Bone mass regulation of leptin and postmenopausal osteoporosis with obesity

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Summary

Background. **Leptin has been known to play a role in weight regulation through food intake and energy expenditure. Leptin also has an important role in bone metabolism. The role of leptin is determined by leptin receptors, either central or peripheral to the bones.**
Design. **We discuss the role of leptin on bone and molecular genetics of osteoporosis in postmenopausal obese women.**
Results. **The role of leptin in bone preserves bone mineral density (BMD) through increased OPG levels leading to bind RANKL, resulting in reducing osteoclast activity. The estrogen role on bone is also mediated by RANKL and OPG. In postmenopausal women who have estrogen deficiency, it increases the rate of RANKL, which increases osteoclastogenesis. Obese individuals who have a high level of leptin will be effected by bone protection.**
Conclusion. **There are similarities in the mechanism between estrogen and leptin in influencing the process of bone remodeling. It may be considered that the role of estrogen can be replaced by leptin. Molecular genetic aspects that play a role in bone remodeling, such as leptin, leptin receptors, cytokines (e.g. RANK, RANKL, and OPG), require further study to be useful, especially regarding osteoporosis therapy based on genetic analysis.**

KEY WORDS: leptin; leptin receptor; estrogen; RANKL; OPG.

Introduction

Leptin, first identified in 1995, is known as an OB (obese) protein which affects food intake and energy expenditure so that the changes in both determine body weight (1). Since then, both leptin and its receptors have been widely studied, and results show that leptin does not only have the role and function of metabolic functions, but also plays a role in neuroendocrine function, immune function, reproduction, and bone metabolism (2-4).
The correlation between leptin and bone metabolism is closely related to the function of leptin in central and peripheral bone, leptin levels and adipose tissue, leptin receptors, and cytokines which influence the leptin function on bone. Therefore, exploration of the correlation between leptin and bone is often associated with these variables. Investigation of leptin and its receptor will be useful for the treatment of bone metabolism diseases, particularly postmenopausal osteoporosis.

Leptin and its receptors

Leptin is a versatile 16 kDa peptide hormone, a member of the family of the long chain helical cytokine. It is mainly produced by adipocytes in proportion to fat size stores (5). Leptin is also produced by brown fat tissue, placenta (syncytiotrophoblasts), ovary, bone marrow, stomach (the lower part of the fundic gland), mammary epithelial cells, pituitary, and liver (6, 7). Leptin is encoded by OB gene (LEP) (OB - obese and LEP - Leptin), according to the name that was first proposed, 'leptos', derived from the Greek word meaning thin, as ob protein is considered to be one of the molecules that regulates energy balance in mice (8). After leptin was identified, research showed the role of leptin in the regulation of body weight when leptin reduced that of ob/ob mice and did not respond in mutant mice. This situation is thought to be resistant to the effects of ob, and leptin replacement demonstrates the improvement of abnormalities in ob/ob mice in the forms of decreasing of body temperature, hyperphagia, decrease of energy expenditure (including activity), decreasing immune function, and infertility (1, 9). Since then, the knowledge which has been obtained from the studies of the role of leptin in body weight regulation has increased. Furthermore, leptin also plays important roles in angiogenesis, hematopoiesis, blood pressure, immune function, lymphoid organ homeostasis, T lymphocyte systems, fertility, and bone formation (6, 7).
Leptin action cannot be separated from the role of the leptin receptor. Since it was first isolated from mouse choroid plexus by expressing cloning, leptin receptors have been identified as a member of the cytokine family of receptors, and it binds leptin with nanomolar affinity (10). There are at least six leptin receptors, namely secretory (Ob-Re), long (Ob-Rb), and short forms (Ob-Ra, Ob-Rc, Ob-Rd, Ob-Rf) (10, 11). Long form leptin receptor (Ob-Rb or also known as Ob-RL) has a long cytoplasma region containing several motifs required for signal transduction (active intracellular signaling domain); therefore it has strong binding affinity because only Ob-Rb encodes all protein motifs capable of activating the Jak-Stat signal transduction pathway (9, 10, 12). The structure of the leptin receptor is similar to the helical cytokine receptor (class I). Leptin receptors form homodimers which are able to activate the Janus kinase (JAK), and Janus kinase is capable of starting as an activator of transcription (STAT - *signal transducer and activator of transcription*). Leptin signals through the activator system of Janus kinase transcription are associated with the form ObRb (isoform long form) which would alter the expression of hypothalamic neuropeptides (5, 13-15).
Ob-Rb is found expressed in high levels in the hypothalamus nu-

clei (10, 12). Ob-Rb activity is thought to play a role in mediating signal transduction by leptin in the hypothalamus, while the other leptin receptor activity (short form) is not strong enough in the function of leptin role (11, 12). The variation of leptin receptor genes which influences its role in body weight regulation was shown in the discovery of leptin receptor gene polymorphism, found exclusively in obese Pima Indians, which proved the variation in the leptin receptor gene in human obesity, and which will show significant changes in the role of leptin (16).

Central and peripheral action of leptin on bone

The definite mechanism influence of leptin on bone metabolism is unclear. The existing literature shows inconsistency and differences. Leptin in mice has been stated as an inhibiting factor of bone formation centrally through the release of a second factor present in fat or hypothalamic relay, the same as its weight control, and leptin was never known to have Ob-Rb on osteoblasts (17).

Central control of leptin on bone acts through by two mechanisms: 1) indirect regulation mechanism, first discovered by Ducy et.al in mutant mice which were unable to produce or respond to leptin; 2) Mice Ob (Lep) - / - have low bone mass because of a lack of leptin (17). When injected with leptin intra cerebroventricular, it caused a decrease in bone mass, because leptin stimulates the brain to release Hypothalamic Osteoblast Inhibitory Factor (HOBIF). Activation ObRb (long isoform of leptin receptor) in the hypothalamus stimulates HOBIF, in which, if secreted, it will reduce the ability of bone matrix formation by osteoblasts (17). Another indirect regulation mechanism is through the neuropeptide Y (NPY) and its receptor (Y2) which stimulate secretion of HOBIF. The loss of Y2 receptor or leptin will reduce productivity and increase osteoblast HOBIF (18-20).

The mechanism of the peripheral role of leptin is indicated in studies which show that expression of leptin receptor in osteoblasts through injection of leptin in mice affects growth and bone development; they are Ob-Rb signaling in osteoblasts and chondrocytes (21). Expression of leptin receptors in rat osteoblasts is determined, and it is able to transduce cell signaling by STAT3 phosphorylation (22). It also proves the activity of leptin receptors in the strong bones. Moreover, reinforced by evidence that leptin has an effect in reducing bone fragility, it contributes to an increase in bone mass and fracture at low levels of obesity (23). Leptin regulates directly on bone through the osteotrophic effect by increasing the differentiation of bone marrow stromal cells (BMSC) in osteoblasts, and inhibits the growth of osteoclasts. Leptin circulation does the penetration into the bone marrow and joins the leptin autocrine / paracrine from the early phase and late phase of osteoblast growth, from mineralized matrix by osteoblasts or osteocytes from the beginning to stimulate production of insulin-like growth factor-1 (IGF-1), in which IGF -1 then stimulates the proliferation of osteoblast precursors to the growth of osteoblastic cells more resistant to apoptosis (23).

Regulation of leptin in mesenchymal progenitor cell (MPC) differentiation and osteoblast function *in vitro* and *in vivo* were determined. Expression of functional leptin receptors by BMSCs was confirmed in an experiment of rapid phosphorylation of Stat3 after leptin treatment of bone marrow stromal cells (BMSCs) from mice with conditional deletion of ObRb in macrophages [(LysM(Cre+F/F)]. When ObRb in primary stromal cells was disrupted by Adenovirus-Cre, it mediated decreased mineralization and increased adipogenesis. Disruption of ObRb in primary stromal cells decreased mineralization and increased adipogenesis. In contrast, BMSCs harvested from leptin-signaling deficient Ob/Ob or Db/Db mice showed increased mineralization (24). The isolated osteoblasts from humans have ObRb receptors. Cultured human osteoblasts showed that leptin stimulates proliferation of osteoblasts, causing BMSC to express alkaline phosphatase, collagen-I, and osteocalcin and matrix mineralization (25).

Mechanism of action of leptin in increasing bone mass is also done by increasing osteoprotegerin (OPG), which would inhibit osteoclastogenenesis through the mediation receptor activator of nuclear factor-kB (RANK) / RANK ligand or OPG ligand / OPG (26). OPG functions as a soluble decoy receptor for RANKL and acts by competing with RANK, which is expressed on osteoclasts and dendritic cells for specifically binding to RANKL. The binding of RANKL to OPG would inhibit RANKL binding to RANK which then inhibits osteoclastogenesis (27).

Leptin, obesity and bone mineral density

Recent research on obesity in humans shows that the overall concentration of *mesangerRNA* (mRNA) of leptin on adipose tissue and serum leptin concentration is positively and closely associated with body fat mass. Leptin in the circulation presents in two forms: the free form (*biologically active form*), and the bound form (*leptin-binding proteins)*. Leptin is secreted in pulsatile with significant diurnal-nocturnal variations (28). Leptin functions regulate body weight as the afferent signal in a negative feedback loop. Weight gain results in an increased plasma leptin level which will respond with a biologic characterized by a state of negative energy balance. Weight loss in lean and obese subjects results in decreased plasma leptin levels, and leads to a state of positive energy balance and a number of other physiologic responses. In humans, the intrinsic sensitivity to leptin and its production rate vary, and both appear to contribute to differences in weight (29). Leptin concentration reflects the amount of energy stored in body fat, and circulating leptin levels are proportional to the amount of body fat. Subjects with a low-calorie diet had significantly lower concentrations of leptin compared to other obese people (30).

Bones as a part of the framework of the human body have a primary function as a strong framework to support, protect, and facilitate the function of soft tissues. Ribs, pelvis, and skull protect the contents of their cavity formations. In addition, ribs are important in breathing, and the long bones are important for the function of locomotion. If the sizes of the framework of all people were equal regardless of weight, some of the bones would certainly have difficulty fulfilling their duties, and it would not be beneficial if the existing order significantly more weight than their needs. In obese individuals, it is necessary to have more powerful bones than in thin individuals. Simply put, fat has a correlation with the frame (bone) (31).

Some studies show a relationship between fat and bone, the size of the bone mass which is commonly known as the bone mineral density (BMD), or bone mineral content (BMC). Data show that excess body weight or high BMI (body mass index) correlated with high BMD or BMC, or, on the contrary, thin or less weight and low BMI, would experience a loss of bone mineral (bone loss). This correlation occurs in both men and women, in all adults, and in almost the entire frame (32-35). This correlation also occurs in children and teenagers; although it is a significant correlation, it still remains unclear why the process of bone acquisition occurs during this period. Some evidence supports the fact that adipose mass acts as a component of total body weight, and is one of the indications of obesity which has the same advantage, namely the effect of increasing bone mass for reducing the risk of osteoporosis. Both during pre- and post-menopause, total body adipose mass is positively associated with BMD at all skeletal levels, and is found in all ethnicities (36, 37). However, fat does not always relate positively with BMD. In other studies, body weight and high percentage body fat were also related to low BMD and vertebral fracture (38).

Some evidence of environmental factors also supports an inverse

correlation between adipose mass and bone. For example, exercise can increase bone mass and decrease adipose mass. Tea and milk consumption is believed to prevent obesity and osteoporosis, and dairy products remain the best source of calcium which can be absorbed. Menopause has also been known to be associated with an increased loss of bone mass, increased adipose mass and decreased muscle mass (36, 37).

Studies on adipose cell function found that adipose tissue is not only a "lazy" and storing energy organ, but also an organ of expression and secretion of active biological molecules, such as estrogen, resistin, leptin, adinopektin and interleukin-6 (IL-6). These molecules have an impact on the homeostasis of human energy, and are involved in bone metabolism, which may contribute to a complex correlation to bone mass of adipose (37). Several mechanisms may explain the complex correlation between adipose mass and bone. One obvious mechanism which can be explained is the mechanism of stress factors. Large adipose mass will provide greater mechanical stress on the bones so that bones respond to it by increasing the power of accommodation. The reality of body adipose mass is only about 27% in men and 38% in white women. Hence, it is explained again by using this mechanism that there is a correlation of body weight, given that adipose mass and gravity increase mechanical stress on the bone (36, 37, 39, 40).

Genetics of leptin receptor gene and osteoporosis

Osteoporosis is defined as a reduction in bone mass and failure, leading to thinning and increased cortical porosity, bone fragility, and fracture risk (41). Bones are able to make what is called bone modeling, ie: the ability to adapt in shape and size in response to mechanical load by shaping and reshaping, using osteoblasts and osteoclast activity (41). Bone mass increases during growth in childhood and adulthood, meaning that during adulthood, bone remodeling is in the dominant process. The process will reach its peak in the third and fourth decade of life, and after that it will decrease progressively, especially in women (41). The accelerative large decline of bone mass occurs in postmenopausal women, due to the decrease of estrogen levels, so that the remodeling process increases (42).

Activities of osteoblasts and osteoclasts are controlled by various hormones and cytokines, as well as by mechanical loading (43). Potential hormonal mechanisms regulate bone loss, including decrease in estrogen and leptin (44). Decreased levels of estrogen in postmenopausal women are considered to be responsible for increasing bone resorption. Estrogen affects the differentiation of osteoblasts, so that the decrease of estrogen levels in postmenopausal women will reduce the activity of osteoblasts differentiation, while the bone resorption process runs as normal (43). Estrogen deficiency at menopause increases remodeling intensity, and a greater proportion of bone is remodeled on its endosteal (inner) surface, and within each of the many sites even more bone is lost as more bone is resorbed, while less is replaced (45). Moreover, it causes an imbalance between formation and resorption and, finally, it will lead to osteoporosis (42). Control of estrogen on osteoblast and osteoclast activity is thought to be by mediation of RANK, RANKL, and OPG. Some studies on the estrogen affect on bone modeling have shown that its mechanism through estrogen receptor estrogen deficiency enhances the ratio of RANKL to OPG and, thus, promotes osteoclastogenesis, accelerates bone resorption, and induces bone loss (46). The mechanism of action of estrogen mediated by cytokines remains unclear. Studies on estrogen deficiency related to RANKL indicated some evidence. Estrogen modulates osteoclast formation both by down-regulating the expression of osteoclastogenic cytokines from supportive cells, and by directly suppressing RANKL-induced osteoclast differentiation (47). RANKL expression on isolated bone marrow correlated directly with the bone resorption markers, which means that upregulation of RANKL on bone marrow cells is an important determinant of increased bone resorption induced by estrogen deficiency (48). In addition, leptin also plays a role in osteoblast differentiation and suppresses resorption by osteoclasts (19, 49).

RANKL is identical to a TNF superfamily ligand that independently is divided into two groups. Synonyms of RANKL are OPG ligand, osteoclast differentiation factor, and TNF-related activation-induces a cytokine. RANKL stimulates the activation, durability, and adhesion of osteoclasts on bone surfaces (50). Osteoprotegerine (OPG) was identified in 1997, and is named according to protective effects on bone (Latin: *os*, bone; *protegere*, protect). Osteoprotegerine is secreted by TNFR without a transmembrane domain containing 401 amino acids with a signal peptide of 21 amino acids required for the homodimer and four characters cysteinerich pseudorepeat (51). Osteoprotegerine works as a receptor for RANKL and ligand as the inducer of apoptosis, associated with TNF, which is a member of the TNF ligand superfamily (51). The role of RANK/RANKL/OPG in osteoclastogenesis mechanisms has been established. Osteoclast formation was regulated by factors expressed by osteoblast/stromal cells. RANKL/RANK signaling regulates the formation of multinucleated osteoclasts from their precursors, as well as their activation and survival in normal bone remodeling and in a variety of pathologic conditions. OPG protects the skeleton from excessive bone resorption by binding to RANKL and preventing it from binding to its receptor, RANK. Thus, RANKL/OPG ratio is an important determinant of bone mass and skeletal integrity (52). The study in murine calvarial bone culture showed that bone resorption modulated by RANKL and OPG leads to changes in osteoblast proliferation, suggesting a feedback mechanism from osteoclasts to osteoblasts. In addition, it was found that RANKL and OPG have more potent effects on osteoclastogenesis than on the activity of mature osteoclasts (53). Histological analysis has shown that RANKL and OPG immunoreactivity were predominantly associated with bone marrow cells. The expression of bone formation markers was activated in the bone formation phase, followed by the stimulation of RANKL/OPG expression in the bone resorption phase. This means that these molecules are key factors linking bone formation to resorption during bone remodeling (54).

It appears that there are similarities between estrogen and leptin in influencing the process of bone remodeling (Figure 1). Estrogen deficiency increases the activity of osteoclastogenesis which is indicated by an increase in RANKL, as well as with leptin. Increased peripheral leptin levels related to the amount of

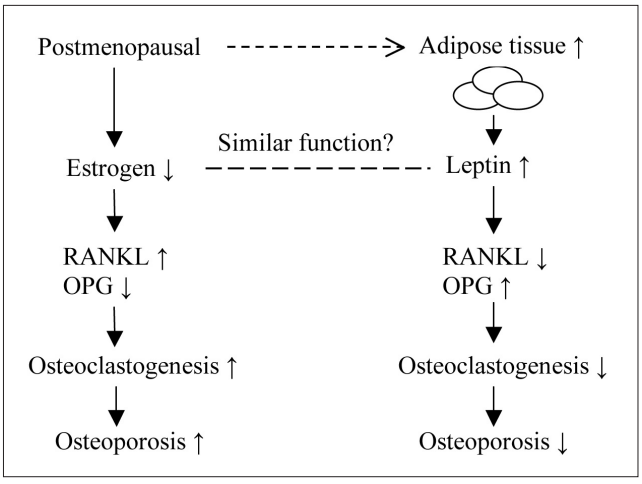

Figure 1 - Conceptual frame.

adipose tissue on individuals with obesity, has been shown to increase BMD through the mediation of RANKL / OPG. In postmenopausal women where estrogen levels decline, could the role of estrogen be replaced by leptin? Molecular genetics aspects that play a role in bone remodeling such as leptin, leptin receptors, and cytokines (e.g. RANK, RANKL and OPG) require further study in order to be useful, especially related to osteoporosis therapy based on a genetic analysis.

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