton—where is the fat?

Despite years of active research, there is still debate over the skeletal effects of leptin. Leptin is a hormone well known for its importance in the regulation of food intake and energy expenditure, as well as neuroendocrine function and both glucose and fat metabolism. However, its effects on bone turnover and bone density are still under investigation. Two articles in this month's edition of *Metabolism* afford insight into the effects of leptin replacement therapy in two unique clinical models: hypothalamic amenorrhea (a state of leptin deficiency) and obesity (a state of leptin tolerance or resistance) [1,2]. In these studies, the efficacy of leptin replacement appeared to vary depending on the body composition and accompanying hormonal profile of the individuals studied. The differing effects of leptin replacement on the skeleton in each setting may answer important questions about leptin physiology and provide new therapeutic insights to guide clinical care.

The role of leptin in skeletal physiology should be considered in the broad context of bone-fat interactions. Bone and energy are related functionally through a complex neuroendocrine circuit involving leptin (derived from fat cells), the hypothalamus, and the sympathetic nervous system [3]. Leptin regulates appetite and energy use, and appears to play a permissive role in reproductive function [4,5]. This adipokine crosses the blood-brain barrier and binds to a receptor within the ventromedial nucleus of the hypothalamus. In mice, absence of leptin results in high bone mass thought to be due to reduced sympathetic tone innervating β2 adrenergic receptors on osteoblasts. However, the system is extremely complex and involves other hypothalamic networks, such as the neuropeptide cocaine- and amphetamine-regulated transcript, melanocortin 4 receptors, the neuropeptide Y receptor system, and the brain-gut peptide neuromedin U [3,6]. Cannabinoid receptors regulating appetite and energy balance also affect bone turnover both centrally and peripherally, primarily by blocking sympathetic innervation [7].

In addition to body composition, the composition of bone marrow is important to consider in understanding bone-fat interactions and the potential contribution of leptin. Within bone marrow, osteoblasts and adipocytes arise from the same mesenchymal stem cell precursors. Entry of these mesenchymal stromal cells into the fat lineage is mediated via activation of peroxisome proliferator-activated receptor γ (PPARγ), a transcription factor involved in the regulation of adipogenesis and osteogenesis. A line of therapeutic agents, the thiazolidinediones, activates the PPARγ complex. These PPARγ agonists promote adipogenesis within marrow and play dual roles: the enhancement of insulin sensitivity and the promotion of stromal cell differentiation to adipocytes. Because of the ultimate fate of stem cells and compromise of osteogenesis, the thiazolidinediones have been associated with bone loss and increased fracture risk [8].

The skeletal effects of leptin are difficult to understand in part because discrepant findings are seen in studies of rodents vs humans and considering whether leptin is administered peripherally or centrally (ie, via the central nervous system). Autonomic function may also be independent of regulation by leptin in humans, potentially explaining the differences in mice and humans [9]. There is consensus that leptin modulates bone metabolism [10,11], although there is debate regarding its influence on bone formation vs resorption. In vitro, leptin stimulates osteoblast and chondrocyte formation and inhibits osteoclastogenesis [12,13]. In vivo, peripheral administration of leptin to leptin-deficient animals results in reduced bone fragility [13]. Intracerebroventricular leptin administration to both leptin-deficient and wild-type mice resulted in restored bone volume in both the femur and vertebrae in one study [14], while leading to decreased bone mass in other models [15,16]. Data are also conflicting from observational and interventional human studies of leptin administration in various low-leptin states. Proof-of-principle studies have been carried out that explore the effect of leptin administration to subjects with either complete leptin deficiency (caused by mutations in the leptin gene) or relative leptin deficiency (lipoatrophy or negative energy balance and neuroendocrine dysfunction, as is seen in young women with exercise-induced hypothalamic amenorrhea or that which stems from anorexia nervosa) [4,5,17]. Varying skeletal effects after leptin administration have been reported, ranging from no change to an increase in bone formation [4,17-20]. The discrepant findings may reflect the heterogeneity of the underlying conditions studied. Results from these clinical studies suggest that metabolic and neuroendocrine function can be corrected by administration of exogenous leptin. Therefore, leptin replacement therapy may hold promise for several conditions, including congenital leptin deficiency, states representing energy deficits (eg, anorexia nervosa and other forms of hypothalamic amenorrhea), in addition to syndromes of leptin resistance (eg, congenital or acquired

lipodystrophy). In the case of lipodystrophy, circulating levels of hormones secreted by adipose tissue, such as leptin and adiponectin, are greatly reduced in subpopulations of these patients, rationalizing the use of leptin add-back replacement therapy or novel agents to augment circulating leptin concentrations (eg, PPARγ agonists). In contrast, states of energy excess such as simple (or so-called garden variety) obesity are associated with hyperleptinemia that reflects either leptin tolerance or resistance [21]. As the data of Conroy et al illustrate [2], these patients appear to be resistant to the skeletal effects of exogenous leptin administration.

The current studies of Sienkiewicz et al [1] and Conroy et al [2] suggest that the skeletal effects of leptin differ depending on whether leptin is administered to an individual who is deficient, tolerant, or resistant to leptin. In the leptin-deficient model (ie, women with hypothalamic amenorrhea), a sensitivity to leptin administration was seen, with evidence of efficacy as assessed by a 4% to 6% increase in spinal bone mineral density (BMD) in the 4 young women followed over 2 years of treatment with metreleptin [1]. The investigators studied 20 hypoleptinemic young women with amenorrhea for at least 6 months. Eleven were randomized to metreleptin and 9 to placebo for 9 months. After a 3-month washout period, 6 subjects continued on open-label metreleptin treatment for another 12 months. Two subjects discontinued the protocol; 4 completed the entire 2-year study. These changes in BMD were accompanied by increases in the bone-trophic factor insulin-like growth factor I and decreases in cortisol and cross-linked C-terminal telopeptides of type 1 collagen, the latter representing markers of bone resorption. Other markers of bone remodeling were not affected, and gains in BMD were not seen at other skeletal sites. In contrast, as studied by Conroy et al [2], leptin administration had no effect on bone biomarkers in the leptin-tolerant or leptin-resistant model of simple obesity. The investigators studied 12 adults who participated in an inpatient weight loss protocol, including 8 women and 4 men of whom 10 were obese. They examined the effects of weight loss and leptin administration on both calciotropic hormones and markers of bone remodeling. As expected, leptin concentrations decreased with weight loss. The bone formation marker bone alkaline phosphatase decreased, whereas the resorption markers N-telopeptides increased, after loss of a 10% weight loss (ie, 10% loss of baseline weight), but remained elevated after leptin administration. Interestingly, leptin did not prevent the uncoupling of bone remodeling that has been shown to accompany weight reduction and fasting [22-24].

What is the typical skeletal response to weight loss? Subjects in the studies of Sienkiewicz et al [1] and Conroy et al [2] illustrate a similar theme: bone formation decreases and bone resorption increases in response to weight loss. Loss of weight induces an alteration of bone turnover or mismatch in bone formation vs bone resorption. As was shown in healthy adults who underwent an acute fast of 4 days, a significant decrease in both serum leptin and concentrations of circulating bone formation markers was seen [25], similar to the findings in the study of underweight and obese adults, respectively, under discussion [1,2]. In malnourished adolescents with anorexia nervosa, the same hormonal profile is found, with low leptin and insulin-like growth factor I accompanying low levels of bone formation markers [26,27]. Even in mature adolescents with this disease, bone formation is reduced and uncoupled to bone resorption in association with a low bone density. A mouse model of caloric restriction [28] exhibited low circulating leptin and insulin-like growth factor I, and evidence of high resorption accompanied by a low bone formation rate. These mice also had increased marrow adiposity, replicating the finding of increased marrow fat in both the appendicular [29] and axial skeleton [30] in emaciated adolescents and young women with anorexia nervosa. As discussed earlier, within bone marrow, adipocytes and osteoblasts arise from the same progenitor cells, the mesenchymal stem cells. In situations of energy deficit such as weight loss, the stem cells may preferentially shift to form adipocytes rather than osteoblasts, leading to a compromise of bone formation. This consequence of energy restriction may ultimately have long-term implications for bone health. Whether leptin plays a role in the modulation of stem cell differentiation would be an important area to explore.

Another debate in the fat/bone field is the effect of obesity on the skeleton. Although some studies suggest that obesity in adults is protective for the skeleton (and low body weight is associated with fractures), data are emerging that obesity has a deleterious effect on a young, growing skeleton with respect to bone strength parameters and bone mineral accrual [31]. In particular, the adolescent skeleton appears to be particularly vulnerable to the structural and hormonal changes that accompany obesity. What is the role of leptin on an obese skeleton? Although obese rodents are less responsive to leptin administration than those of a normal weight, it is unclear whether obese human subjects exhibit leptin resistance [32]. Data from studies of obese and normal-weight adults have brought disappointment, showing little effect of leptin administration on body weight and suggesting either leptin tolerance or resistance in these individuals [33]. In the current study of Conroy et al [2], even after a 10% weight loss, the majority of subjects were still obese. As the authors discuss, these same protocol subjects were shown in other reports [34,35] to be responsive to leptin with respect to energy expenditure, decreased activity of the sympathetic nervous system, and changes in thyroid function, even though no effect on bone turnover markers was noted in the current report. Collective data from these protocols suggest that obesity represents a state of either leptin tolerance or resistance, at least with regard to bone metabolism. The findings also raise the intriguing possibility that differing thresholds of leptin resistance may exist for the varying physiological functions of this hormone.

Limitations of the articles under discussion merit consideration. Each was a pilot study of a small sample size. Therefore, larger trials are needed to confirm the preliminary findings found in each report. These studies also followed surrogate markers of bone turnover and obtained skeletal measures using dual-energy x-ray absorptiometry, each with inherent limitations. Dual-energy x-ray absorptiometry provides a 2-dimensional measurement of bone density and affords little information about skeletal strength, structure, or

geometry. Dual-energy x-ray absorptiometry measures in obese patients are also fraught with problems due to the projectional nature of these assessments. The bone remodeling markers have a low precision, and their utility is less clear in studies of adolescent and premenopausal women. In addition, the trends noted in these surrogate markers may not translate into ultimate changes in bone density in the populations studied, a point especially relevant to the report by Conroy et al [2]. The relatively short duration of the studies is noteworthy; and in particular, it is important to consider the small number of subjects in the study by Sienkiewicz et al [1] who were able to complete the 2-year protocol. Replication of results in larger studies of longer duration will be necessary, but the current data are compelling and set the stage for larger and more detailed studies. Lastly, use of skeletal assessment tools that capture changes in bone microarchitecture and strength will be needed in future studies to understand the fracture risk of these patient groups, both at baseline and in response to leptin administration.

Fat-derived leptin regulates energy homeostasis and metabolic, neuroendocrine, and reproductive function. As discussed, the importance of leptin in human physiology is highlighted by findings from recent "proof of concept" trials that test the effect of recombinant human leptin in subjects with congenital leptin deficiency, hypoleptinemia associated with energy-deficient states, and hyperleptinemia associated with simple obesity [21]. The pilot studies highlighted in this month's edition of *Metabolism* demonstrate that weight reduction is associated with bone loss in both lean and obese adults. The studies also provide evidence for efficacy in the leptin-deficient state and a lack thereof in the face of leptin tolerance or resistance. Because most obese individuals appear to be resistant to the skeletal effects of leptin, the therapeutic use of this agent does not appear to be a viable treatment option to prevent bone loss that accompanies weight reduction in these patients. However, the development of leptin sensitizers is under way and represents an exciting new focus in pharmaceutical research. Whether combination therapy with leptin and potential leptin sensitizers will prove effective in the management of simple obesity represents a gap in knowledge and an important area for further investigation. In contrast, leptin administration is an appealing option for use in energy-deficient states to restore neuroendocrine function and normalize bone turnover, as suggested by the current data of Sienkiewicz et al [1]. However, the long-term safety and efficacy of leptin treatment of hypothalamic amenorrhea are under investigation and merit confirmation in larger longitudinal trials. If proven to be both safe and efficacious in these future studies, leptin replacement could emerge as a therapy to preserve bone mass in the large number of young women with hypothalamic amenorrhea from eating disorders, as well as that induced by exercise. If shown to be viable as a longer-term therapy (>1 year), this new therapy could have important public health implications, given the high incidence of this problem among adolescent girls and adult women. Lastly, results from these ongoing trials will provide important scientific insights on leptin biology and its regulation of fat/bone interactions.

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