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Mini Review

An evolving integrative physiology: skeleton and energy metabolism

*Na Kyung Lee**

Department of Biomedical Laboratory Science, College of Medical Science, Soonchunhyang University, Asan 336-745, Korea

The adipocyte-derived hormone leptin regulates appetite and bone mass. Recent research demonstrates that reciprocally, osteoblasts have a role in controlling energy metabolism. Several genes expressed in osteoblasts are involved in this process, and one of them is the *Esp* gene. The remaining genes regulate *Esp* gene expression. OST-PTP, the protein name of *Esp*, regulates the carboxylation of osteocalcin secreted from osteoblasts, thus affecting insulin sensitivity and insulin secretion. This review provides evidence for a novel interpretation of the connection between bone and energy metabolism and expands our understanding of the novel physiology of bone beyond its classical functions. [BMB reports 2010; 43(9): 579-583]

INTRODUCTION

Bone remodeling occurs in a constant and balanced manner in the body throughout adulthood. This process includes destruction of the mineralized bone matrix by osteoclasts and bone formation by osteoblasts (1, 2). Considering that osteoporosis due to an increase in bone resorption (3) invariably follows gonadal failure (4) and obesity offers protection from osteoporosis (5, 6), these observations suggest that body weight (or appetite), reproduction, and bone mass might be connected through the same hormone(s).

If this hypothesis is true, it implies that glucose and fat metabolism might be regulated by bone in a feedback loop. Indeed bone remodeling is a process requiring a considerable amount of energy (7), and breakage of energy homeostasis in the body hampers skeletal metabolism (8, 9). By providing evidence supporting these hypotheses, recent mouse genetic studies and molecular approaches have enhanced our knowledge and understanding of the reciprocal regulation of bone and energy metabolism.

*Corresponding author. Tel: 82-41-530-3036; Fax: 82-41-530-3085; E-mail: nlee@sch.ac.kr
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REGULATION OF BONE FORMATION BY LEPTIN

Leptin is a good candidate for investigating the existence of a common regulatory link between bone remodeling and energy metabolism. An adipocyte-derived hormone, leptin inhibits appetite and favors energy expenditure and fertility (10, 11). Mice lacking either leptin (*ob*/*ob*) or its receptor (*db*/*db*) are obese and hypogonadal (12).

Leptin-deficient *ob*/*ob* mice exhibit high bone mass due to a massive increase in bone formation parameters. This leptin-less animal model provides strong experimental evidence that leptin uses a central (presumably hypothalamic) relay to control bone mass because leptin treatment through intracerebroventricular (ICV) infusion in *ob*/*ob* mice fully corrects the bone phenotype caused by the leptin deficiency (13). In addition, the infusion does not cause any detectable leak of leptin into the general circulation (10, 14).

Two distinct mechanisms explain the regulation of bone resorption by leptin. Symphathetic signaling favors osteoclast differentiation by inducing the receptor activator of NF-kappaB ligand (*Rankl*), a true osteoclast differentiation factor (1), in osteoblasts. In contrast, leptin inhibits *Rankl* expression through the cocaine- and amphetamine-regulated transcript (CART) in the ventromeidal hypothalamus (VMH) (15). Brain regulation of bone mass has been verified in other groups experimentally (10, 16-18).

High bone mass of the *ob*/*ob* mice is a consequence of their lack of leptin - not obesity - because a *Leptin* transgene can correct the high bone mass of fat-free mice, which are lean and leptin-deficient because of the virtual absence of adipocytes (13).

Together these studies suggest that leptin is the adipocyte-derived gene product responsible for the bone phenotype and is an important factor mediating functional connections between fat and bone.

REGULATION OF ENERGY METABOLISM BY THE SKELETON

These results beg the question: are osteoblasts in turn regulating any aspect of energy metabolism through a feedback regulation? Luckily there are very few genes expressed in osteoblasts that are not expressed in fibroblasts (13); one of the can-

didates is osteocalcin.

Osteocalcin, a low molecular weight (5,700 Da) protein produced by osteoblasts, is synthesized as a pre-promolecule with three glutamic acid residues (Glu) and is secreted into blood. Glutamic residues are gamma carboxylated during post-translational modifications into Gla residues by a vitamin K-dependent gamma carboxylase; hence, another name for osteocalcin is Bone Gla Protein (BGP) (19-21).

Although until recently, no receptor has been reported for osteocalcin it demonstrates some features of a hormone. Primarily, it is cell-specific and secreted into blood. The observation that *Osteocalcin*$^{-/-}$ mice exhibited abnormally increased visceral fat (P. D. and G. K., unpublished data) also raises the possibility that osteocalcin may regulate energy metabolism. Moreover, in humans, when osteocalcin levels are decreased or osteocalcin is poorly carboxylated, the blood glucose level decreased in a manner similar to that shown in mice (22-24).

Osteocalcin$^{-/-}$ mice have higher blood glucose levels and lower serum insulin levels than WT mice. Ki67 immunostaining showed that β-cell proliferation decreased in the pancreas of *Osteocalcin*$^{-/-}$ mice; however, fat mass increased in *Osteocalcin*$^{-/-}$ mice (25).

The bioactivity of osteocalcin is regulated by osteotesticular protein tyrosine phosphatase (OST-PTP), which has the gene name *Esp* (26), and its expression is restricted to embryonic stem (ES) cells, Sertoli cells, and osteoblasts; it is not expressed in the pancreas or in fat (25, 27). *Esp*$^{-/-}$ mice have a relative increase in uncarboxylated osteocalcin compared to the carboxylated form in the serum when hydroxyapatite beads are used; however, it failed to regulate osteocalcin expression (25). A triple enzyme-linked immunosorbent assay (ELISA) system for quantification of mouse total, carboxylated, and uncarboxylated osteocalcin was developed, and it verified that carboxylation of osteocalcin decreased in the serum of *Esp*$^{-/-}$ mice (28).

Esp-deficient mice display a metabolic phenotype that is the mirror image of the one present in the *Osteocalcin*-deficient mice; for example, a low blood glucose level and hyperinsulinemia compared to wild-type (WT) littermates. Since OST-PTP is a transmembrane protein and is not secreted (25, 27), this implies that OST-PTP functions by regulating osteocalcin bioactivity. Indeed removing one allele of *Osteocalcin* from *Esp*$^{-/-}$ mice is sufficient to rescue the *Esp*$^{-/-}$ mice phenotype, which suggests that OST-PTP and osteocalcin are in the same regulatory pathway and *Esp*$^{-/-}$ mice are models of increased osteocalcin bioactivity (25).

To verify whether uncarboxylated osteocalcin is the bioactive form that regulates energy metabolism, recombinant osteocalcin was produced bacterially; this yields an uncarboxylated form only because there is no expression of gamma carboxylase in bacteria. Its treatment induced the expression of *insulin* and *cyclinD1*, a cell cycle marker, in islets (25). Osteocalcin pumps used to determine the direct effect of a 0.3 ng/h dose for 1 month demonstrated improved glucose handling (29). It increased serum insulin levels, thus decreasing blood glucose levels in WT mice. An increase in β-cell proliferation and *insulin* expression was observed at low concentrations (0.3 ng/ml); however, insulin sensitivity and energy expenditure was exhibited at high concentrations (10 or 30 ng/ml). Although there is a difference in dose response (29), these results support the role of osteocalcin in the regulation of energy metabolism.

Recently it was verified that expression of the *Esp* gene is regulated by ATF4 (30) favoring the expression of *Rankl* in osteoblasts, and by FoxO1 (31), a forkhead family transcription factor. Although *Atf4* is a broadly expressed gene (32), *Atf4*$^{-/-}$ mice primarily show phenotypic abnormalities in the skeleton. ATF4 regulates terminal differentiation and virtually all functions of the osteoblast related to the control of bone mass (15, 32). Yoshizawa *et al*. (2009) showed that mice overexpressing *Atf4* in osteoblasts display a decrease in insulin secretion and are insulin insensitive. The *α1(I)Collagen-Atf4* transgene corrected the energy metabolism phenotype of *Atf4*$^{-/-}$ mice and could significantly lower fat mass and blood glucose levels. Mice lacking *Atf4* only in osteoblasts (*Atf4*$_{osb}^{-/-}$) presented the same metabolic abnormalities as *Atf4*$^{-/-}$ mice. ATF4 is a known regulator of the expression of osteocalcin, and the metabolic phenotypes of *Atf4*$^{-/-}$ mice are similar to that of *Esp*$^{-/-}$ mice, raising the possibility that ATF4 could regulate the expression of *Esp*, hampering the metabolic function of osteocalcin.

Analysis of the *Esp* promoter revealed the existence of cAMP-responsive element (CRE) at -340, and a sequence between -600 and -300 including the CRE of the *Esp* promoter is necessary for its activity. ChIP assays confirmed that ATF4 binds to the CRE element in the *Esp* promoter. These results indicate that ATF4 directly regulates *Esp* expression in osteoblasts. Likewise, the percentage of the bioactive form of osteoclacin decreased in the serum of *α1(I)Collagen-Atf4* mice; however, it increased in *Atf4*$_{osb}^{-/-}$ mice, even though the level of serum total osteocalcin decreased in *Atf4*$_{osb}^{-/-}$ mice (30). This genetic and molecular evidence strengthens the perspective that ATF4 achieves its function on metabolism through regulation of *Esp* expression.

In addition to ATF4, FoxO1 is another regulator of *Esp* gene expression. Since FoxO1 regulates several key aspects of glucose homeostasis in diverse cells including β cells, hepatocytes, and adipocytes (33-36), Rached *et al*. (2010) examined its function in osteoblasts. An increase in pancreatic β cell proliferation and insulin secretion was observed in mice lacking *Foxo1* only in osteoblasts (*Foxo1*$_{osb}^{-/-}$). The insulin tolerance test (ITT) showed increased insulin sensitivity caused by increased *Adiponectin* gene expression in *Foxo1*$_{osb}^{-/-}$ mice. The resemblance of metabolic phenotypes of *Foxo1*$_{osb}^{-/-}$ mice to that of *Esp*$^{-/-}$ mice led them to analyze the expression levels of *Esp* and *Osteocalcin*. The ability of an osteoblast-specific *Foxo1* deficiency to affect metabolic homeostasis was due to increased *Osteocalcin* expression and decreased expression of

Esp, which is responsible for decreasing the bioactivity of osteocalcin. As expected, uncarboxylated osteocalcin increased in the serum of *Foxo1*$_{osb}^{-/-}$ mice compared to that of WT mice (37).

By showing that osteoblasts contribute to glucose homeostasis by controlling the expression of *Esp* and the bioactivity of osteocalcin this increasing body of evidence strongly suggests a tight link between bone and energy metabolism.

OSTEOBLAST-DEPENDENT LEPTIN REGULATION OF INSULIN SECRETION

Another factor enhancing *Esp* expression in osteoblasts is leptin (38). In contrast to uncarboxylated osteocalcin, leptin plays a role in inhibiting insulin secretion, in part through a direct effect on β-cells (39). This finding suggested the possibility that leptin may inhibit insulin secretion through indirect mechanisms to fulfill the function (e.g. via osteoblasts because it affects osteoblast functions) (40).

Indeed, it was verified that leptin upregulates symphathetic tone, which favors *Esp* expression in osteoblasts. Using *Lepr*$_{syn}^{-/-}$ mice (in which the leptin receptor is deleted in all neurons) researchers showed that leptin uses a neural pathway to regulate insulin secretion. In addition, a decrease in serum epinephrine, serum norepinephrine, and *Ucp1* expression in brown fat was observed in these mice, reflecting a low sympathetic tone (38). To investigate whether the sympathetic tone under the control of leptin regulates insulin secretion through osteoblasts, *Adrβ2*$_{osb}^{-/-}$ mice (in which the adrenergic receptor is deleted only in osteoblasts) were generated (38). As expected, *Adrβ2*$_{osb}^{-/-}$ mice showed an increase in serum insulin levels. Long-term (1 wk) intracerebroventricular (ICV) infusion of leptin decreased glucose-stimulated insulin secretion in WT but not in *Adrβ2*$_{osb}^{-/-}$ mice, supporting the idea that sympathetic signaling in osteoblasts contributes to leptin regulation of insulin secretion.

In osteoblasts, isoproterenol, an agonist of the β-adrenergic receptor, upregulated *Esp* expression four-fold; however, the expression of osteocalcin did not change (41). Accordingly, although the serum levels of total osteocalcin were normal, the amount of uncarboxylated osteocalcin increased in *Adrβ2*$_{osb}^{-/-}$ mice. Studies using *Adrβ2*$_{osb}^{+/-}$; *Atf4*$^{+/-}$ mice showed that ATF4 mediates the sympathetic regulation of insulin secretion through its expression in osteoblasts because serum insulin levels and β cell proliferation increased in these mice (41). These results support the importance of the crosstalk existing between adipocytes and osteoblasts for the regulation of glucose homeostasis (Fig. 1).

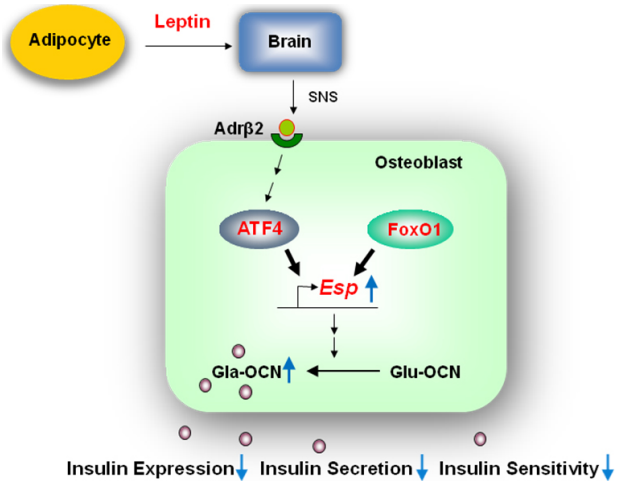

Fig. 1. Integrative representation of glucose handling by leptin, ATF4, and FoxO1 through up-regulation of *Esp* gene expression.In the presence of leptin, sympathetic tone stimulates the expression of *Esp*, a gene inhibitingbioactivity of osteocalcin, through Adrβ2 and ATF4 in osteoblasts. FoxO1 also controls the expression of *Esp*, thus decreasing osteocalcin bioactivity. This finally causes negative effects on the expression, secretion, and sensitivity of insulin.

CONCLUSION

Recently, mouse genetic experiments and molecular results uncovered novel physiology of the skeleton, highlighting a link between bone and glucose metabolism. A similar relationship is true in humans. Growing clinical evidence supports the relationship between osteocalcin and glucose levels; for example, abnormal levels of osteocalcin (or its carboxylation ratio in serum) reflect changes in glucose metabolism (42-44). Although a specific receptor for osteocalcin has not been identified, these observations demonstrate that bone performs completely different roles as an endocrine organ, by showing how integrative and tremendous skeleton physiology is. This insight into bone physiology leads us to investigate the skeletal system for therapeutic targets of type II diabetes as well as osteoporosis.

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