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A Unified Model for the Action of Leptin on Bone Turnover

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Abstract Leptin has been advocated as a centrally acting factor responsible for inhibiting accumulation of bone mass. However, recent investigations unequivocally establish leptin as a local (autocrine) factor expressed by osteoblasts. Exogenously added leptin causes osteoblastic cell proliferation and differentiation, while also rendering osteoblasts more efficacious in terms of mineralization. Leptin acts as an anti-apoptotic agent, and augments messages responsible for the remodelling of bone tissue, i.e., mRNAs for osteoprotegerin (OPG) and the interleukin IL-6. Furthermore, leptin message is readily expressed in osteoblasts subjected to mechanical strain. In this respect, osteoblasts, which are unilaterally stretched proliferate and differentiate, a phenomenon being potentiated by exposure of the cells to differentiating humoral factors. This article discusses a unified model of dually acting leptin through the central nervous system and the mechanostat principle applied to osteoblasts. The proposed model may account for the finely tuned bone homeostasis maintained within rather narrow limits, depending on exposure to humoral factors and the prevailing mechanostat usage mode. J. Cell. Biochem. 88: 706–712, 2003.

Key words: leptin; osteoblast; bone

Early after its discovery in 1994, leptin was acknowledged as an adipocyte-derived signaling polypeptide, which limited food intake and increased energy expenditure via specific receptors located in the central nervous system (CNS) [Thomas and Burguera, 2002]. When acting on the hypothalamus, leptin stimulates the sympathetic nervous system, thus evoking attenuation of appetite, enhancement of lipolysis in adipocytes with increased expression of uncoupling proteins, and eventually the generation of heat [Thomas and Burguera, 2002].

Leptin retrieved in the plasma of adult individuals originates mainly from adipose tissue, although it is also expressed in, and secreted from, other tissues like muscle, gastric, and breast epithelium, placental trophoblasts, arterial wall cells, normal pituicytes, and different types of pituitary adenomas [Reseland and Gordeladze, 2002]. In this context, leptin has unequivocally been shown to emanate from osteoblasts, which synthesize and mineralize bone matrix proteins [Reseland et al., 2001].

Consequently, the picture is becoming increasingly complex, and leptin is now regarded as a multi-potent cytokine eliciting both indirect, central and direct, peripheral effects in different organs and tissues such as bone.

RATIONALE FOR ADVOCATING LEPTIN AS A CENTRALLY ACTING BONE REGULATOR

Osteoporosis is characterized by low bone mass with an increased risk of fractures following trauma. It has been observed that osteoporosis is triggered or worsened by cessation of gonadal function, and that obesity protects the individual from bone loss [Haberland et al., 2001]. Secondly, the condition of increased weight-bearing, as experienced by adipose subjects, is known to further stimulate bone growth. Finally, it is well known that estrogen is produced by and stored in adipose tissue. Hence, researchers sought for a candidate, that might ensure a proper regulation of both

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gonadal function and body weight, as well as bone mass.

Leptin was launched as such a candidate [Karsenty, 1999; Ducy et al., 2000a,b]. The proposal was based on experiments carried out in mice deficient in either leptin production (ob/ob type animals) or devoid of functional leptin receptor expression (db/db and fa/fa type mice). These animals are obese, hypogonadic, and displayed a markedly elevated bone mass [Haberland et al., 2001]. It was argued on a genetic basis that leptin may account for the control of bone formation, which is not secondary to any endocrine abnormalities (since ob/+ and db/+ phenotypes are devoid of such abnormalities). These animals produce more bone, but exhibit the same amount of osteoblasts as normal mice. Animals deprived of white fat, and thus leptin, also appeared to display high bone mass [Haberland et al., 2001]. Failure to demonstrate leptin and leptin receptor expression in bone tissue was interpreted to advocate a role for leptin as a neurogenic stimulus to maintain bone mass at a normal level.

INNERVATION OF BONE TISSUE

One interesting question to ask is whether bone cells, via a functional neuronal network, are connected to the parts of the hypothalamic nuclei (arcuate nucleus) expressing leptin receptors and/or neuropeptide Y, known to mediate the effect of leptin on bone [Gordeladze et al., 2001; Reseland and Gordeladze, 2002; Thomas and Burguera, 2002]. Bone tissue is innervated by fibers containing substance P, vasoactive intestinal peptide (VIP), calcitonin gene-related peptide, and glutamate. During long bone development, both sensory and sympathetic fibers are observed in intimate connection with bone marrow cells, osteoblasts, and osteoclasts. These fibers do not form typical synapses in the nerve endings, so it is unclear how they transmit and receive signals from bone cells. It has been shown that denervation can affect bone remodeling and mechanically induced osteogenesis. Finally, VIP has been shown to inhibit bone resorption in vitro [Turner et al., 2002].

Glutamate is a key neurotransmitter involved in learning and memory in reflex loops and the hippocampus. It is argued that bone cells exhibit habituation (desensitization) to repeated mechanical stimulation and sensitization to mechanical loading by parathyroid hormone (PTH). Acquired long-term memory to a mechanical loading environment may influence the responsiveness of bone tissue to external stimuli [Turner et al., 2002]. For example, bone tissue from the skull shows markedly different responses to several stimuli, for example, mechanical loading, disuse and PTH, compared with long bones. However, glutamate synapses have not been located in bone, where the main bone remodeling cells constitute a mesh consisting of more or less "loose" osteoblasts and osteoclasts surrounded by interconnected osteocytes. Bone tissue has been shown to express a glutamate/aspartate transporter (GLAST) (in osteocytes) and also functional NMDA and metabotropic glutamate receptors (in osteoblasts and osteoclasts). The glutamate receptor may be induced upon mechanical stimulation, implicating glutamate signaling as an important factor in the regulation of steady state level settings of bone mass. Hence, it may well be so that leptin serves as an important factor in neurogenic regulation of bone modeling and remodeling [Mason et al., 1997; Laketic-Ljubojevic et al., 1999].

The intrathecal and intraventricular adminstration [Whitfield, 2001; Yaksh et al., 2002], and lumbar spinal infusion [Dunbar et al., 1997] of leptin may affect several nervous relay systems, which interfere with the neurogenic control of skeletal muscle contraction and/or autonomic nervous system regulation of organs allegedly being indirectly involved in the regulation of bone mass. Intracerebro-ventricular adminstration of leptin to normal rats resulted in mean arterial blood pressure (MAP) increase. Blood flow decreased in the iliac and superior mesenteric arteries, but not in the renal artery. Both lumbar and renal sympathetic nervous activities (SNA) were increased. Plasma glucose and insulin were unchanged. It was concluded that leptin enhanced MAP by decreasing arterial flow to the skeletal muscle and the splanchnic vascular bed, and hypothesized that centrally acting leptin might serve as a link between obesity, hyperinsulinism, and hypertension [Dunbar et al., 1997]. From this study, one may postulate that muscle tone might be reduced, since it has not been discussed whether leptin administration to the CNS might affect the neurochemistry of neuronal networks leading to a loss of muscle tone. As a plausible explanation for this phenomenon, it may be stated

that leptin affects the steady state of the so-called mechanostat by tilting it into the mode of non-usage (see below).

HUMORAL REGULATION OF BONE CELLS AND THE MECHANOSTAT PRINCIPLE

It has been shown that OB-R1 receptor mRNA is expressed in pig hypothalamus, cerebral cortex, amygdala, thalamus, cerebellum, area postrema, and anterior pituitary as well as in adipose tissue, liver, kidney, pancreas, adrenal gland, heart, spleen, lung, muscle, and bone [Lin et al., 2000]. Therefore, the outlined concept of leptin's main effect on bone being mediated through the CNS suffers firstly, from the lack of acknowledging that leptin, through nervous input, might interfere with other organ systems, indirectly intervening with bone homeostasis, and secondly, from the ignorance of published data strongly advocating leptin-induced local effects on bone tissue. It is therefore necessary to briefly review how humoral and mechanical factors affect recruitment and differentiation of bone cells.

Osteoblasts are derived from bone marrow progenitor cells of the mesenchymal lineage [Lian et al., 1999]. The multipotent stem cells give rise to osteoblasts, the colony-forming unit fibroblast (CFU-F), as well as fibroblastic stromal cells, chondrocytes, adipocytes, and muscle cells. Several factors are involved in the differentiation and regulation of osteoblasts in terms of proliferation and mineralization [Lian et al., 1999; Gordeladze et al., 2001]. Some of these components are stimulators of collagen synthesis and cell proliferation (transformin growth factor β = TGF β, insulin, PTH, and insulin like growth factor = IGF-I), osteocalcin synthesis enhancers (TGFβ, the vitamin D-receptor and glucocorticoid responsive elements = GREs), and osteocalcin synthesis inhibitors (insulin, growth hormone, and IGF-I). Furthermore, the interleukins IL-1, IL-6 and others (via stimulation by PTH, and through the release of IGF-I) augment osteoclastic bone resorption and bone remodeling [Lian et al., 1999; Gordeladze et al., 2001].

Imposed on the effects of humoral and local factors, is the mechanical strain, promoting modulation of the skeleton to make it suitable for its load. It turns out that obese people have greater bone strength and mass than otherwise comparable, but slender people [Frost, 1999; Gordeladze et al., 2001]. Past efforts to explain this view invoked genetic, biochemical, endocrine, and other non-mechanical factors. Some investigators have also suggested that body weight could have some direct effect on bone mass, however, some obese individuals exhibit overt osteopenia. Information and ideas contained in a new paradigm of skeletal biology suggest an additional explanation.

In summary, it can be asserted that making bone stronger usually requires more bone tissue and better architecture. The largest loads on bone (and the strains they cause) increase bone strength and mass. Physiologically, such impact can only be achieved by skeletal muscle contraction. If body weight is not moved (as in bed-ridden patients) the reduced physical activity and muscle strength can cause osteopenia, regardless of how great the weight is. The first disusage stage causes osteopenia, the second maintains it, and the changes take place slowly from the first to the second stage. The pure states lie at opposite ends of a smoothly changing continuum. The muscular forces, needed to move obese bodies around during work and play, increase bone strength, as well as bone mass. If obese individuals become inactive with weak muscles, their bone strength and mass decrease as they do in non-obese individuals. Thus, we may obtain a plausible new explanation for the puzzle mentioned above.

This is called the mechanostat principle. A characteristic stress–strain curve is comprised of thresholds like the remodeling, modeling, and the microdamage threshold (MESr, MESm, MESp) with intervals denominated the disuse window (DW), adapted window (AW) or comfort zone, mild overload window (MOW) as in children who grow, and the pathologic overload window (POW). Optimally, one should aim at controlling the mechanical load on bone to remain in the AW (100–1000 μstrain) when pre-exposure of the skeleton to loads in the MOW (1000–3000 μstrain) has secured an optimal bone mass and architecture [Frost, 1999]. It might be postulated that the thresholds of the mechanostat are altered by local and/or circulating hormones. It is also conceivable that the mechanostat principle may be superimposed on other types of thresholds like maximal hormone levels, intermittent hormonal exposure, receptor autoregulation, receptor desensitization, receptor internalization, endogenous GTP-binding protein levels/activation,

cross-talk phenomena, signaling cascade assembly in lipid rafts, and protein phosphorylation/dephosphorylation status [Gordeladze et al., 2001].

The skeleton's primary mechanical function is to provide rigid levers for muscles to act against as they hold the body upright in defiance of gravity. The mechanisms for adaptation involve a complex cellular, reversible mechanodeformation process including: 1) mechanocoupling (i.e., conversion of mechanical forces into local mechanical signals), such as fluid sheer stresses, that initiate a response on bone cells, 2) biochemical coupling (i.e., transduction of a mechanical signal to a biochemical response involving pathways within the cell membrane and cytoskeleton, and 3) cell-to-cell signaling from sensor cells (probably osteocytes and bone lining cells) to effector cells (osteoblasts and osteoclasts) to initiate either bone formation or resorption to secure appropriate architectural changes [Frost, 1999]. These microstructural alterations occur in order to adjust and improve the bone geometry to its prevailing mechanical environment.

Structural changes can be predicted to some extent from three fundamental rules: 1) bone adaptation is driven by dynamic rather that static loading, 2) extending the loading duration has a diminishing effect on further bone adaptation, and 3) bone cells accommodate to a mechanical loading environment, making them less responsive to routine or customary loading signals. According to this model, the maintenance or gain of bone mass are associated with the presence of circulating or locally synthesized hormones and/or heavy mechanical load, which might comprise blunt obesity and/or physical activity [Frost, 1999; Gordeladze et al., 2001].

One of the many hormones that might account for the link between energy and bone metabolism is leptin. This hormone might, as evidenced by data demonstrating both osteoblastic origin and direct effects on bone, serve as a modulator of the reciprocal effect of humoral factors mechanical forces on bone turnover. In light of this hypothesis, it is interesting to note that plasma leptin concentrations are decreased upon increased physical activity when adjusted for changes in body fat mass [Reseland et al., 2001], whereas the level of circulating IL-6 is acutely augmented [Pedersen et al., 2001]. Mechanical stimulation of human osteoblasts in culture does in fact enhance cellular expression of leptin, while also augmenting proliferation rates, osteoblastic differentiation and the expression of osteoprotegering (OPG) and IL-6 mRNAs [Gordeladze et al., 2002].

EVENTS MEDIATED BY THE LEPTIN RECEPTOR IN OSTEOBLASTS

The last question to ask is whether osteoblastic cells possess the receptor apparatus necessary to convey the leptin signal. An early and most likely pivotal event evoked by subspecies of the cytokine family of "hormones" is the activation of one or more members of the Janus (or JAK) family of tyrosine kinases. The activated JAK kinases, which form a complex with the cytokine receptor subunits, induce auto-phosphorylation as well as phosphorylation of the receptor. These phosphorylated tyrosines form binding sites for various signaling molecules, which are themselves thought to be phosphorylated by JAK kinases, including signal transducers and activators of transcription (STATs) [Vaisse et al., 1996], which regulate transcription by adaptor proteins, thereby initiating the mitogen activated protein kinase (MAPK) pathway. They also comprise insulin receptor substrate (IRS) proteins, which are believed to regulate metabolic events in the cell. Most interestingly, osteoblasts express a functional leptin receptor capable of conducting an active signal through the Jak/Stat pathway. This is a strong piece of evidence that leptin, in fact, plays a major, direct role in bone metabolism. Leptin has been found to exert functional effects in osteoblastic cells, by increasing proliferation, differentiation, and mineralization both in primary osteoblasts and osteosarcoma cells [Reseland et al., 2001; Gordeladze et al., 2002]. Leptin has also been demonstrated to induce prolonged life span of human primary osteoblasts by inhibiting apoptosis [Gordeladze et al., 2002]. The generation of cultured osteoclasts differentiated from peripheral blood mononuclear cells (PBMCs) and murine spleen cells are inhibited by leptin [Holloway et al., 2002], indicating that leptin act locally to increase bone mass and may contribute to linkage of bone formation and resorption.

Maor et al. [2002] found that chondrocytes in the growth centers contain specific binding sites for leptin, which stimulated the width of the chondroprogenitor zone at low concentrations,

whereas higher concentrations had an inhibitory effect. They also demonstrated that leptin induced both proliferation and differentiation activities in the mandibular chondyle. Furthermore, leptin also increased the abundance of the IGF-I receptor protein and IGF-I receptor mRNA within the chondrocytes and the progenitor cell population. Their results indicate that leptin acts as a skeletal growth factor with a direct peripheral effect on skeletal growth centers.

MORE EVIDENCE SUPPORTING LEPTIN AS A DIRECT MODULATOR OF BONE METABOLSIM

However, in parallel with the experiments conducted by M Amling, P Ducy, G Karsenty, and others, ample evidence have been accumulated to postulate a local and direct effect of leptin on bone: leptin seemed to correlate with bone area and change in bone area in peripubertal women [Matkovic et al., 1997], serum levels of leptin and bone mass is correlated in non-obese women [Pasco et al., 2001], resting metabolic rate (RMR) and low leptin levels correlate with low BMR in ballet dancers [Kaufmann et al., 2002], anorexia nervosa patients on nutritional rehabilitation displayed increased circulating levels of IGF-I and leptin, which correlated to C-telopeptide production rates [Heer et al., 2002], leptin is advocated as an independent predictor of whole body and femoral neck BMD in postmenopausal women [Blain et al., 2002], intraperitoneal leptin administration increased plasma osteocalcin in male ob/ob mice and prevented its fall during 24 hr fasting and 5 days of food restriction in normal mice [Goldstone et al., 2002], and leptin effectively reduced trabecular bone loss, trabecular architectural changes, and periosteal bone formation in overiectomized rats [Burguera et al., 2001]. Furthermore, it appeared that leptin enhanced mRNA OPG/RANKL-ratio in humans [Holloway et al., 2002], and leptin inhibits osteoclast generation in peripheral blood monocytes (PBMCs) incubated on bone with hM-CSF and sRANKL. Finally, leptin enhanced OPG mRNA expression in PBMC, suggesting that the inhibitory effect was mediated via the RANKL/RANK/OPG system.

Earlier, leptin was demonstrated to induce differentiation of stromal cells into osteoblasts, and not to adipocytes [Thomas et al., 1999]. Leptin receptor mRNA expression in and protein secretion from [Reseland et al., 2001] osteoblasts in culture strongly indicate a direct effect of leptin on bone metabolism.

OSTEOBLASTIC CELL DIFFERENTIATION AND FUNCTION RELATED TO LEPTIN

Leptin is expressed in normal human osteoblasts (from the iliac crest), but not in human osteosarcoma cells. The leptin receptor (with the intracellular long arm) is expressed in all osteoblastic cells, but to a variable degree. Leptin enhances the expression (as estimated by by RT-PCR) of TGFβ, IGF-I, Collagen Iα, alkaline phosphatase (ALP) and osteocalcin, while also stimulating collagen synthesis and proliferation of such osteoblasts. It appears that leptin promotes increased mineralization of osteoblasts, but probably only after prolonged exposure.

Subsequent to leptin exposure, expression of the osteoblastic marker gene *OSF-2* and the osteocytic marker gene *CD44* is diminished. Both the expressions of IL-6 and OPG, which are signals that stimulate and block osteoclastic resorption, respectively, are enhanced upon leptin exposure. Leptin counteracts retinoic acid-induced apoptosis in osteoblasts. The hormone also reduces the expression of the apoptosis-inducing gene *Bax-α*, while the antiapoptotic gene *Bcl-2* is upregulated.

The expression of leptin in osteoblasts is not acutely auto-regulated [Reseland et al., 2001], however, after prolonged incubation, leptin down-regulates its own expression. Contrastingly, the leptin-receptor (OB-R1) mRNA is not affected. Besides hormones and the sympathetic nervous system, specific nutrients are also capable of regulating leptin in most tissues. Polyunsaturated fatty acids (PUFAs) like eicosapentaneoic acid (EPA) markedly reduced leptin expression in placental cells [Reseland et al., 2001]. EPA also alters leptin expression in osteoblasts in culture, while EPA and other PUFAs affect novo collagen synthesis and osteocalcin expression.

Leptin mRNA is augmented upon mechanostimulation of the cells by 1200 μE (microstrain = alteration of the cell diameter by 0.12%) for 1800 cycles at 1 Hz. This effect is potentiated by preincubating the osteoblasts in a differentiating medium [Gordeladze et al., 2002], which

renders the osteoblasts capable of expressing and secreting leptin.

A UNIFIED MODEL FOR THE CENTRAL AND PERIPHERAL ACTION OF LEPTIN

All of the above collected data indicate that leptin is expressed in osteoblasts, possibly at a late stage of the osteoblastic development, and that leptin may be one of several regulators of bone metabolism. The observations also indicate that leptin may facilitate osteoblastic phenotype development, as well as ensuring the survival of osteoblasts to become osteocytes with their mechanosensing properties. Noticeably, leptin is expressed upon mechanostimulation in the so-called anabolic mechanical usage mode, suggesting that leptin may be produced locally when needed to ensure proper bone architecture and strength.

It is therefore conceivable that leptin, when perfusing the local modeling or remodeling bone unit may play a role in osteoblastic cell differentiation and function, as well as osteoclastic resorption. Furthermore, leptin may turn out to be a common denominator of input from nutraceutical fatty acids and mechanostimulation via osteocytes. As for the influence of PTH on bone, it may well be that the effect of leptin on bone is dependent of concentration and mode of exposure, determined by the source, from which leptin is reaching the bone cells. The central effect of leptin may act like a brake on the anabolic actions of hormones, local factors, and mechanosensing on bone mass, where leptin, derived from the bone itself and/or bone marrow or peripheral adipocytes, belongs to the anabolic factors produced locally or reaching the interior of bone tissue from the circulation.

Thus, the dual action of leptin may ensure a proper balance of bone mass in response to nutritional status, where the anorectic like patient devoid of circulating leptin and/or locally produced leptin avoids the trauma of extensive bone loss, while the adipose individual exposed to high levels of leptin and wight-related bone cell strain does not produce too much bone tissue (normally inferior in architecture and strength).

Hence, it may be of interest to determine under which conditions (hormonal, nutritional, mechanical, and/or cell developmental), locally produced or peripherally derived leptin turns out to be detrimental to the synthesis, deposition, and mineralization on new bone matrix, and at which stage of cellular develoment leptin serves as an autocrine, anabolic and/or auxiliary factor to ensure an increase in bone matrix production and mineralization.

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