# Energy regulation by the skeleton

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*Bones of the skeleton are constantly remodeled through bone resorption by cells called osteoclasts and bone formation by cells called osteoblasts. Both cell types are under multi-hormone control. New research findings demonstrate that bone formation by osteoblasts is negatively regulated by the hormone leptin, which is secreted by adipocytes and acts through the leptin receptor in the central nervous system and ultimately through the sympathetic nervous system. Leptin deficiency leads to increased osteoblast activity and increased bone mass. Reciprocally, expression of the Esp gene, exclusive to osteoblasts, regulates glucose homeostasis and adiposity through controlling the osteoblastic secretion of the hormone-like substance osteocalcin. An undercarboxylated form of osteocalcin acts as a regulator of insulin in the pancreas and adiponectin in the adipocyte to modulate energy metabolism. Osteocalcin deficiency in knockout mice leads to decreased insulin and adiponectin secretion, insulin resistance, higher serum glucose levels and increased adiposity.*

## INTRODUCTION

An unexpected reciprocal relationship has been discovered between mammalian bone and energy metabolism whereby leptin, an appetite-regulating hormone secreted by adipocytes, influences osteoblast function and bone formation rates and, in turn, osteocalcin, produced by osteoblasts, influences energy metabolism. Most of the discoveries described here relating to the regulation of energy metabolism by the skeleton have been made in the laboratory of G. Karsenty.[1]

The bones of the skeleton are constantly turned over through bone remodeling, a process consisting of two normally balanced phases of bone resorption followed by bone formation. Bone resorption, carried out by cells called osteoclasts, is partially under endocrine control.[2] Bone formation, a function of cells called osteoblasts, lays down new bone matrix in place of bone resorbed by osteoclasts. Imbalance between the two processes whereby osteoclast activity outpaces osteoblast activity, as in aging due to a decline in gonadal hormone secretion, leads to decreased bone mineral density and an increased risk of osteoporotic bone fracture. The fact that remodeling of bone takes place simultaneously in many locations throughout the skeleton made it seem likely that not only osteoclast, but also osteoblast, activity was under endocrine control.

## LEPTIN: A NEGATIVE REGULATOR OF BONE FORMATION

Leptin is a hormone closely linked to obesity through suppression of appetite. The clinical literature suggested that obesity appeared to protect the body from osteoporosis. For example, Tremollieres et al.[3] observed that obesity was accompanied by reduced bone loss and greater bone mass in post-menopausal women. This relationship led to the hypothesis proposed by Ducy et al.,[4] that leptin, a hormone secreted by adipocytes and connected to obesity, may be controlling osteoblast activity.

To explore this hypothesis, Ducy et al.[4] studied bone formation in leptin-deficient ob/ob mice and leptin-receptor deficient db/db mice that were obese. They found that ob/ob mice that were deficient in leptin, and

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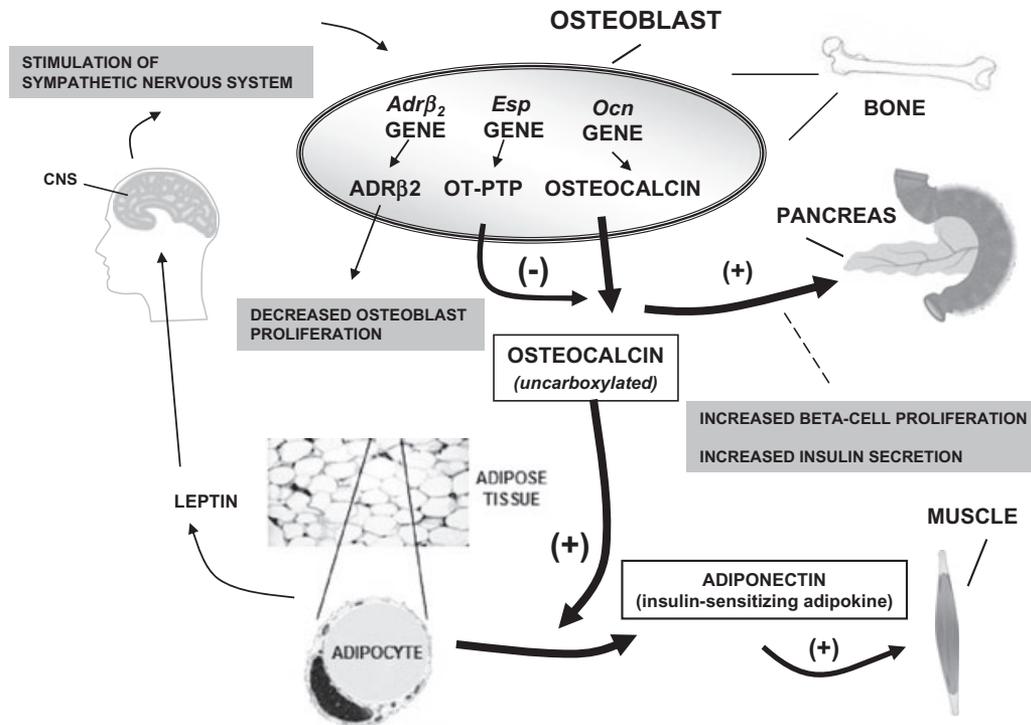
*Figure 1* **Endocrine regulation of energy metabolism by the skeleton.** Adipocytes secrete the adipokine leptin that influences bone metabolism. Leptin binds to leptin receptors in the brain. The leptin signal causes stimulation of the sympathetic nervous system and activation of the β2-adrenergic receptor gene (Adrβ2) in bone, which decreases osteoblast proliferation and bone formation. The osteoblast, in turn, influences energy metabolism by expressing osteotesticular protein tyrosine phosphatase (OT-PTP), a product of the Esp gene. OT-PTP apparently influences the vitamin K-dependent γ-carboxylation of osteocalcin, an osteoblast-specific protein that acts in a hormone-like manner to affect adipocytes and β cells in the pancreas. Uncarboxylated osteocalcin increases β cell proliferation and insulin secretion in the pancreas, and further influences energy metabolism by affecting adipocyte secretion of adiponectin, an insulin-sensitizing adipokine.

therefore obese, showed a "massive increase in bone formation parameters", similar to obese db/db mice that lacked the hypothalamic leptin receptor. That this was a direct effect of leptin, and not a consequence of obesity, was shown by the observation that bone mass was still increased in leptin-deficient *non-obese* heterozygote (ob+/ob−) mice. The increase in bone mass and bone volume of ob/ob mice was reversed and restored to normal by infusion of leptin into the intra-cerebroventricular (ICV) space. No leakage of leptin into the circulation was detectable. Therefore, the effect of leptin acting as an inhibitor of bone formation is somehow mediated though the central nervous system.

Further study indicated that the mediator of the leptin signal on bone formation was the sympathetic nervous system acting through the β2 adrenergic receptor (*Adr*β₂) (Figure 1). The importance of the adrenergic receptor in regulating bone mass was confirmed by Elefteriou et al.,[5] who found that *Adr*β₂-deficient mice, which are not obese, had increased bone mass and their skeleton was resistant to ICV infusion of leptin. The conclusion from these experiments was that leptin binds to β2 adrenergic receptors on ventromedial hypothalamic neurons, which signal through the sympathetic nervous system and act negatively on osteoblasts.[5]

### INFLUENCE OF OSTEOBLASTS ON ADIPOCYTE METABOLISM

Lee et al.[6] argued that "a remarkable feature of most hormone regulations is that they are controlled by feedback loops such that a cell type affected by a hormone sends signals influencing the hormone-producing cell". In the case of osteoblast regulation by the adipocyte hormone leptin, the authors[6] proposed that osteoblasts might, in their turn, be able to influence the adipocytes in a feedback loop.

In their search for a hormone-like molecule secreted by osteoblasts, the authors[6] explored the properties of osteocalcin (OC), a low-molecular-weight (5700 D) potential hormone-like peptide that is produced by the osteoblast, where it is synthesized as a prepro-molecule and secreted into the circulation. Osteocalcin is post-translationally modified by a vitamin K-dependent

γ-carboxylation of two of its glutamic acid residues on the pre-osteocalcin molecule secreted by osteoblasts. The synthesis of osteocalcin can be induced by the vitamin D metabolite 1,25-dihydroxy-vitamin $D_3$ (calcitriol).[7] Surprisingly, although osteocalcin is produced by osteoblasts in the course of bone remodeling and is the most abundant non-collagenous protein (about 15%) in bone,[7] it was shown long ago by Ducy et al.[8] that it is not involved in bone formation. The authors generated OC knock-out mice (OC$^{-/-}$) and found that the animals actually had *increased* bone formation, compared to wild-type (WT) mice. This was unexpected because one would expect that, because of OC's high affinity for the bone mineral hydroxyapatite, due to the presence in the molecule of the γ-carboxyglutamic acid residues, it would have a role in bone mineralization.

Murshed et al.[9], in continuation of the work of Ducy et al.[8] with OC$^{-/-}$ knockout mice, confirmed that OC has no role in bone formation.

## INVOLVEMENT OF Esp GENE WITH REGULATING DEVELOPMENT OF GLUCOSE INTOLERANCE AND OBESITY

As mentioned by Lee et al.,[6] Ducy and Karsenty made a chance observation that OC$^{-/-}$ knockout mice exhibited abnormally high amounts of visceral fat; this led them to the hypothesis that "skeleton may regulate energy metabolism".[6] They set about to determine how the skeleton, in particular the osteoblasts, might be involved in the regulation of energy metabolism.[6] They studied *Esp*, one of the few genes expressed exclusively in osteoblasts. This gene encodes a signaling protein named osteotesticular protein tyrosine phosphatase (OT-PTP). The authors[6] generated Esp$^{-/-}$ knockout mice and surprisingly found that such mice showed a 60–300% increase in pancreatic β cell proliferation and increased β cell mass, suggesting that this gene is somehow involved in the negative regulation of these pancreatic processes. In consequence, there was a twofold rise in serum insulin, a threefold reduction in blood glucose, higher glucose tolerance, increased uptake of glucose by muscle and liver, increased insulin sensitivity, a threefold increase in adiponectin secretion by adipocytes, decreased serum triglycerides, and decreased visceral fat, compared to WT mice. The osteoblastic gene *Esp* regulated glucose homeostasis by its action on insulin production in the pancreas.

### Esp gene knockout protects against development of diabetic symptoms

To explore this phenomenon further, the authors[6] showed that the Esp$^{-/-}$ knockout mice were protected from obesity and glucose intolerance and, therefore, that expression of the osteoblastic gene *Esp* was in some way involved in the development of obesity and glucose intolerance. To confirm this important conclusion, the authors[6] performed three experiments:

1) Both WT and Esp$^{-/-}$ mice were injected with gold thioglucose into their ventromedial hypothalamus. This procedure was known to cause lesions in the hypothalamus leading to hyperphagia both in WT and Esp$^{-/-}$ mice. After three months, the WT mice were obese, glucose intolerant, and insulin resistant, with high levels of serum triglyceride; whereas the Esp$^{-/-}$ knockout mice were lean, glucose tolerant, and insulin sensitive, with normal serum triglyceride levels.

2) WT and Esp$^{-/-}$ knockout mice were fed a high-fat diet for six weeks. The Esp$^{-/-}$ knockout mice gained less weight compared to the WT mice and did not develop glucose intolerance and insulin resistance.

3) WT and Esp$^{-/-}$ mice were injected with streptozotocin, which caused destruction of the majority of their pancreatic β cells. In consequence, both groups of mice showed low serum insulin levels and decreased pancreatic insulin content. Half of the WT mice injected with streptozotocin died after eight days, and the survivors in this group had the expected high glucose levels, whereas the glucose level of the Esp$^{-/-}$ knockout mice treated with streptozotocin was normal, and they did not excrete glucose in their urine, as the WT animals did.

Clearly, lack of the osteoblast *Esp* gene protected the mice from diabetes. In other words, expression of *Esp* was required under these circumstances for development of obesity and diabetes in mice.

### Overexpression of *Esp* in the osteoblast causes diabetic symptoms

To confirm this conclusion, following the loss-of-function (*Esp* knockout) experiments, the authors[6] performed *Esp* gain-of-function experiments. Transgenic mice were generated, over-expressing *Esp* cDNA specifically in osteoblasts. In these animals with high osteoblastic *Esp* expression there was decreased pancreatic β-cell proliferation and cell mass, hypoinsulinemia, decreased insulin secretion when challenged by glucose, and decreased serum adiponectin levels, compared to WT mice, leading to hyperglycemia, glucose intolerance, and insulin resistance.

The experiments described led the authors to conclude that the known receptor-like protein OST-PTP, downstream from *Esp* expression, leads to secretion of an unknown molecule from the osteoblast that regulates glucose homeostasis[10] (Figure 1).

## IDENTIFICATION OF OSTEOCALCIN AS THE OSTEOBLAST-DERIVED SIGNALING MOLECULE REGULATING ENERGY METABOLISM

In vitro culture experiments confirmed the conclusion that the osteoblast cell produced a factor that affected pancreatic β cells and adipocytes. The authors[6] co-cultured WT osteoblasts with pancreatic islets and found enhanced insulin secretion by the islet cells, compared to islets cultured without osteoblasts. This enhancement of insulin secretion was also significantly increased if the osteoblasts were derived from Esp^{−/−} knockout mice. Incubation in cultures of WT osteoblasts with adipocytes caused increased adipocyte adiponectin expression, compared to cultures without osteoblasts. This expression was increased twofold if the adipocytes were co-cultured with osteoblasts from Esp^{−/−} knockout mice. The same effect with both islets and adipocytes was achieved by incubation with supernatant medium from WT and Esp^{−/−} knockout osteoblasts. Clearly, the osteoblasts secreted something into the medium that affected both islets and adipocytes and appeared to regulate glucose homeostasis.

What is this hormone-like osteoblast-secreted molecule that is involved in the regulation of glucose homeostasis? Osteocalcin, a peptide hormone-like substance, is specifically produced and secreted by osteoblasts. The above-mentioned observation made by the authors, that OC knockout mice exhibited abnormal amounts of visceral fat,[6] led them to propose the hypothesis that the hormone secreted by osteoblasts that affects glucose homeostasis might be OC. Early clinical observations by Rosato et al.[11] revealed significantly lower serum OC values in patients suffering from type 2 diabetes compared to healthy persons, and restoration of glycemic control resulted in increased OC levels.

Lee et al.[6] found that OC^{−/−} knockout mice showed higher serum glucose compared to WT mice. Insulin secretion, insulin sensitivity, glucose tolerance and energy expenditure were decreased. Expression of insulin target genes in liver and muscle (*Fox a2, Pgc-1α, Nrft, Mcad*) were all decreased, while *Pepck* expression was increased. The pancreatic β-cell mass and insulin content decreased twofold, while fat mass, adipocytes, and serum triglycerides increased. Significantly, adiponectin gene expression by adipocytes declined severely, as did serum adiponectin. Clearly, these effects closely resemble those observed in mice *over*-expressing the *Esp* gene and support the authors' hypothesis that OC is the osteoblast hormone that regulates glucose homeostasis[6] (Figure 1).

In further experiments, insulin expression was induced in cultures of pancreatic β-cells when treated with bacterially produced recombinant OC (3 ng/ml).[6] Control fibroblasts similarly treated did not secrete

insulin. Extending these experiments to in vivo tests, the researchers observed that injecting recombinant OC into OC^{−/−} mice stimulated insulin secretion, so that their serum insulin levels were nearly doubled 120 min after injection, while serum glucose declined.[6]

### Mechanism of osteocalcin action on insulin secretion and insulin sensitivity

Searching for a mechanism of OC action on insulin secretion and insulin sensitivity, Lee et al.[6] generated heterozygote OC^{+/−}/adiponectin^{+/−} mice. In such heterozygotic mice, serum adiponectin decreased dramatically, whereas insulin secretion and serum glucose remained normal, even though insulin sensitivity decreased greatly. This experiment showed that, at least partly, the OC effect on insulin sensitivity occurred through its action on adiponectin secretion. As stated by the authors, "OC is a molecule secreted by osteoblasts that can increase insulin and adiponectin expression".[6]

Attention was also drawn to the fact that the metabolic phenotype of the OC^{−/−} knockout mice was the mirror image of the Esp^{−/−} knockout mouse phenotype.[6] This notion implies that the metabolic changes observed in the Esp^{−/−} knockout mice should be reversed by decreasing OC gene expression. This proved to be the case, as shown by the following ingenious experiment: Esp^{−/−} knockout mice were deprived of one OC allele; then, comparing WT, Esp^{−/−} knockouts, OC^{+/−} heterozygotes and the Esp^{−/−}/OC^{+/−} double heterozygote, it was found that serum glucose, serum insulin and serum adiponectin levels were all corrected to normal (WT) from the low serum glucose, high serum insulin, and high adiponectin levels. The authors[6] take these observations to show that Esp and OC gene expressions lie on the same metabolic pathway, and that the effects observed in the Esp^{−/−} knockout mice are caused by a *gain* of expression of OC.

### Uncarboxylated form of osteocalcin regulates glucose homeostasis

A curious aspect of the properties of OC is its high affinity for the bone mineral, hydroxyapatite. The presence of two γ-carboxyglutamic acid residues in the molecule make it a potent chelator for calcium. Lee et al.[6] found that 90% of serum OC was bound to hydroxyapatite, whereas in Esp^{−/−} knockout mice only 74% was bound. The authors suggested that the protein regulated by the *Esp* gene, OST-PTP, controls the γ-carboxylation of OC and proposed that the uncarboxylated form of OC regulates glucose homeostasis. Their hypothesis was confirmed by the following two experiments:

1) Osteoblasts in culture were treated with warfarin, a vitamin K-antagonist that prevents OC

γ-carboxylation. The culture medium of these cells contained a much smaller percentage of OC bound to hydroxyapatite compared to osteoblasts not exposed to warfarin. When those warfarin-treated osteoblasts were then co-cultured with adipocytes, they showed a one- to fivefold increase in the release of adiponectin by the adipocytes, compared to warfarin-untreated cells.

2) Adipocytes were exposed to fully carboxylated OC and bacterially produced recombinant OC that was uncarboxylated. The authors[6] found that adipocytes exposed to uncarboxylated OC produced twice the amount of adiponectin compared to adipocytes exposed to normal (carboxylated) OC. Similarly, cultured pancreatic islet cells, treated with uncarboxylated OC expressed 1.5-times the amount of insulin and 2.5-times the amount of cyclin D (an indicator of cell proliferation), compared to islet cells exposed to normal OC. It appears, therefore, that the uncarboxylated molecule of OC is the active form.

## CONCLUSION

In summary, the laboratory of Karsenty[6] has described a reciprocal relationship between bone and energy metabolism. The release of leptin from adipocytes results in inhibition of bone formation and, conversely, the release of osteocalcin from bone osteoblasts results in increased insulin and adiponectin secretion.

Lee et al.[6] speculate on the possible evolutionary origin of the phenomenon whereby the skeleton participates in the regulation of energy metabolism. They propose that the large surface of the skeleton would present an "excellent site of hormone synthesis", though this would seem somewhat too broad a concept to offer much of an explanation of the phenomenon.

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