ACTION OF LEPTIN ON BONE AND ITS RELATIONSHIP TO MENOPAUSE

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Background: Leptin a cytokine protein secreted by adipose tissue raises considerable interest as a potential mediator of the protective effects of fat mass on bone tissue. After menopause heavier women conserve bone mass better than those with lower body weight. The protective effect of obesity on bone mass has been ascribed to a high body fat content. As Leptin levels reflect the body fat content it has emerged as a possible mediator of these protective effects.

Methods: A search of the available literature focused on the role of leptin on bone tissue.

Results: Both peripheral and central action of leptin on bone metabolism have been proposed. In vitro and in vivo evidence supports the hypothesis that leptin can act directly or indirectly on bone remodelling by modulating both osteoblast and osteoclast activities. However, studies in humans have not yet been able to confirm these actions possibly because of the shifting balance between stimulatory direct action and suppressive indirect action of leptin on bones via the hypothalamus. The effects of oestrogen decline and deficiency during natural or artificially induced menopause and administration of hormone replacement therapy has on leptin production remains controversial. Various studies have shown differences in leptin values in pre- and postmenopausal women. The existing clinical data on this issue are discordant.

Conclusion: Larger clinical studies are necessary to clarify leptin's role in vivo and to assess the contribution of the central and peripheral role of leptin in the overall maintenance of bone turnover in human beings.

INTRODUCTION

Leptin, a member of the helical cytokine family is the soluble 16-kDa product of the *OB* (*Lep*) (*Ob* for obese and *Lep* for leptin) gene on human chromosome 7q31.3 and chromosome 6 in mouse¹. Leptin is mainly produced by white fat cells, it is also produced by brown adipose tissue, placenta (syncytiotrophoblasts), ovaries, skeletal muscle, stomach (lower part of fundic glands), mammary epithelial cells, bone marrow, pituitary and liver¹. Leptin first emerged as a component of a regulatory loop linking fat mass to food intake and energy expenditure but later also raised considerable interest as a potential mediator of the protective effects of fat mass on bone tissue². The protective effect of obesity on bone mass has been ascribed to a high body fat content. Leptin is produced by adipose cells, with its levels reflecting the amount of adipose tissue, so leptin logically emerged as a possible mediator of protective effect of fat tissue on bone. Leptin exerts its effects through interaction with six types of receptors. Leptin receptors are divided into secretory (*ObRe*), long (*ObRb*) and short forms (*ObRa*, *ObRc*, *ObRd*, *ObRf*). (*ObRe*) acts as a binding protein for leptin in the plasma in humans and mice and is important for leptin transfer into the brain. *ObRb* is the only receptor isoform that contains active intracellular signaling domains. This receptor is found in a number of hypothalamic nuclei where it exerts its effects. *ObRb* receptors are also found in other cells including osteoblasts. Leptin binds to the Ventral Medial nucleus of the hypothalamus, known as the "satiety center" where it signals to the brain a sensa-

tion of satiety. Leptin also works by inhibiting the activity of neurons that contain the neuropeptide Y (*NPY*) and agouti-related peptide (*AgRP*), and stimulating neurons expressing α -melanocyte-stimulating hormone (α -*MSH*). The *NPY* neurons are a key element in the regulation of appetite. Once leptin has bound to the *ObRb* receptor, it activates the *stat3* molecule, which is then phosphorylated and travels to the nucleus, where it is presumed, to effect changes in gene expression. One of the main effects on gene expression is the down-regulation of the expression of the endocannabinoids, responsible inter alia for increasing appetite. There are several other intracellular pathways activated by leptin, but less is known about how they function in this system.

Mechanism of leptin action on bone

The exact influence of leptin on bone metabolism has not yet been clarified. The literature is inconsistent and conflicting. Leptin is emerging as central in two entirely different bone-controlling mechanisms. The first is the indirect one. It was discovered by Ducy and al.^{3–8} in mutant mice and rats that either cannot produce or cannot respond to, leptin. Leptin secreted from fat cells is carried by the *ObRa* receptors of vascular endothelial cells across the blood-brain-barrier where it activates *ObRb* receptors in the hypothalamus. These signals then stimulate expression of *HOBIF* (hypothalamic osteoblast inhibitory factor) which when released, lowers the matrix-making ability of osteoblasts^{2–8}. These authors discovered what they believed to be the reason why obese *Ob(Lep)*^{-/-} mice, which should have low bone mass because of their lack

of leptin, and thus estrogen, actually have an abnormally high bone mass³⁻⁸. They found that intracerebroventricular injections of leptin actually caused the bone mass to decrease. It is assumed that leptin stimulates the brain to release HOBIF. Another part of central control mechanism of leptin is the NPY and its Y2 receptor, which may stimulate HOBIF release. In mice lacking neuropeptide 2 (NPY2) receptors in the hypothalamus deletion of hypothalamic NPY2 receptors resulted in increased bone mass without any obvious endocrine imbalance that would affect bone homeostasis, which shows that these receptors regulate bone formation by altering autonomic function⁹. A loss of either Y2 receptors or leptin should reduce HOBIF and increase osteoblast productivity⁹. Eleftheriou et al.¹⁰ found that NPY-deficient mice have normal bone mass, which is in contrast to the mice deficient in NPY2 receptor. NPY4 receptor deficient mice have normal bone mass, whereas double knockout of NPY2 and NPY4 receptors leads to a greater increase in bone volume than in mice deficient only in the NPY2 receptor¹¹. Both receptors modulate sympathetic outflow in the brain stem. Augmented bone formation in the double knockout mouse suggests synergistic activity between NPY2 and NPY4 receptor pathways¹¹. The second direct mechanism of leptin exerts its osteotrophic effects by promoting differentiation of bone marrow stromal cells into osteoblasts¹² and by inhibiting osteoclast generation¹³. Circulating leptin penetrates the bone marrow, where it joins the autocrine/paracrine leptin from early cells of the osteoblast lineage and from late-stage, matrix-mineralizing osteoblasts or from early osteocytes to stimulate the production of IGF-1. IGF-1 in turn stimulates the proliferation of osteoblast precursors makes the osteoblastic lineage cells more resistant to apoptosis, and further enhances bone formation through suppression of osteoclast generation by causing osteoblastic stromal cells to make less RANKL (NF-kappaB ligand) which stimulates osteoclast generation, and by stimulating the production of the osteoclast-suppressing OPG (osteoprotegerine) the osteoclast suppressor. Osteoblasts freshly isolated from adult humans have functional ObRb receptors¹⁴. Bassilana et al.¹⁵ have found that human mesenchymal stem cells begin expressing both the nonfunctional, leptin transvascular-transporting ObRa, the fully functional long ObRb signal-transducing receptors, and leptin itself during osteoblastic differentiation. Reseland et al.¹⁶ have found that human osteoblasts start making and secreting leptin when they are either in the late, matrix-mineralizing stage or when changing to osteocytes. Leptin has also stimulates the proliferation of cultured human osteoblasts¹⁷, and it has been shown to cause human bone marrow stromal cells to express alkaline phosphatase, collagen-I, and osteocalcin and to mineralize matrix¹².

Although data from cultured human bone cells point to leptin as a direct stimulator of bone growth and a suppressor of bone resorption, the correlation between the serum leptin level and BMD (bone mineral density) in humans is so far equivocal, possibly because of a shifting balance between the cytokine's stimulatory direct action and sup-

pressive indirect action on bones via the hypothalamus. In human beings, positive, negative, or no associations between serum leptin levels and bone mineral density have been reported. Pasco et al.¹⁸ have reported that serum leptin levels are positively correlated to the bone mineral content. Results of Blain et al.¹⁹ demonstrated that leptin is an independent predictor of whole body and femoral neck BMD in postmenopausal women. Yamauchi et al.²⁰ showed that in postmenopausal women plasma leptin levels were positively correlated with BMD values. Moreover, plasma leptin levels were significantly lower in women with vertebral fractures than in those without fractures. The study of Di Tomaselli et al.²¹ also confirms a significant correlation between serum leptin and BMD in early postmenopausal women, but the correlation was lost during progression of the postmenopausal period. Thomas et al.²² have reported that BMD is positively correlated to fat mass and the serum leptin level in women. Z. Nagy et al.²³ found a negative correlation between serum leptin levels and radial and femoral BMD in postmenopausal women. On the other hand P. Hadji et al.²⁴ reported that bone mass is not correlated with the serum leptin level in pre or postmenopausal women. Goulding et al. found no significant correlations between plasma leptin and dynamic markers of bone resorption or formation²⁵. Rauch et al. also failed to find a relationship between bone mass and serum leptin levels by examining total and trabecular bone density at the distal radius in adult women and reported no correlation, between plasma leptin level and bone turnover markers in adult women²⁶.

Differences in leptin levels between men and women

Leptin possesses dimorphism, it is higher in women than in men. Leptin pulse amplitude is two to three times higher in women than in men and the expression rate of leptin mRNA in subcutaneous fat tissue is significantly higher in females than in males²⁷. Moreover, in vitro, the leptin secretion rate of men's subcutaneous adipose tissue is 66 % that of women's²⁸. This would suggest a dependence of leptin on estrogen. Some authors admit the difference may result not from higher estrogen level in women, rather from a suppressive effect of high levels of circulating androgens in men on leptin production^{29, 30}. It has been suggested that androgens reduce the regulation of leptin secretion^{29, 31}. Administration of testosterone reduces the expression of leptin mRNA in men. However, the influence of circulating androgens is not supported by other studies^{33, 34}. In these studies, leptin levels were inversely correlated with androgen levels.

Leptin and menopause

Postmenopausal women, whose estrogen levels naturally or artificially decline, are at the highest risk for developing osteopenia and osteoporosis. The influence of hormonal changes during menopause on leptin levels is still poorly understood. It is well documented that the serum concentration of leptin secreted by adipocytes correlates positively with the amount of fat mass and BMI³⁵⁻³⁸. It appears that percent body fat in postmenopausal

women may be the best adiposity-related predictor of serum leptin³⁹. As a consequence of the decline in endogenous estrogens and reduced energy during menopause women tend to gain body fat⁴⁰. After menopause heavier women conserve bone mass better than those with lower body weight. The protective effect of obesity on bone mass has been ascribed to a high body fat content. As a result of menopause transition the levels of circulating estrogens decrease whereas the adrenal glands continue to secrete androgen precursors. Inactive steroid precursors are aromatized to estrogens in adipose tissue. The relative increase in the androgen/estrogen ratio is likely to be important for the fat distribution shift⁴¹. Postmenopausal women manifest shift to a abdominal fat distribution (android type of obesity), which is associated with a more androgenic profile with increased free testosterone levels and lower sex hormone-binding globulin (SHBG) levels. The shift to abdominal adiposity shows itself in the changes of WHR (waist to hip ratio). The connection between serum leptin concentration and WHR is controversial. Some authors have shown that serum leptin levels are higher in android obese women than in gynoid ones⁴², but other studies demonstrate a lack of correlation between serum leptin concentration and the type of obesity. Martinez-Carpio et al. found no correlation between leptin and WHR in postmenopausal women, but they found significant correlations between leptin and weight, BMI and chest, waist and hip circumferences⁴³. This is in agreement with the results of Tupikowska and Minocci et al.^{34, 44}. They also showed that WHR values were not correlated with leptin concentrations⁴⁴. They suggest that subcutaneous abdominal fat is a determinant of leptin concentration, independently of the amount of fat mass, whereas the contribution of preperitoneal visceral fat is not significant.

Various studies have shown differences in leptin values in pre- and postmenopausal women. Some authors suggest that serum leptin concentration are lower in the post-menopausal period. Rosenbaum et al.²⁹ Shimizu et al.⁴⁵ They report that pre-menopausal women have higher leptin production than postmenopausal women. However there are also reports indicating that decrease in estrogen levels in postmenopausal status has no direct effect on adipose tissue leptin production or concentrations^{31, 34, 45-48}. There are only few studies comparing the leptin level in women with artificially induced menopause before and after ovariectomy. When women with previously normal cycles before surgery were studied during the fourth post-operative day period after bilateral ovariectomy a significant reduction in leptin concentrations was found. There was a positive correlation of leptin values with estradiol and progesterone before and after the operation⁴⁹.

Several authors have investigated plasma leptin levels in postmenopausal women during administration of HRT (hormone replacement therapy). Some results show that estrogens can regulate leptin production and its serum concentration⁴⁵. Some studies reported no differences in serum leptin levels between users and non - users of hormonal replacement therapy, while others showed both de-

creased and increased leptin levels. Existing clinical data addressing this issue are discordant. Di Carlo et al.⁵⁰ found increased serum leptin level in untreated postmenopausal women decreased to premenopausal values after hormonal treatment. These results suggested that sex hormone replacement therapy can modulate leptin levels. In obese and non-obese postmenopausal women studied before and after HRT administration, plasma leptin levels showed an increase following treatment which was more pronounced in obese women⁵¹. Also intranasal and oral HRT had the effect of increasing the levels of leptin after 24 weeks in healthy postmenopausal women⁵². Other authors indicate that neither endogenous E2 nor sex hormone replacement therapy has any effect on serum leptin concentration³⁴. These results support the hypothesis that the expression of leptin in adipocytes and circulating levels of this hormone are not related to the estrogen levels and type of hormonal therapy used. Hickey et al. analyzed data on postmenopausal women taking part in a cardiovascular risk factor study and found that leptin concentrations were similar in sex hormone users and non-users⁵³. Also the results of the San Antonio heart study also showed no relation between serum leptin levels and menopausal status or postmenopausal sex hormone use⁵⁴. Gower et al. compared HRT-treated postmenopausal women with untreated controls. There were no significant determinants of serum leptin levels⁵⁵. In a randomized placebo-controlled study in postmenopausal women with type 2 diabetes mellitus treated with transdermal E2 +NETA (estradiol + norethisterone acetate), no significant alterations in leptin levels were found⁵⁶. The group of Di Tomaselli evaluated serum leptin levels in healthy HRT treated and untreated postmenopausal women. In women taking HRT, serum leptin levels did not change significantly throughout the study period⁵⁷. In an large sample of healthy postmenopausal users and non-users of hormone replacement therapy, Hadji et al. showed that neither sex hormone supplementation nor serum estrogen concentrations had any effect on leptin concentration, even after adjustment for BMI²⁴. Other investigators take the possibility into account that differences in leptin level may depend on the type and dose of HRT used⁵⁸. Gower et al. measured leptin in women with an untreated spontaneous cycle and with a cycle treated with different types of HRT. Women treated with estradiol plus progesterone had elevated serum leptin concentrations, but when estradiol was administered alone, leptin concentrations were not affected⁵⁶. Serum leptin levels were increased in postmenopausal women treated with short-term HRT independent of changes in fat mass; in the same women, progesterone administration alone did not influence leptin levels. Some authors suggest that only a supraphysiological estrogen concentration can act on adipocytes and modulate leptin production. It is possible that different doses of estradiol exert a more pronounced effect on circulating leptin⁵⁹. Also studies in women with artificial menopause show similar results. Messinis et al. studied leptin levels in ovariectomized normal women treated with estradiol plus progesterone⁶⁰. They found that HRT prevented the ovariectomy-induced decrease in lep-

tin levels^{59, 61}. On the other hand administration of HRT to ovariectomized women starting 15 days after surgery did not modify leptin levels⁶².

Leptin administration as a treatment for osteopenia

Leptin might be used to treat osteopenia only if it can be engineered to avoid suppressive indirect effects on bones via the hypothalamus – avoid the hypothalamus and refrain from stimulating HOBIF expression. Only a very small group of humans is known to possess homozygous mutations for the leptin gene. Up to date, two United Kingdom families of Pakistani origin carrying a frameshift/premature mutation, and one Turkish family carrying a missense mutation, have been described²¹. This mutation leads to a constant demand for food, resulting in severe obesity. This condition can be successfully treated by the administration of recombinant human leptin and theroretically administration of leptin could be used to treat osteopenia in these subjects.

CONCLUSION

Although data from cultured human bone cells point to leptin as a direct stimulator of bone growth and a suppressor of bone resorption, understanding of leptin's role in human bone-remodelling is not yet defined. The exact role of leptin on bone mass remains unclear. The correlation between the serum leptin levels and BMD in humans is so far equivocal, possibly because of a shifting balance between the cytokine's stimulatory direct action and suppressive indirect action on bones via the hypothalamus. The effect that oestrogen decrease and deficiency during menopause and administration of hormone replacement therapy has on leptin production and contribution of leptin on emergence of osteopenia remains controversial. Existing clinical data addressing this issue are discordant. Larger clinical studies are therefore necessary to clarify leptin's role in vivo and to assess the contribution of the central and peripheral role of leptin to the overall maintenance of bone turnover in human beings and how leptin contributes to the mechanism of loss of bone mass during artificial and natural menopause.

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