Convergence between bone and energy homeostases: Leptin regulation of bone mass

Review

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The observation that obesity protects from osteoporosis suggested that energy metabolism and bone mass could be regulated by the same hormones. Testing this hypothesis revealed that leptin regulates bone mass through a hypothalamic relay and using two neural mediators, the sympathetic tone and CART, both acting on one cell type the osteoblast. This review summarizes the genetic and molecular bases of this regulation and discusses its potential clinical implications.

Although it is mainly known for, and tightly associated with, the control of appetite, the adipocyte-derived hormone leptin is more than an antiobesity molecule. Indeed, leptin is also a major regulator of reproduction and bone mass. This article will review the genetic and molecular evidence establishing that leptin controls, through a regulatory loop including hypothalamic neurons and two neural outputs, the proliferation and function of the osteoblast and thereby bone mass. This body of work provides the first evidence of a convergence between bone and energy metabolisms.

Skeleton is a late acquisition during development; this explains why in most cases a very high degree of conservation exists between mice and humans in the function of genes involved in skeletal biology. Indeed, whether those are regulatory molecules such as transcription factors, kinases, cell membrane receptors, secreted molecules, or structural proteins, inactivation of genes encoding these molecules results in essentially identical phenotypes in both species (Karsenty and Wagner, 2002). This high degree of conservation between mice and human explains why mouse genetics has become so central to our understanding of human skeletal biology and in uncovering novel modes of regulation of bone mass.

The biological relevance of bone remodeling

Bone is constantly turned over through bone remodeling (Rodan and Martin, 2000). This is a biphasic process occurring throughout the skeleton over a period of approximately 3 months in rodents. It includes destruction (resorption) of pre-existing bone, a function exerted by a specialized bone-specific cell, the osteoclast, followed by de novo bone formation, a function of another bone-specific cell, the osteoblast. Normally, resorption and formation of bone occur not only sequentially but in a balanced manner in order to maintain bone mass nearly constant during most part of adulthood. This qualifies bone remodeling as a true homeostatic function, a feature that, as explained below, has important molecular implications.

What is the biological importance of bone remodeling? Nowadays the maintenance of a constant bone mass is the aspect of bone remodeling we are the most familiar with because osteoporosis, the most frequent bone disorder, is a bone remodeling disease (Cooper and Melton, 1996). Clearly, the rising incidence of this degenerative disease suffices to justify the research effort in uncovering molecular bases of bone remodeling. Osteoporosis is caused by a relative increase of bone resorption over bone formation (Raisz, 2005), an event most often triggered by gonadal failure (Riggs et al., 1998; Riggs and Melton, 1986). Thus, it is a disease of people 50 years and older, and a disease that became a public health concern during the 20th century, when people began to outlive their own skeleton. The relatively recent appearance of osteoporosis makes it an unlikely justification for the conservation of bone remodeling during evolution.

In contrast, another aspect of bone remodeling has been of critical importance for the survival of vertebrates throughout evolution. Specifically, it is the ability of bones, through constant cycles of destruction and formation, to repair micro- and macrodamages without surgical intervention and thereby to preserve mobility. This aspect of bone remodeling is a survival function for vertebrates, not only in the dark ages of evolution, but also until few centuries ago.

Experimentally, bone remodeling can be studied directly using bone histology or indirectly by using bone mineral density and measure of relevant biological markers in serum and urine. In humans, for obvious ethical reasons, the study of bone remodeling relies only on indirect assays. In contrast, using the mouse as an animal model presents the advantage that bone remodeling can be studied histologically. One can measure not only bone mass but also the number and activity of osteoblasts and of osteoclasts in vivo through histomorphometry, a technique providing a more dynamic view of bone remodeling. In addition, cell-based assays using cells derived from genetically modified animals can be performed. This set of methodologies has become over the years the gold standard to study bone physiology.

A common endocrine control of body weight, reproduction, and bone mass

Although remarkable progress has been made, the consensus in the field is that we still do not know all the genetic and molecular mechanisms influencing bone remodeling. Uncovering novel regulators of this process requires looking from both a conceptual and experimental point of view. Conceptually, because resorption and formation of bone belong to the same physiological function, this implies that the same classes of molecules should regulate both of them. In agreement with this assumption, transcription factors and cytokines of various sorts affect osteoclast as well as osteoblast differentiation and function (Olsen et al.,

2000). However, one of the most prominent regulation of bone remodeling is the hormonal regulation of bone resorption exerted by, among others, sex steroids and the parathyroid hormone (Potts and Juppner, 1998; Riggs et al., 1998).Yet surprisingly, until recently the existence of an hormonal regulation of bone formation had not been established. Such a discrepancy in the regulation of two aspects of the same physiological function appeared unlikely.

An experimental argument further supported the hypothesis that bone formation must be regulated by hormones. In a mouse model of inducible osteoblast ablation relying on cell-specific expression of the *Thymidine-kinase* gene, gancyclovir treatment resulted in the death of all osteoblasts and therefore in virtually empty bones (Corral et al., 1998). The most informative aspect of this experiment happened when the gancyclovir treatment ceased. Remarkably, in a short period of time according to bone remodeling standard (one month), the bone mass of these transgenic mice returned to normal with a surprising precision. The rapidity of this recovery indicated that osteoblasts could sense when bones were empty and that they had to work at full capacity to replenish them. The precision of this recovery implied that osteoblasts could sense when bone mass had been restored and that they could decrease their output. Such an ability of osteoblasts, regardless of their location in the body, to modulate their function depending on what bone mass is could only be explained if hormonal and/or neural outputs were regulating their function.

How could one identify hormone(s) regulating bone formation without relying on a large genetic screen? As it is most often the case in physiology, an answer to this question came from the clinical literature. Two major clinical features of osteoporosis are that: (1) osteoporosis invariably follows gonadal failure, and (2) obesity protects from it. Taken together, these two observations suggest the existence of a common regulation of body weight (or appetite), reproduction, and bone mass. Although this hypothesis sounds nonspecific, it is in fact rather restrictive. Indeed, since appetite and reproduction are by and large governed by the hypothalamus, this hypothesis implies that the control of bone remodeling may also, in part, originate from the hypothalamus. Although novel, this concept should not be surprising, since most homeostatic functions are subjected to hypothalamic regulation, and bone remodeling is a prototypical homeostatic function.

Regulation of bone formation by leptin

Determining whether a common endocrine control of appetite, reproduction, and bone mass exists required studying the influence of hormone(s) known to regulate appetite and reproduction on bone remodeling. As it turned out, only one hormone significantly influences these two functions: leptin (Ahima, 2004; Spiegelman and Flier, 2001). Leptin inhibits appetite and favors reproductive function; consequently, mice lacking either leptin (*ob*/*ob*) or its receptor (*db*/*db*) are obese and sterile. From a physiological and molecular point of view, leptin is a privileged hormone to study for two reasons. First, there is only one ligand and one receptor, making interpretation of any experiment simpler (Tartaglia et al., 1995; Zhang et al., 1994). Second, during the 40 years or so that passed between the identification of the (*ob*/*ob*) and (*db*/*db*) mouse strains and the cloning of leptin and its receptor, an impressive amount of information has been gathered about the various phenotypic abnormalities present in these mutant mice. This knowledge proved to be invaluable in the study of leptin regulation of bone mass.

The sterility (or hypogonadism) of *ob*/*ob* and *db*/*db* mice should increase their bone resorption. Indeed, osteoclast numbers and bone resorption parameters are increased in leptin signaling-deficient (*ob*/*ob* and *db*/*db*) mice (Ducy et al., 2000). Despite this, leptin signaling-deficient mice display a higher bone mass than wild-type (WT) mice. This high bone mass, affecting all bones in the body, is due to a massive increase in bone formation parameters (Ducy et al., 2000). To date, leptin signaling-deficient mice are still the only animal models in which hypogonadism and high bone mass coexist. High bone mass was also observed in a patient harboring an inactivating mutation of the leptin gene (Elefteriou et al., 2005). Although this finding was based on noninvasive approaches, it is profoundly important since it represents the best available evidence that the absence of leptin affects the function of osteoblasts in the same manner in human and mice.

The high bone mass of *ob*/*ob* or *db*/*db* mice cannot be explained by their obesity since mice lacking adipocytes (''fat-free'' mice) display the same phenotype (Ducy et al., 2000). Moreover, a leptin transgene can correct the high bone mass of fat-free mice, indicating that leptin is the adipocyte-derived gene product responsible for their bone phenotype (Elefteriou et al., 2004). Similarly, lipodystrophic patients present objective evidence (advanced bone age) of an increase in the function of osteoblasts, further indicating that this regulatory pathway is conserved between mouse and human (Elefteriou et al., 2004). It is worthwhile to note that glucocorticoid serum levels are elevated in *ob*/*ob* mice but not in lipodystrophic mice. Likewise, glucocorticoid serum levels are normal in lipodystrophic leptin-deficient patients. Yet, they all display an increase in bone formation activity, demonstrating that the bone phenotype of leptin signaling-deficient mice and humans is not linked to their level of circulating glucocorticoids.

In full agreement with the restriction implied by the initial hypothesis, intracerebroventricular (ICV) infusion of leptin in leptin-deficient mice, at a rate that does not result in any detectable leak of leptin in the general circulation, completely corrects their high bone mass (Ducy et al., 2000). This experiment, in a ''leptin-less'' animal, is strong experimental evidence that leptin uses a central (presumably hypothalamic) relay to control bone mass, as it does to mediate its other functions (Ahima et al., 2000; Ahima, 2004). In addition, the complete rescue of the bone phenotype following leptin ICV infusion argues against any other mode of action of leptin; if this were so, the rescue would only have been partial. This is an important point, as addition of supraphysiological amounts of leptin to WT animals do have consequences not observed in the privileged context of loss-of-function models (Cornish et al., 2002; Martin et al., 2005). Several experimental arguments also indicate that leptin does not act directly on osteoblasts. First, Stat3 phosphorylation cannot be detected following treatment of primary osteoblast cultures with physiological doses of leptin; an effect of leptin was observed only when supraphysiological doses of the hormone and osteoblastic cells differentiated in vitro were used (Ducy et al., 2000; Thomas et al., 1999). Likewise, studies of peripheral leptin injection in mice employed very high doses, which are likely to induce leptin resistance and therefore bone loss (Cornish et al., 2002; Martin et al., 2005). Second, if leptin action were local, the high bone mass observed in *db*/*db* mice (which

lack its receptor) would by implication stem from an osteoblast defect. Yet, cultured osteoblasts from *db*/*db* mice did not produce any more extracellular matrix than WT osteoblasts (Ducy et al., 2000). Third, transgenic mice expressing leptin in osteoblasts have no overt bone abnormalities (Takeda et al., 2002). Thus, at the present time, there is no convincing evidence of a direct action of leptin on osteoblasts in vivo.

Anatomical and molecular bases of leptin's control of bone formation

The identification of leptin-sensitive neurons controlling bone formation has relied on chemical lesioning in WT and leptin signaling–deficient mice, and on the use of other genetically modified mouse models (Takeda et al., 2002). Monosodium glutamate (MSG) treatment of WT mice lesions neurons of the arcuate nuclei, resulting in increased appetite and obesity, but with no effect on bone formation parameters. Likewise, mice lacking the melanocortin 4 receptor (Mc4R), a receptor expressed on arcuate neurons and involved in leptin's control of appetite, have, throughout their life, normal bone formation parameters (Huszar et al., 1997; Vaisse et al., 1998). In contrast, lesioning neurons of the ventromedial hypothalamic (VMH) nuclei using gold thioglucose (GTG) induces an increase in bone mass due to an increase in bone formation parameters similar to the increase observed in *ob*/*ob* mice (Takeda et al., 2002). These experiments established that hypothalamic neural networks regulating bone formation exist. Yet, since they were performed in WT mice, they did not prove that VMH-sensitive neurons regulate bone formation in a leptin-dependent manner. The proof came when *ob*/*ob* mice with destroyed arcuate or VMH neurons received leptin ICV infusion (Takeda et al., 2002). In the *ob*/*ob* mice whose VMH neurons had been lesioned, leptin ICV infusion decreased body weight but did not affect bone formation parameters or bone mass. Conversely, leptin failed to decrease body weight but decreased bone mass and bone formation in *ob*/*ob* mice whose arcuate neurons had been lesioned. Thus, VMH neurons, or more precisely GTG-sensitive neurons, regulate bone formation under the control of leptin. Recent efforts to determine whether *Sf1*-expressing neurons located within the VMH nuclei regulate bone mass were inconclusive, probably because no histological analysis was performed (Dhillon et al., 2006).

What is the mediator of leptin's regulation of bone formation? An answer could be inferred from classical physiological studies performed in *ob*/*ob* mice, as well as from clinical observations. It has long been known that *ob*/*ob* mice have low sympathetic activity (Bray and York, 1998). This observation naturally led to the assumption that the sympathetic nervous system must mediate leptin regulation of body weight and/or reproduction. This supposition was challenged however by the fact that *Dbh*-deficient mice, unable to produce epinephrine and norepinephrine, were neither obese nor sterile (Thomas and Palmiter, 1997). Two explanations could account for the discrepancy between the appetite and fertility phenotypes of the *ob*/*ob* and *Dbh*-deficient mice. First, in the absence of sympathetic signalling, another pathway might mediate leptin regulation of appetite and reproduction; although conceivable, this is a rather difficult hypothesis to test. A second, simpler explanation could be that the low sympathetic tone observed in *ob*/*ob* mice is not responsible for their obesity or their sterility, but rather relates to another phenotype caused by leptin deficiency, for instance high bone mass. In other words, the sympathetic tone would not be primarily a mediator of leptin regulation of appetite or reproduction. The testability of this hypothesis made it attractive. A clinical observation gave it further credence: patients with reflex sympathetic dystrophy, a disease characterized by localized high sympathetic activity, develop a severe and localized osteoporosis that can be improved by β blockers (Kurvers, 1998). That osteoporosis develops in these patients without impairment of energy expenditure provides in vivo evidence that the sympathetic tone regulates bone mass independently of any influence it may have on energy expenditure.

If indeed sympathetic tone mediates leptin regulation of bone mass selectively, then a series of inferences could be made and tested: (1) *Dbh*-deficient mice should have high bone mass, (2) this high bone mass should be resistant to leptin ICV infusion, and (3) this infusion should decrease fat and body weight. All these hypotheses turned out to be correct (Takeda et al., 2002). More importantly, restoring sympathetic activity in *ob*/*ob* mice had no measurable effect on food intake and body weight but led to a 45% decrease in bone mass. Taken together, these lines of evidence demonstrate that in animals fed a normal diet the sympathetic nervous system mediates only leptin's regulation of bone mass.

Molecular bases of leptin control of bone formation

Fortunately only one adrenergic receptor, the β2 adrenergic receptor (Adrβ_2), is expressed in osteoblasts (Takeda et al., 2002), and mice lacking this receptor had already been generated (Chruscinski et al., 1999). As implied by the data presented above *Adr*β_2-deficient mice are not obese, are fertile, and have none of the metabolic abnormalities seen in *ob*/*ob* and *db*/*db* mice (Chruscinski et al., 1999; Elefteriou et al., 2005). Yet, they display an increase in bone formation and in bone mass that cannot be rescued by leptin ICV infusion. This latter experiment established genetically that the sympathetic nervous system, via Adrβ_2, mediates leptin regulation of bone mass (Elefteriou et al., 2005; Takeda et al., 2002). Because *Adr*β_2-deficient mice have no overt endocrine abnormalities, it also formally establishes that the high bone mass observed in absence of leptin signaling is not secondary to any metabolic perturbations. Subsequent bone marrow transplantation experiments demonstrated that the sympathetic regulation of bone formation directly occurs at the level of the osteoblasts (Elefteriou et al., 2005).

How does sympathetic signaling in osteoblasts affect their function so profoundly? The homeostatic nature of bone remodeling was a pivotal element in formulating a testable hypothesis. Most homeostatic functions are regulated in a circadian manner (Lowrey and Takahashi, 2004; Perreau-Lenz et al., 2004), raising the hypothesis that bone remodeling is also subjected to circadian regulation and therefore regulated by the molecular clock. Consistent with this, the secretion of the two most abundant proteins made by osteoblasts, Type I collagen and Osteocalcin, cycles during a 24 hr period (Gundberg et al., 1985; Simmons and Nichols, 1966).

Anatomically, the circadian clock includes a central component located in the suprachiasmatic nucleus of the hypothalamus and subordinate components present in peripheral tissues (Morse and Sassone-Corsi, 2002). Molecularly, both clock components comprise key genes regulating each other's expression. Schematically, ''the engine'' of the clock is composed of Bmal1 and Clock, two bHLH-PAS transcription factors that heterodimerize to regulate the expression of other core circadian

genes such as the *Period* (*Per*) genes, the *Cryptochrome* (*Cry*) genes, *Ror*α, and *Rev-erb*α. PER and CRY form a complex that inhibits the expression and function of *Bmal1* and *Clock* (Reppert and Weaver, 2002; Schibler and Naef, 2005). Mutant mice lacking molecular clock components have been generated, and most of them exhibit disruption of circadian rhythmicity (Okamura et al., 1999; Zheng et al., 2001). Most of these mutant mice, such as the *Per*$_1$*; Per*$_2$-deficient or *Cry1; Cry2*-deficient mice, have a normal appetite and body weight, are fertile, and display no overt endocrine or metabolic perturbations when fed a normal diet. Because of this absence of metabolic abnormalities, mice with a disrupted circadian rhythm are ideal models to determine whether the molecular clock regulates bone formation directly.

Multiple mutant mouse strains lacking one or several clock genes were analyzed; they all displayed a similar increase in bone formation parameters (Fu et al., 2005). For various reasons *Per*-deficient mice were used as a model of choice for subsequent work. The normal serum levels of free leptin and normal sympathetic activity of *Per*-deficient mice ruled out that the molecular clock acts upstream of leptin to regulate bone formation. In contrast, leptin ICV infusion of *Per*-deficient mice increases bone mass, while leptin decreases bone mass in WT mice. This suggests that components of the molecular clock mediate, in osteoblasts, the leptin-dependent sympathetic regulation of bone formation. Accordingly, most components of the molecular clock are expressed in osteoblasts, where their expression cycles during a 24 hr period and is regulated by the sympathetic tone (Fu et al., 2005). Furthermore, osteoblast-specific deletion of the *Per* genes results in high bone mass due to an increase in bone formation. Altogether these observations support the hypothesis that components of the molecular clock mediate, in osteoblasts, the leptin-dependent sympathetic regulation of bone formation.

Per-deficient and *Adr*β*2*-deficient osteoblasts progress faster through the G_1 phase of the cell cycle than WT osteoblasts (Fu et al., 2005). Expression of all *D-type Cyclin* and of *Cyclin E* is increased in *Per*-deficient as well as in *Adr*β*2*-deficient osteoblasts, and sympathetic tone inhibits *G*$_1$ *cyclin*, *D-type Cyclin* and *Cyclin E* expression in WT osteoblasts. While there is no evidence that Bmal and Clock regulate *Cyclin D*$_1$ expression directly, they inhibit the activity of the promoter of *c-myc,* an important regulator of *Cyclin D*$_1$ expression. This decrease in *c-myc* expression probably results in decreased *Cyclin D*$_1$ expression.

The similarity between *Adr*β_2-deficient and *Per*-deficient mice suffers one significant exception: ICV infusion of leptin increases osteoblast numbers in *Per*-deficient, not in *Adr*β_2-deficient mice. This aspect of leptin regulation of bone formation could not have been observed by the study of *ob*/*ob* and *Adr*β*2*-deficient mice. Indeed, this discrepancy between *Adr*β_2-deficient and *Per*-deficient mice implies that sympathetic signaling exerts two influences on osteoblast proliferation, one negative and dominant through the molecular clock, and one positive, visible only upon disruption of the molecular clock. Subsequent experiments showed that c-*fos* (a critical regulator of bone remodeling (Jochum et al., 2001)) and other members of the AP-1 family are overexpressed in osteoblasts lacking either *Per* or *Adr*β_2. This in turn favors expression of *c-myc* and thereby osteoblast proliferation via increased *Cyclin D*$_1$ expression (Fu et al., 2005). In agreement with this regulatory cascade, AP-1 gene expression in WT osteoblasts is regulated by both leptin and the sympathetic tone. This regulatory loop is not apparent in *ob*/*ob* mice because the molecular clock inhibits AP-1 gene expression in these animals.

Together, genetic and molecular studies provide a detailed picture of how leptin regulates bone formation (Figure 1). Following binding to its receptor on VMH neurons leptin uses sympathetic signaling as its only identifiable mediator to negatively act on osteoblasts. In these cells sympathetic signaling exerts two actions: one through AP-1 favors osteoblast proliferation, and another one, through the molecular clock, inhibits osteoblast proliferation by affecting both *D type cyclin* and *AP-1* gene expression (Figure 2).

Regulation of bone resorption by leptin

The work presented above was prompted by the assumption that identical classes of molecules must regulate the two aspects of bone remodeling, formation, and resorption. Demonstrating that bone formation is hormonally regulated verified this hypothesis but also uncovered a neural regulation of bone formation. The logic of the original hypothesis would then predict that bone resorption should be regulated by neural means.

The demonstration that this indeed was the case came again from the analysis of the *Adr*β_2-deficient mice (Elefteriou et al., 2005). Quite surprisingly, these mutant mice not only present an increase in bone formation, but also a decrease in bone resorption parameters (Figure 3). This latter abnormality, which contributes to the *Adr*β_2-deficient mice high bone mass, cannot be corrected by leptin ICV infusion indicating that leptin via the sympathetic tone regulates bone resorption.

The differentiation of the osteoclast, the bone resorbing cell, is determined by osteoblasts which produce the two main regulators of osteoclast differentiation: M-CSF, a survival factor for osteoclast progenitor cells, and RANKL, a true osteoclast differentiation factor (Teitelbaum and Ross, 2003). Coculture of osteoblasts and osteoclast precursors revealed that sympathetic signaling regulates osteoclast differentiation by regulating expression of *Rankl* in osteoblasts (Elefteriou et al., 2005). The biological importance of this regulation was demonstrated through gonadectomy, a procedure that increases bone resorption and decreases bone mass. Following gonadectomy, bone resorption parameters and bone mass remained unaffected in *Adr*β_2-deficient mice, indicating that the integrity of the sympathetic nervous system is required for the bone loss that follows gonadal failure.

This experiment uncovered another aspect of the complex regulation of bone mass by leptin. Indeed, from a bone biology perspective gonadectomized *Adr*β_2-deficient mice should be a phenocopy of *ob*/*ob* mice since both mouse models are hypogonadic and have a low sympathetic tone. Yet, they differ in a major way: while gonadectomized *Adr*β_2-deficient mice have normal bone resorption activity, *ob*/*ob* mice have high bone resorption activity (Elefteriou et al., 2005). The increase in bone resorption observed in *ob*/*ob* mice therefore cannot be due to their hypogonadism; otherwise, gonadectomized *Adr*β_2-deficient mice would also have an increase in bone resorption. This difference could be explained by a speculative ability of leptin to regulate the expression of gene(s) controlling osteoclast differentiation.

The fact that all the molecules identified as mediating leptin regulation of bone mass mediate only this function suggested that this specificity might also apply to leptin-dependent control

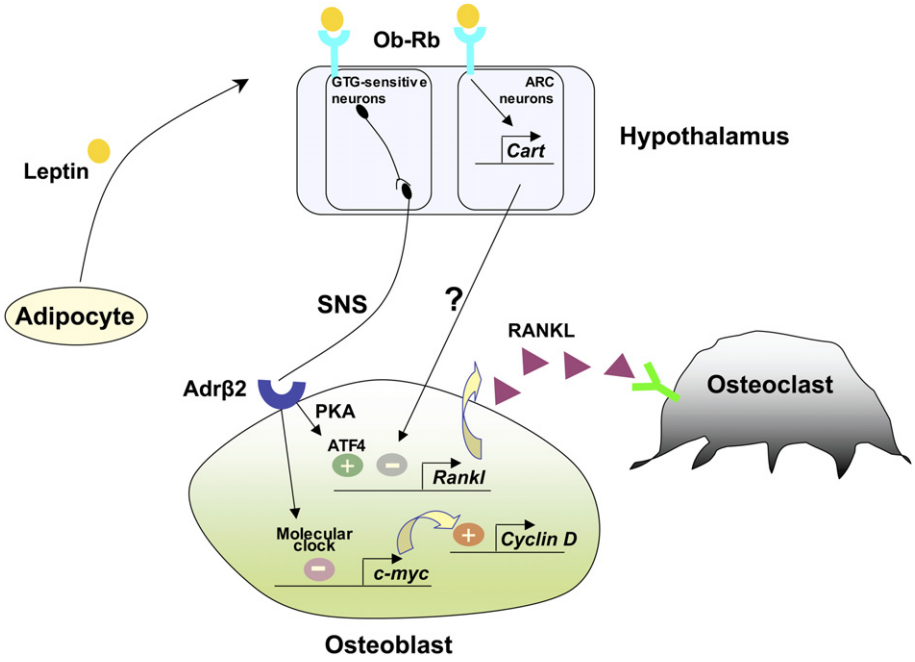

Figure 1. Current model of the leptin-dependent regulation of bone mass

Leptin binds to its hypothalamic receptors and induces two cascades to control bone mass. In the arcuate nuclei, it increases *Cart* expression that in turn regulates, via an unknown mechanism, *RankL* expression by osteoblasts and thereby bone resorption. Leptin also binds to receptors on the GTG-sensitive neurons of the VMH nuclei, inducing an increase in sympathetic activity which signals to osteoblasts via the β2 adrenergic receptors present at their surface. Two distinct molecular cascades downstream of this receptor are then activated. One inhibits osteoblast proliferation via the molecular clock regulation of *c-myc* and *Cyclin-D* expression. The other, mediated by PKA phosphorylation of ATF4, promotes *Rankl* expression and thereby bone resorption.

of osteoclast differentiation. One gene expressed in various parts of the brain and whose expression is increased by leptin and decreased in *ob/ob* mice, is *Cart* (cocaine amphetamine regulated transcript) (Elias et al., 1998; Kristensen et al., 1998). *Cart*-deficient mice have a normal appetite and are fertile, but display an osteoporosis phenotype due to an isolated increase in bone resorption parameters (Asnicar et al., 2001; Elefteriou et al., 2005) (Figure 4). Two other lines of evidence, one coming from mouse genetics the other from human genetics, underscored CART's biological importance as a regulator of osteoclast differentiation. First, *Mc4R*-deficient mice, that show a 2-fold increase in hypothalamic *Cart* expression, present a high bone mass with an isolated decrease in bone resorption parameters (Elefteriou et al., 2005), a phenotype corrected by simply deleting *Cart* (Ahn et al., 2006). Remarkably, *Cart* deletion did not improve the metabolic abnormalities observed in the $Mc4R^{-/-}$ mice, adding further credence to the notion that *Cart* is only a mediator of leptin regulation of bone mass. Second, patients deficient in *MC4R* have been reported to display an increase in bone mineral density, an indirect measure of bone mass (Farooqi et al., 2000). Biochemical analysis of the serum of these patients showed that they have an increase in CART levels and in biomarkers of bone resorption activity (Ahn et al., 2006). Thus, both loss- and gain-of-function experiments

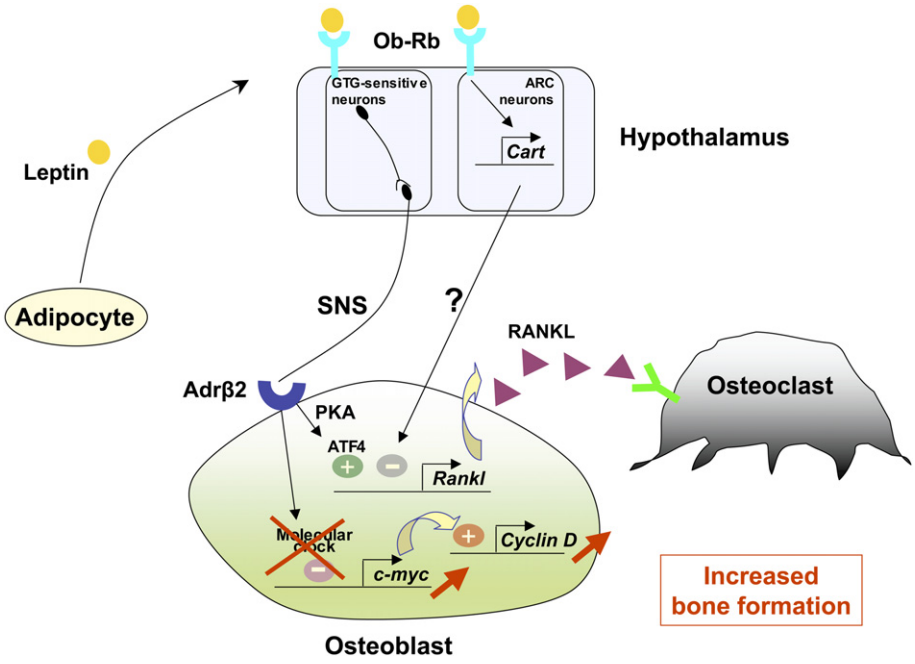

Figure 2. Schematic representation of the modifications caused by *Per1/2* or *Cry1/2* inactivation on the leptin-dependent regulation of bone mass

Upon inactivation of the molecular clock, the leptin/SNS arm regulating osteoblast proliferation via c-myc and CyclinD is overactivated, leading to an increase in bone formation and thus a high bone mass phenotype.

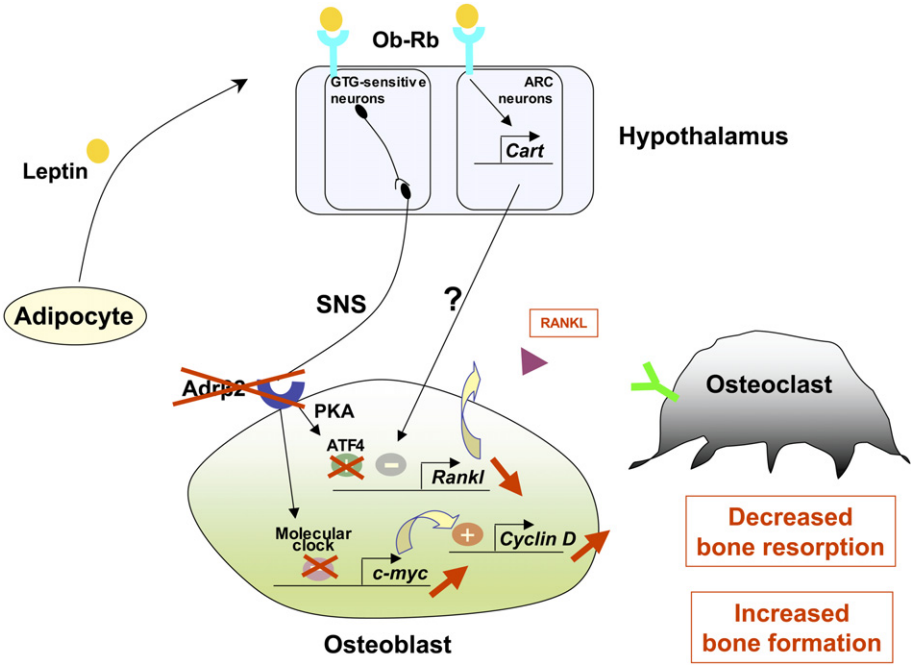

Figure 3. Schematic representation of the modifications caused by *Adrβ2* inactivation on the leptin-dependent regulation of bone mass

Inactivation of β2 adrenergic signaling leads to high bone mass. It increases bone formation by inhibiting the negative regulation exerted by the molecular clock on osteoblast proliferation. In addition, it decreases bone resorption by limiting the ATF4-mediated regulation of *Rankl* expression.

support the notion that CART is a regulator of bone resorption in rodents and in humans. This latter point was further confirmed by the finding that *CART* polymorphism affects bone mass in post-menopausal women (Guerardel et al., 2006). These human genetic data all support the notion that the leptin-dependent regulation of bone mass is conserved between mice and humans.

In summary, as in the control of bone formation, leptin regulates bone resorption through two antagonistic pathways (Figure 1). On the one hand, leptin favors resorption through the sympathetic nervous system, while on the other hand, it inhibits this function through CART (Figure 4). The absence of CART probably explains the increased bone resorption observed in the *ob/ob* mice (Elefteriou et al., 2005).

Summary and perspectives

This body of work illustrates the power of mouse genetics to study biological functions in adult animals, to uncover unappreciated regulatory loops and to provide the molecular bases of these regulatory loops. As mentioned before, because leptin and bony skeleton appear together during evolution the function of several key components of this pathway such as leptin itself, the sympathetic tone, or CART could be confirmed directly or indirectly in humans. Despite the importance of this regulatory

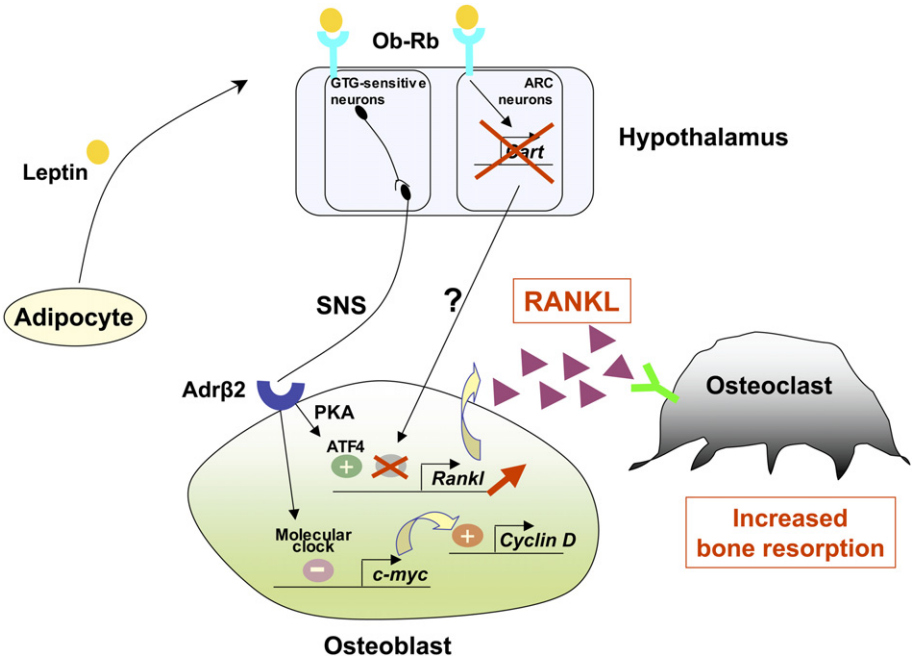

Figure 4. Schematic representation of the modifications caused by Cart inactivation on the leptin-dependent regulation of bone mass

Cart absence leads to a decrease in bone mass because of an isolated increase in osteoclast activity following *Rankl* overexpression by osteoblasts.

loop, it cannot however explain the pathophysiology of all bone metabolic diseases from osteoporosis to renal osteodystrophy. Rather, it provides one additional piece of knowledge that may influence our understanding of osteoporosis pathophysiology.

The notion that an adipocyte-derived hormone regulates bone mass through a hypothalamic relays and two neural outputs raises a novel series of questions. First, how do osteoblasts, the ultimate target cell of leptin regulation of bone mass, dictate to adipocytes or neurons how much leptin, catecholamine, or CART they should release? A second question, closer to leptin regulation bone resorption, is to elucidate the molecular details of CART signaling, to the level achieved for sympathetic regulation of osteoblast functions. A third question, which has already begun to be addressed, is whether other adipokines or neuropeptides regulate bone mass.

Finally, the regulation of bone mass by leptin has clinical implications. Indeed, the observation that gonadectomized *Adr*β_2-deficient mice fail to develop osteoporosis suggests that the integrity of the sympathetic nervous system is necessary for bone loss following menopause. The clinical significance of this stems, in part, from the existing use of β blockers as generic drugs with little toxicity. Several retrospective studies have suggested that inhibition of sympathetic signaling may protect osteoporotic women from bone fracture (Pasco et al., 2004; Rejnmark et al., 2006; Schlienger et al., 2004; Turker et al., 2006). For now, only one prospective clinical trial of limited scope has been reported, and its results look encouraging if not promising (Turker et al., 2006). Such an extension of this basic science work, into the realm of clinical science, will take several years to reach a firm conclusion.

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