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Co-dependence of bone and energy metabolisms

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Abstract

The growing number of genetically modified mouse models available but also of the possibility to delete one or several genes at will in a defined time frame or in a specific cell type or tissue(s) has open new possibilities for the study of whole animal physiology. This in vivo approach has been especially successful in uncovering a regulatory loop linking the control of energy metabolism and the regulation of bone remodeling. This review is intended to summarize the key events that led to the identification and the characterization of the different steps and molecules constituting this regulatory network.

Keywords

Bone Formation; Bone Resorption; Leptin; Serotonin; Osteocalcin; Sympathetic Nervous System

Introduction

Bone constantly renews itself through remodeling, the coordinated system whereby an equal amount of bone extracellular matrix resorbed by osteoclasts is laid down by osteoblasts. This process presents three remarkable features that themselves carry particular meanings in terms of physiology. First, resorption and formation of bone occur not only sequentially but also in a balanced manner in order to maintain bone mass nearly constant during most part of adulthood, establishing bone remodeling as a true homeostatic function. Since most homeostatic functions are centrally controlled this suggests that bone remodeling too should be, at least partly, controlled via a central relay. The second particularity is that the destruction/construction activity characterizing bone remodeling occurs constantly and simultaneously in thousands of sites, thus requiring a large and constant supply of energy to bone cells. This metabolic demand suggests that bone remodeling could have a mean to regulate how glucose, and more generally energy, is handled by the body. Lastly, bone remodeling's ability to repair micro-damages has been essential to preserve mobility until our modern days and thereby this function can be considered not as a mere particularity of vertebrate physiology but as a survival function for this branch of evolution. If one puts together these three observations and their biologic ramifications an hypothesis appears whereby a coordinated regulation of bone remodeling and energy metabolism, most likely involving a central component, would exist

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and would have appeared with vertebrates i.e. with bone remodeling not with the control of energy metabolism. Indeed, much like during development in which genes are activated when their function is needed, not before or after, evolution is likely to have selected genes when it needed them, not before or after. Testing the different elements of this hypothesis led not only to its validation but also to opening new fields of research in bone, fat and pancreas physiology, some of them having the potential to establish novel concepts in drug development for diseases as common as osteoporosis and diabetes.

1. Regulation of bone mass by adipocytes, a leptin-dependent cascade of events

In the search for mechanisms that could mediate a co-regulation of bone and energy homeostasis the first clue originated from two well-known clinical observations. The first one is that bone remodeling is dysregulated upon sex-steroid depletion, the second one is that obesity protects against the bone loss associated with this dysregulation. If these two classical observations are considered together they suggest, in molecular terms, that bone mass, body weight and gonadal function could be regulated by one or several common molecules. From this point of view, an excellent hormonal candidate appeared to be leptin, a 16 kDa hormone secreted by adipocytes [1]. Indeed, leptin is a major metabolic regulator, it inhibits appetite and favors energy expenditure, and of reproduction [2,3]. As a result *ob/ob* mice, a natural mutant strain where the leptin gene is inactivated, present a morbid obesity and are sterile [1]. Likewise, mice lacking the leptin receptor (*db/db* mice) are obese and sterile [4]. Aside from this obvious link between metabolism and reproduction, leptin fulfills two other criteria that places it at the top of the list for candidate molecules involved in a co-regulation of bone and energy metabolisms. First, years of analysis of leptin's mode of action on appetite and reproduction have demonstrated unequivocally that these functions are centrally mediated [2, 5]; it was therefore quite likely that leptin's regulation of bone, if it existed, would also be mediated by the brain. Second, while the controls of feeding, energy storage or reproduction have been acquired quite early during evolution leptin only appeared with vertebrate biology and more specifically only with vertebrates able to remodel bone. This could be a coincidence. Yet, if the only function of leptin was to inhibit feeding, its most obvious function because of the dramatic phenotype existing in the *ob/ob* mice, one would wonder either why it did not appear with the concept of feeding or why it appeared at a time when scarcity of food was much more a concern than over-abundance. On the other hand if leptin was meant to be a co-regulator of bone mass and energy metabolisms it seemed logical that evolution would have “created” it at the time these two functions would have first co-existed, regardless of the control of appetite by itself. The identification of leptin as a full fledged regulator of both arms of bone remodeling gave a new meaning to this speculative argument.

1.1. Regulation of bone mass by leptin: going from adipose tissue to the central nervous system

Analysis of both *ob/ob* and *db/db* mice by histomorphometry, the technique allowing the most comprehensive, since it can assess not only bone mass but also the relative contribution of bone formation and bone resorption to a phenotype, as well as most objective evaluation of bone parameters, since its results do not need to be adjusted for differences of lean/fat mass between samples, showed that trabecular bone volume was nearly 2-fold increased in absence of leptin signaling compared to wild-type littermates [6]. This phenotype was observed in both sexes, in growing and remodeling animals, and in long bones as well as in vertebrae. It turned out to be caused by an increase in the rate of bone formation so massive that it could override the increase in bone resorption caused by the central hypogonadism and the decrease in cocaine amphetamine regulated transcript (CART, see below) existing in these mice [6-9]. That the

absence of leptin signaling could result in a high bone mass despite a menopause-like condition suggested that the regulation of bone mass accrual could be a premium function of leptin.

This notion was further established by the analysis of a third genetic model. When leptin binds to its receptor it activates Jak2, this results in the phosphorylation of several tyrosine residues on the leptin receptor [10]. One of these, Y285, then binds Socs3 which attenuates the amplitude of leptin signaling. Hence, if this particular tyrosine residue is mutated Socs3 cannot bind and leptin intracellular signaling is amplified [11]. A few years ago such mutation had been engineered by substituting Y285 by a Leucine, which is not phosphorylated, and introduced by homologous recombination in ES cells. This resulted in a mouse model with a gain-of-function in leptin signaling, the *l/l* mice [12]. Remarkably, these *l/l* mutant mice demonstrated normal appetite on a normal diet, normal energy expenditure and normal reproductive functions [12]. Yet, they have a low bone mass phenotype [13]. The fact that bone mass accrual is the only function of leptin that is affected by a partial gain-of-function mutation in leptin signaling is consistent with the notion that the regulation of bone mass is a critical function of leptin, even if this has a less dramatic manifestation than its function on appetite.

1.2. Regulation of bone mass by brain serotonin: the central relay

As mentioned above, leptin's functions on appetite and reproduction are considered to be primarily mediated by a central relay. It turned out that leptin's regulation of bone remodeling did not make exception to this rule. Indeed, 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 the high bone mass of these mice [6]. The most compelling evidence, however, that leptin acts on bone centrally and not directly on osteoblasts came once again from genetic experiments. On the one hand, overexpressing leptin in osteoblasts or inactivating the leptin receptor in osteoblasts does not cause a bone phenotype [13,14]. On the other hand, mice in which the leptin receptor is inactivated in all neurons or only in neurons of the brainstem, the site of leptin's action in brain (see below), have a high bone formation phenotype similar to the one of the *db/db* mice [13,15].

The notion that leptin could be acting centrally originally came from two observations. First, the leptin receptor is expressed at high levels in two hypothalamic locations, the neurons of the arcuate (ARC) and of the ventromedial hypothalamic (VMH) nuclei. Second, rats in which the neurons of the VMH nuclei or of the ARC nuclei had been chemically destroyed using monosodium glutamate or gold thioglucose, respectively, led to hyperphagia and as a result to profound obesity. Repeating these classical lesioning experiments revealed that only destruction of the VMH nuclei could cause a massive increase in bone mass and bone formation parameters similar to the one observed in *ob/ob* mice [14]. Most importantly, when such lesioning was performed in *ob/ob* mice leptin ICV infusion could still decrease body weight but could not anymore affect bone formation parameters or bone mass [15]. These experiments thus identified the VMH neurons as the mediators of leptin's regulation of bone formation.

Subsequent studies, however, appeared to cast doubts on the hypothalamic actions of leptin on both bone mass and metabolism. Indeed, mice harboring a conditional deletion of the leptin receptor in VMH neurons did not develop a bone phenotype nor they showed increased appetite [16]. Likewise, inactivation of the leptin receptor in ARC neurons did not cause the metabolic abnormalities that chemical lesioning of these neurons did [17]. The apparent contradiction between the chemical and genetic experiments could be viewed as a demonstration that genetic experiments are much more precise and reliable than pharmacological ones. Although this assumption bears truth in most cases, these differing results could also be viewed as different pieces of the same puzzle. If one look at them without any bias all the lesioning experiments indicate is that the ARC and VMH are structures required for leptin's functions but they do not mean that they are the sites of its action. Likewise, the genetic experiments only show that

leptin do not need to signal through its receptor in the ARC and VMH neurons to affect appetite and bone formation, they do not question that these structures can be required for leptin's function in brain. When combined, however, these two sets of data raise the hypothesis that leptin could bind to its receptor in other brain structures than the ARC and VMH nuclei where it would control the expression or activity of a secondary messenger that would then use these nuclei to mediate its own function on bone mass and appetite. This hypothesis turned out to be exact, to fulfill these two functions leptin makes use of a secondary messenger, brain serotonin [15]. Instead of acting directly on hypothalamic neurons, leptin signals to its receptor expressed on serotonergic neurons present in the brainstem and this binding causes a decrease in the production of serotonin [15]. Because the role of brain-derived serotonin is reviewed in details in a separate paper of this issue, only the main lines of this functional cascade are presented here. Serotonergic neurons project both to the VMH and ARC neurons. Via the VMH serotonin causes a decrease in sympathetic output thereby blocking its dual regulation of bone formation and bone resorption (see below). In contrast, through its action on ARC neurons, serotonin favors appetite and energy expenditure (Figure 1).

1.3. Regulation of bone mass by the sympathetic nervous system, NeuromedinU and CART: going from the central nervous system to the skeleton

The next step was to identify how the central leptin/serotonin signals could reach bone cells. At the present time three mediators have been identified molecularly as well as genetically (Figure 2).

The first mediator to have been identified, and whose function is still the best understood to this day, is the sympathetic nervous system (SNS). Three key experiments identified the sympathetic tone as a major regulator of bone formation as well as bone resorption downstream of the leptin/serotonin central cascade. First, mice deficient in dopamine β -hydroxylase, an enzyme necessary to produce norepinephrine and epinephrine, the catecholamine ligands produced by the SNS, have a high bone mass and this high bone mass persists upon leptin ICV infusion [14]. Second, while *ob/ob* mice have a low sympathetic tone and a high bone mass phenotype, treating them with an adrenergic agonist or restoring their sympathetic activity to normal levels by decreasing brain serotonin production decreases their bone mass [14,15]. Third, analysis of mice deficient in the β 2 adrenergic receptor (*Adrb2*), the only adrenergic receptor expressed at significant levels in osteoblasts, reproduce the high bone mass/high bone formation phenotype observed in *ob/ob* mice, and this phenotype cannot be rescued by ICV infusion of leptin [9]. Further studies of the *Adrb2*-deficient mice allowed to define the molecular mechanisms taking place in osteoblasts that regulate bone formation as well as bone resorption in response to leptin/serotonin central signaling (Figure 2). In particular, they showed that the bone formation phenotype of the mutant mice is caused by an increase in osteoblast number due to an increase in these cells proliferation ability. This phenotype results from the combination of two parallel regulations: on the one hand *Adrb2* signaling induces AP-1 genes to favor osteoblast proliferation, and on the other hand it inhibits this process by decreasing, through the molecular clock, the expression of both D-type Cyclins and AP-1 genes [18]. Interestingly, the bone resorption phenotype of the *Adrb2*-deficient mice is also mediated by the osteoblast. Indeed, sympathetic signaling increases protein kinase A phosphorylation of ATF4, a cell-specific CREB-related transcription factor essential for osteoblast differentiation and function, which induces *Rankl* expression and thereby increases osteoclast differentiation and bone resorption [9,19].

A second mediator of leptin's action on osteoblasts is CART, a 116-amino acid long protein whose production in the hypothalamus is positively regulated by leptin but that is also present in the general circulation probably due to its secretion by pituitary glands and pancreatic islets [20,21]. This pattern of expression raises the possibility that CART might not be acting

exclusively as a neuromediator, and indeed, peripheral overexpression of CART in transgenic mice increases bone mass by reducing osteoclastogenesis. While CART-deficient mice do not present body weight or reproduction abnormalities they display a late onset low bone mass phenotype caused by a sharp increase in bone resorption [9,22]. Again, this phenotype is mediated by an osteoblastic relay, as CART inhibits bone resorption by decreasing RANKL expression by osteoblasts [9,23] (Figure 2). The low bone mass/high resorption phenotype observed in absence of CART is similar to the one observed mice deficient in MC4R, another known target of leptin in the hypothalamus [24]. As a matter of fact, CART was identified as the downstream mediator of MC4R in its control of bone resorption [24]. However, no change in hypothalamic *Cart* expression was observed in mice deficient in brain serotonin suggesting that leptin regulates its expression at this site independently of serotonin [15]. Whether the blood levels of CART are also normal in these mutant mice remains to be determined.

The third mediator of leptin known to act on bone is Neuromedin U, a neuropeptide produced by central neurons as well as nerve cells of the small intestine, whose gene expression is regulated by leptin [25]. NeuromedinU-deficient mice present a high bone mass/high bone formation phenotype similar to the one displayed by *ob/ob* mice that cannot be rescued by ICV infusion of leptin [26]. Additional analyses demonstrated that Neuromedin U is involved in the leptin-dependent negative regulation of bone formation by the sympathetic tone and the molecular clock [26].

2. Regulation of energy metabolism by osteocalcin, the skeleton as an endocrine organ

The work presented above established two aspects of the initial hypothesis that bone and energy metabolism could be cross-regulated via a central component, fat, a tissue pivotal in energy homeostasis, via leptin and brain serotonin controls both sides of bone remodeling through the osteoblast. Still, to fully validate this hypothesis a signal needed to be found that originates from bone and regulates energy metabolism. Unexpectedly, this signal turned out to be osteocalcin, a well-known molecule specifically secreted by osteoblasts.

2.1. Osteocalcin as a regulator of glucose homeostasis

Osteocalcin was first identified as a major component of the bone extracellular matrix where it binds to hydroxyapatite thanks to three γ -carboxylated Glu residues (Gla residues) [27,28]. It was then shown that osteocalcin is synthesized by osteoblasts as a pre-pro-molecule containing Glu residues and that it is γ -carboxylated and cleaved before it is secreted in the bone microenvironment as well as in the general circulation. Osteocalcin-deficient mice were generated more than 10 years ago and showed a mild and very late onset increase in bone formation [29]. Surprisingly considering its ability to bind the mineralized matrix this study as well as subsequent studies of osteocalcin overexpression in transgenic mice showed that osteocalcin does not play a role in bone mineralization [29,30]. In contrast, osteocalcin-deficient mice have a metabolic phenotype caused by a decrease in insulin secretion and sensitivity as well as by decreased β -cell proliferation and decreased energy expenditure [31]. As a result they are glucose intolerant and insulin resistant by 3 months of age. Conversely, infusing recombinant osteocalcin in wildtype mice increased β -cell proliferation, insulin secretion and insulin sensitivity, and this effect could prevent the development of glucose intolerance and insulin resistance when the mice were fed a high fat diet [32]. Importantly, cell culture experiments demonstrated that osteocalcin acts directly on β -cells and adipocytes to regulate insulin secretion and/or gene expression [31,32]. These studies also showed that it is the uncarboxylated form of osteocalcin that is bioactive and, accordingly, injecting mice with warfarin, a well-known inhibitor of Vitamin K-dependent γ -carboxylation, caused a decrease in blood glucose levels [31,33].

2.1. Regulation of osteocalcin bioactivity

As mentioned above, osteocalcin activity on energy metabolism depends on its uncarboxylated form. Although its link with the γ -carboxylation process is not yet understood one protein was found to modify osteocalcin carboxylation, the intramembranous OST-PTP tyrosine phosphatase. OST-PTP is encoded by the *Esp* gene, which is expressed specifically in embryonic stem cells, Sertoli cells of the testis and osteoblasts [34,35]. Inactivation of *Esp* in all cells or in osteoblasts only causes a metabolic phenotype mirroring the one observed in absence of osteocalcin [31]. Moreover, as mice infused with recombinant osteocalcin, *Esp*-deficient mice are protected from the development of glucose intolerance when fed a high fat diet [31]. A more formal link between *Esp* and osteocalcin was established by genetic experiments showing that placing the *Esp*-deficient mice on an *Osteocalcin* haploinsufficient background was sufficient to correct their metabolic phenotype [31]. Molecularly, it was also determined that although Osteocalcin gene expression and serum concentration were unchanged in absence of *Esp*, the level of circulating undercarboxylated osteocalcin was significantly decreased [33]. These observations indicate that the absence of *Esp* promotes osteocalcin bioactivity and that the *Esp*-deficient mice are a gain-of-function model for osteocalcin (Figure 3).

Linking this regulation with the one occurring downstream of leptin/serotonin central signaling on osteoblast functions, the sympathetic tone was subsequently found to regulate *Esp* expression in osteoblasts and thereby osteocalcin carboxylation/bioactivity (Figure 3). Both *ob/ob* mice and mice lacking the β 2-adrenergic receptor specifically in osteoblasts show an increase in uncarboxylated osteocalcin in serum which induces an increase in insulin secretion [33]. As treatment of osteoblasts with the β -agonist isoproterenol demonstrated, this change is associated with an isolated increase in *Esp* expression in osteoblasts [33]. Hence, there is a regulatory loop in which adipocytes via leptin, brain serotonin and sympathetic signaling in osteoblasts regulate both sides of bone remodeling as well as glucose homeostasis via osteocalcin. It had been shown that one of the transcriptional mediator of the sympathetic regulation of bone resorption by osteoblasts is ATF4, a transcription factor already known to regulate osteocalcin expression [9,19] (Figure 3). The same is true for the β 2-adrenergic regulation of osteocalcin bioactivity. Indeed, ATF4-deficient mice display an increase in insulin production and glucose tolerance that could be traced to a decrease of *Esp* expression [36]. Conversely, overexpressing ATF4 in osteoblasts is associated with glucose intolerance, increased *Esp* expression and decreased levels of uncarboxylated osteocalcin [36]. This effect of ATF4 on *Esp* expression was direct as chromatin immunoprecipitation experiments revealed that this factor binds to the *Esp* promoter.

Another transcription factor, FoxO1, was found to modulate both osteocalcin gene expression and its bioactivity through *Esp* regulation (Figure 3). Indeed, a recent study showed that osteocalcin serum levels are increased 30% in osteoblast-specific Foxo1-deficient mice due to up-regulation of Osteocalcin gene expression [37]. In addition, expression of *Esp* is reduced 75% in these mutant mice causing a significant increase in the amount of undercarboxylated osteocalcin present in the serum. As a result, osteoblast-specific *Foxo1*-deficient mice show an increase glucose tolerance and insulin sensitivity that was, as previously observed with the osteocalcin infusion and *Esp*-deficient mice, sufficient to protect them from the development of diabetes and obesity induced by a high fat diet [37].

3. Clinical relevance of the cross-regulation between bone and energy metabolisms

The importance of any biologic discovery is relative to its relevance to human development, physiology or pathology. It is therefore important to mention, even briefly, in this review that

many steps and players of this cross-talk between bone and energy metabolisms primarily identified in mice have since been identified and/or validated by clinical studies.

Patients harboring an inactivating mutation of the leptin gene or lipodystrophic patients, whose serum leptin levels are extremely low due to the absence of adipocytes, display advanced bone age and in some cases osteosclerotic lesions, an evidence that, as in mice, the absence of leptin increased osteoblasts function [38,39]. Likewise, patient with mutations in MC4R display a decrease in bone resorption parameters causing a late onset high bone mass phenotype associated, as in mice, an increase in CART serum level [24,40]. The role of sympathetic signaling in human bone biology has also been confirmed by several clinical studies. For instance, analysis of large cohorts from the Geelong Osteoporosis Study (Australia), the General Practice Research Database (UK) and the Study of Osteoporotic Fractures (USA) showed that use of β -blockers was associated with a statistically significant decreased risk of fracture [41-43].

A large body of clinical studies has also demonstrated the connection between glucose homeostasis and osteocalcin in human. Indeed, circulating osteocalcin levels have been reported to be inversely associated with measures of insulin resistance (fasting insulin and glucose levels and homeostasis model assessment of insulin resistance [HOMA-IR]) and adiposity (body mass index [BMI] and fat percentage) [44-47]. It was also reported that patients with type 2 diabetes have lower levels of osteocalcin than healthy controls and that osteocalcin concentration increases upon improvement of their glycemic control [48]. Lastly, a recent study showed that in two large cohorts of different ethnicity (blacks and non-hispanic whites) serum osteocalcin levels were significantly higher in participants who had fewer traits of the metabolic syndrome [47].

Perspectives

Based on the hypothesis that bone and energy metabolisms should be subjected to coordinated regulations this body of work has identified adipocytes via leptin, brain serotonin and sympathetic signaling in osteoblasts as regulators of both arms of bone remodeling, and osteoblasts via osteocalcin as regulators of glucose homeostasis. Viewed more globally, this also means that the endocrine function of one organ, fat, is mediated by the endocrine function of another organ, skeleton. If one now considers that osteocalcin regulates insulin production as well as the sensitivity to insulin of peripheral organs such as muscle, adipose tissue and liver, this network further gains in complexity since the endocrine function of pancreas becomes part of the loop. Yet, one can predict that this complexity is only going to increase since other connections might exist that constitute feedback or feed-forward loops within this same network or even link these regulations to other tissues. Future studies based on the concept of integrative physiology that led to the findings summarized in this review will most likely uncover such novel branches. In doing so they should provide a better understanding of how organs can work together to maintain homeostasis as well as to succeed in adapting life to novel environmental conditions.

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Figure 1.
Leptin use a central relay to regulate bone mass and energy metabolism.

Figure 2.
The osteoblast is the peripheral mediator of all function of leptin on bone mass accrual.

Figure 3.
The osteoblast regulates glucose homeostasis through osteocalcin.