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Searching for additional endocrine functions of the skeleton: genetic approaches and implications for therapeutics

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Abstract

Our knowledge of whole organism physiology has greatly advanced in the past decades through mouse genetics. In particular, genetic studies have revealed that most organs interact with one another through hormones in order to maintain normal physiological functions and the homeostasis of the entire organism. Remarkably, through these studies many unexpected novel endocrine means to regulate physiological functions have been uncovered. The skeletal system is one example. In this article, we review a series of studies that over the years have identified bone as an endocrine organ. The mechanism of action, pathological relevance, and therapeutic implications of the functions of the bone-derived hormone osteocalcin are discussed. In the last part of this review we discuss the possibility that additional endocrine functions of the skeleton may exist.

Keywords

Osteoblast; osteocalcin; glucose homeostasis; reproduction; cognition and brain development

Introduction

As a highly mineralized fibrous tissue, bone, one of the two tissues that form the skeleton, carries out several important physiological functions, such as providing structural support for the whole body, protection to internal organs, attachment sites for skeletal muscle and being the main reservoir of calcium and phosphorus. Moreover, by producing FGF23, a hormone that acts on the kidneys to decrease the reabsorption and to increase excretion of phosphate, bone plays an active role in regulation of whole-body mineral metabolism, a physiological process that is fundamental for bone health [1–9]. Recent studies had revealed

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that bone provides bone marrow niches essential for adult hematopoietic stem cells [10–12]. Based on these crucial functions, bone could already been viewed as a vital organ.

Bone also has a unique cell biology feature: the ability that constantly renews itself through a physiological process called bone (re)modeling. During childhood bone modeling allows bone mass to be built up whereas during adulthood bone remodeling maintains bone mass by repairing micro-damages caused by regular physical activities and macro-damages resulted from injuries such as fractures [13]. Bone (re)modeling comprises two successive steps: resorption of preexisting mineralized bone extracellular matrix by osteoclasts followed by bone formation by the osteoblasts [14]. Since bone (re)modeling occurs on a daily basis in many locations in an organ that covers a large surface in the body, it is reasonable to assume that this process should be a high energy consuming process. This assumption is verified by clinical observations: Patients with anorexia nervosa, a disease characterized by very limited food intake, often develop growth arrest in childhood and osteoporosis in adulthood [15–25]. These observations led to the hypothesis that there may be a coordinated regulation between bone and energy metabolism.

The initial phase of testing this hypothesis, through mouse genetic studies led to the demonstration of the existence of a leptin-dependent central regulation of bone mass accrual. Leptin, an adipokine also controlling food intake and whole-body energy metabolism, inhibits bone mass accrual through its actions in the central nervous system involving serotonergic neurons, and the sympathetic nervous system [26–32]. While these studies strongly supported the notion that energy metabolism regulates bone mass accrual, they also raised the question of whether a feedback regulation from bone to energy metabolism may exist. Genetic studies aiming at addressing this question resulted in identification of a novel bone-derived hormone and several novel endocrine functions of bone that are the topic of this review [33–35].

Osteocalcin, an osteoblast specific hormone, regulates glucose homeostasis

Osteocalcin, an osteoblast specific protein, was first identified on the basis that it is the most abundant non-collagenous protein of the bone extracellular matrix. Because it contains three γ -carboxylated Glu residues (Gla residues), which confer to protein high-affinity for minerals such calcium phosphate hydroxyapatite crystals, osteocalcin had long been suspected to play a role during mineralization of the bone extracellular matrix [36, 37]. However, analyses of *Osteocalcin*^{−/−} mice as well as transgenic mice overexpressing *Osteocalcin* failed to detect any role of osteocalcin, in vivo, during the mineralization of the bone extracellular matrix [38, 39]. Instead, the unexpected observation that *Osteocalcin*^{−/−} mice had visibly much more visceral fat than wild-type littermates, caught the attention of the investigators and led to the suspicion that osteocalcin may rather influence some aspects, yet to be defined at that time, of energy metabolism [40]. As it turns out, *Osteocalcin*^{−/−} mice are glucose intolerant and insulin resistant because of a decrease in β -cell proliferation, in insulin secretion and in insulin sensitivity. In addition, *Osteocalcin*^{−/−} mice experience a decrease in energy expenditure at rest [33]. In contrast, continuous infusion or intermittent

injections of recombinant osteocalcin in wild type mice significantly improved their glucose tolerance and insulin sensitivity [34, 41]. Cell-based assays revealed that osteocalcin directly acts on pancreatic β -cells to regulate insulin gene expression and secretion [33, 34]. Hence, these data indicate that osteocalcin is a bone-derived hormone favoring whole-body glucose homeostasis (Figure 1).

Regulation of osteocalcin bioactivity

The newly synthesized osteocalcin in osteoblasts is carboxylated on three glutamic acid residues through an intracellular process that requires two enzymes, the γ -carboxylase (GGCX) and vitamin K epoxide reductase (VKORC1) [36, 42]. In the general circulation, both carboxylated and undercarboxylated forms of osteocalcin can be found [43]. It is the latter form of osteocalcin that is able to stimulate insulin synthesis and secretion in pancreatic β -cells [33, 34]. Consistent with these observations, an osteoblast-specific deletion of GGCX or VKORC1 results in a massive increase of undercarboxylated osteocalcin levels and an improved glucose tolerance [44, 45]. These experimental facts may explain why warfarin, a well-known inhibitor of Vitamin K-dependent γ -carboxylation, decreases osteocalcin carboxylation and blood glucose levels [33, 34, 46]. If the uncarboxylated osteocalcin is the active hormone then the question becomes how is the bioactivity of osteocalcin, i.e. its decarboxylation, regulated physiologically?

The answer to this question came from the studies of another osteoblast-specific gene, *Esp*, which encodes OST-PTP, an intramembranous tyrosine phosphatase [47, 48]. Mice lacking this gene globally or in an osteoblast-specific manner had better glucose tolerance and insulin sensitivity than control mice. Since mice lacking *Esp* in all cells or in osteoblasts only had the same metabolic phenotype, it indicated that OST-PTP fulfills this metabolic function through its presence in osteoblasts. This is particularly important since the metabolic phenotype of mice lacking *Esp* in osteoblasts only was the mirror image of the one seen in *Osteocalcin*^{-/-} mice [33]. In fact genetic experiments did demonstrate that the improvement in glucose homeostasis observed in the *Esp*^{-/-} mice was secondary to an increase of the circulating levels of undercarboxylated osteocalcin and therefore an increase in osteocalcin functions [33]. Expression of *Esp* in osteoblasts is positively regulated by two transcription factors, ATF4 and FoxO1, and osteoblast-specific deletion of these genes in mice led to a significant decrease in *Esp* expression and thereby an increase in undercarboxylated osteocalcin levels, glucose tolerance and insulin sensitivity [49–51]. Hence, these studies together indicate that *Esp*-deficient mice are a gain-of-function model for osteocalcin.

How an intracellular tyrosine phosphatase like OST-PTP could regulate the carboxylation status of osteocalcin that is an extracellular molecule? Biochemical and molecular investigations demonstrated that neither osteocalcin, GGCX nor VKORC1 was substrate of OST-PTP but rather that OST-PTP dephosphorylates the insulin receptor in osteoblasts. This explains why insulin signaling is so much enhanced in *Esp*^{-/-} osteoblasts [35]. These observations implied that the *Esp*^{-/-} mice were a model of gain-of-function of insulin signaling in osteoblasts or in other words, that insulin signaling is upstream of osteocalcin bioactivity. In agreement with this notion, mice lacking the insulin receptor specifically in

osteoblasts (*InsR_{osb}*^{−/−}) have reduced circulating levels of the uncarboxylated and active form of osteocalcin, are glucose intolerant and insulin resistant just as seen in *Osteocalcin*^{−/−} mice [35, 52]. The analysis of the *InsR_{osb}*^{−/−} mice revealed that insulin signaling in osteoblasts inhibits the expression of *Osteoprotegerin* (*Opg*), a decoy receptor for RANKL and an inhibitor of osteoclast differentiation and bone resorption [53]. As a result of this up-regulation of *Osteoprotegerin*, bone resorption is hampered in the *InsR_{osb}*^{−/−} mice [54]. Since an acidic pH is the only means that can decarboxylate proteins outside the cells, these observations prompted the investigators to ask if insulin signaling in osteoblasts favors osteocalcin bioactivity because it increases bone resorption and therefore the decarboxylation of osteocalcin. Exactly as surmised, pharmacological approaches and genetic analyses in mice and in humans demonstrated that insulin signaling in osteoblast activates osteocalcin by favoring bone resorption and thereby the decarboxylation of this hormone [35] (Figure 2).

The notion that bone resorption favors osteocalcin activation has now been verified by several studies analyzing mouse models harboring either an increase or a decrease in osteoclastic resorption. First of all, *oc/oc* mice, a spontaneous loss-of *Tcirg1* mutation mouse model [54–56], develop a severe osteopetrotic and a glucose intolerance phenotype associated with a massive decrease in the circulating levels of undercarboxylated osteocalcin and insulin [35]. Likewise, ablating osteoclasts through transgenic expression of *Diphtheria toxin fragment A* (*DTA*) in osteoclasts specifically, caused a reduction in osteocalcin bioactivity and glucose tolerance [57]. In contrast, *Osteoprotegerin-deficient* mice, a mouse model of osteoporosis because of an increase in the number of osteoclasts [53], displayed a higher circulating levels of undercarboxylated osteocalcin and an improved glucose homeostasis when compared to WT littermates [57]. Consistent with the clinical observations that high *Opg* levels and low osteocalcin levels are positively associated with insulin resistance in type 2 diabetes patients (see review [58]), these *in vivo* genetic evidences further support the notion that osteoclastic bone resorption is a physiological means to regulate glucose homeostasis.

Mutual dependence between bone and glucose homeostasis

The finding that bone is an endocrine organ regulating glucose homeostasis brought up another question that is to know why would bone have this function? Addressing this question allowed the field to make significant progress. The original hypothesis of a coordinated regulation of bone mass and energy metabolism was based on the assumption that bone may be an organ requiring large amounts of energy or nutrients to achieve bone (re)modeling. However, this assumption had never been tested experimentally *in vivo*. In addressing this question, we recently found that, just as it is the case for neurons, glucose is the main nutrient of osteoblasts and it is transported in osteoblasts in an insulin-independent manner through a facilitative glucose transporter, *Glut1*. Mice lacking *Glut1* specifically in osteoblasts displayed a severe delay in skeletogenesis prenatally and developed osteopenia and glucose intolerance postnatally because of a compromised osteoblast differentiation, a decrease in bone formation and reduced circulating levels of insulin [59]. Further molecular and genetic investigations revealed that to achieve its multiple functions, glucose inhibits two distinct functions of AMPK. On the one hand by inhibiting AMPK, glucose uptake in

osteoblasts favors mTORC1 pathway and promotes synthesis of the main bone matrix protein, type I collagen, i.e. bone formation. On the other hand glucose also suppresses the ability of AMPK to activate, through phosphorylation, Smurf1, an E3 ubiquitin ligase mediating proteosomal degradation of the master regulator of osteoblast differentiation and function Runx2. Since Runx2 is the main transcriptional regulator of *Osteocalcin* expression, by promoting Runx2 accumulation, glucose uptake in osteoblasts favors *Osteocalcin* expression [60, 61] and thereby whole-body glucose homeostasis. The relationship between Runx2 and glucose uptake goes a step further since Runx2 in turn regulates *Glut1* expression in osteoblasts [59]. By uncovering an amplification chamber in which Glut1, Runx2 and osteocalcin signaling pathway in coordinating osteoblast differentiation, bone formation and whole-body glucose homeostasis, these findings certainly provide one rationale for why bone would regulate glucose homeostasis (Figure 3).

Bone regulates male fertility through osteocalcin

The exciting discovery of the endocrine nature of osteocalcin and its ability to regulate glucose metabolism opened up a new avenue for investigation of novel endocrine functions of bone. Among many endocrine hormones that regulate bone physiology [62–67], the sex steroid hormones produced by gonadal glands exert the most powerful hormonal regulation to maintain bone mass [68–75]. The critical importance of this hormonal regulation of bone physiology naturally raises the prospect that there might be a feedback regulation of bone on reproduction. The identification of osteocalcin as a hormone allowed for testing this question. This analysis began with the observations that testosterone production was markedly increased in testis explants co-cultured with WT but not with *Osteocalcin*^{−/−} osteoblasts [76]. From then on, systemic investigations of loss-of and gain-of function models for osteocalcin (*Osteocalcin*^{−/−} and *Esp*^{−/−} mice), revealed that *Osteocalcin*^{−/−} male mice, but not females, have a compromised fertility phenotype associated with a significant decrease in testes, epididymides and seminal vesicles weights as well as sperm count, whereas these parameters were increased in *Esp*^{−/−} male mice. Cell-based assays established that osteocalcin promotes Leydig cell maturation and testosterone production by these cells, but has no effect on the production of testosterone, estradiol and progesterone in ovary [76]. Thus these studies indicate that bone through osteocalcin can affect gonadal functions in male (Figure 4).

The identification that osteocalcin bioactivity is regulated by osteoclastic bone resorption [35], raised the question as to know if this aspect of bone physiology could also affect reproductive functions. This hypothesis was tested using loss-of-function and gain-of-function mouse models of bone resorption. Mice lacking the *insulin receptor* specifically in osteoblasts (*InsR_{osb}*^{−/−}) that have less active osteocalcin due to a decrease in bone resorption [35] show a decrease in testes size and weight, sperm count and circulating testosterone levels [77]. Likewise, ablation of osteoclasts by osteoclast-specific expression of *DTA* causes a 2-fold decrease in the levels of undercarboxylated osteocalcin and thereby a significant reduction in sperm count, circulating testosterone levels and testes weight [77]. In contrast, *Osteoprotegerin-deficient* mice that have a substantial increase in bone resorption [53], demonstrate a significant increase in circulating levels of active osteocalcin and testosterone, sperm count and testes, epididymides, and seminal vesicle weights [77]. This

bone-testis axis exists also in humans since several studies have demonstrated that circulating levels of osteocalcin is positively correlated with serum levels of testosterone in men [78–83].

Gprc6a, a G-protein coupled receptor mediates osteocalcin signaling in Leydig cells and pancreatic β -cells

How does osteocalcin act molecularly? A pre-requisite to answer this question is to identify the receptor(s) of osteocalcin. The observation that osteocalcin can stimulate cAMP production in Leydig cells suggested that the osteocalcin receptor could be a G protein coupled receptor (GPCR)[76]. Taking advantage of the fact that osteocalcin regulates male, not female fertility, a gene expression screening, looking for *GPCRs* whose expression is enriched in testis and Leydig cells but not in ovary, resulted in the identification of Gprc6a, an orphan GPCR that had been proposed to be a receptor of osteocalcin [76, 84–87]. Several lines of biochemical and genetic evidence verified that Gprc6a indeed is a bona fide receptor of osteocalcin in Leydig cells as well as in pancreatic β -cells [76, 88]. First, osteocalcin can directly bind to WT but not *Gprc6a*^{−/−} Leydig cells or β -cells; second, osteocalcin can not enhance cAMP and testosterone productions in *Gprc6a*^{−/−} Leydig cells and can not induce insulin synthesis and secretion in *Gprc6a*^{−/−} islets; Third, mice lacking *Gprc6a* specifically in Leydig cells or β -cells, resulted in compromised fertility characterized with low testosterone production or hypoinsulinemia and glucose intolerance due to a decrease in pancreatic β -cell proliferation, insulin synthesis and secretion as seen in *Osteocalcin*^{−/−} mice. Fourth, compound heterozygous mice lacking one allele of *Osteocalcin* and one allele of *Gprc6a* specifically in Leydig cells or β -cells developed reproduction or metabolic phenotypes that resemble to those of *Osteocalcin*^{−/−} and *Gprc6a*^{−/−} mice [76, 88]. Subsequent molecular analysis revealed that osteocalcin activates the transcription factor CREB to up-regulate the expression of a set of key enzymes of testosterone biosynthetic pathway in Leydig cells, such as StAR, Cyp11a, Cyp17, 3 β -HSD, Cyp19, and HSD-17, and the expression of *Insulin* and *Cyclin D1* in β -cells [76, 88, 89] (Figure 4). Gprc6a is also highly expressed in various tissues and cell types [86, 87, 90, 91], thus its identification as an osteocalcin receptor paves the way to search for additional target cells and functions of osteocalcin.

Conserved functions of osteocalcin in humans

An important issue raised by these mouse genetic studies is to determine whether osteocalcin acts also as a hormone in humans. The identification of Osteocalcin/Gprc6a cascade in regulation of fertility and glucose homeostasis provided a toolkit to search for mutations in human patients displaying similar phenotypes to those of *Osteocalcin*^{−/−} or *Gprc6a*^{−/−} mice. A human disease called peripheral testicular failure mimics the fertility phenotype of the male *Osteocalcin*^{−/−} mice: low testosterone, high luteinizing hormone and sterility [92–95]. Sequencing *GPRC6A* in these patients revealed the existence of a point mutation in exon 4 of *GPRC6A* in two unrelated patients [77]. This point mutation results in a missense mutation of a highly conserved residue located in the N-terminal region of GPRC6a (F464Y mutation) [77]. More importantly, this mutation acts as a dominant

negative inhibitor, since when co-expressed with WT GPRC6a *in vitro*, the F467Y mutant blocks osteocalcin signaling in a dose dependent manner; likewise, intratesticular infection with a lentivirus expressing F467Y mutated form of Gprc6a decreased expression of osteocalcin target genes that mediate testosterone synthesis *in vivo* [77]. Of note, both patients were also glucose intolerant. Thus, these data indicated that osteocalcin signaling through GPRC6A fulfills conserved endocrine functions between human and mouse.

A reciprocal interaction between bone and brain

It is now established through the studies of the leptin-serotonin-dependent central control of bone mass accrual that brain plays a fundamental role in regulating bone physiology [26–29, 31]. The existence of the mutual dependence between bone, energy metabolism and gonads together with the observation that patients with Cleidocranial dysplasia (CCD), a disease caused by loss-of function for the master regulator of osteoblast differentiation and function, Runx2, often manifest cognitive deficit [96–98], raised the hypothesis that bone may in turn communicate to brain and affect its functions.

Since Runx2 is the main regulator of *Osteocalcin* expression [60, 61], and *Osteocalcin*^{−/−} mice appeared to be much less active compared to WT littermates, it was natural to ask if osteocalcin affects brain functions. These investigations demonstrated that indeed osteocalcin could signal in the brain. The active form of osteocalcin crosses the blood-brain barrier, specifically binds to neurons of the brainstem, midbrain, and hippocampus, favors the expression of genes necessary for serotonin and catecholamine synthesis and inhibits the expression of genes involved in GABA biosynthesis [99] (Figure 5). Accordingly, adult *Osteocalcin*^{−/−} mice have low levels of serotonin, dopamine, and norepinephrine and high levels of GABA in their brains, leading to anxiety and depression-like behaviors and impaired spatial learning and memory [99].

Moreover and rather unexpectedly, maternal osteocalcin also regulates fetal brain development [99]. First, circulating osteocalcin can be detected in *Osteocalcin*^{−/−} fetus carried by *Osteocalcin*^{+/-} mothers but not in the ones originated from *Osteocalcin*^{−/−} mothers. Second, osteocalcin can cross the placenta and its expression is not detected in the placenta. Third, the compromised fetal neurogenesis due to a 2-fold increase of apoptotic neurons in the hippocampus was only seen in *Osteocalcin*^{−/−} fetus carried by *Osteocalcin*^{−/−} mothers but not in the ones carried by *Osteocalcin*^{+/-} mothers. Fourth, when *Osteocalcin*^{−/−} mothers had been injected with recombinant osteocalcin daily throughout pregnancies, their *Osteocalcin*^{−/−} offspring showed a nearly complete rescue of both the compromised fetal neurogenesis and the impaired learning and memory in adulthood. Collectively, these data indicate that maternal osteocalcin, through affecting fetal brain development, is required for optimal spatial learning and memory in the adult offspring (Figure 5).

Interestingly, mice lacking *Gprc6a*, the only known osteocalcin receptor functioning in Leydig cells and pancreatic β -cells, although having similar metabolic and reproductive abnormalities to *Osteocalcin*^{−/−} mice, do not manifest any molecular and histological defects in their brains as the ones seen in *Osteocalcin*^{−/−} mice and behave normally [99]. In fact, *Gprc6a* is not expressed in regions of the brain where osteocalcin signals. This

observation suggests that osteocalcin may signal through other receptor(s) in the brain to fulfill its functions. The identification of such receptors is now needed to better define all the functions of osteocalcin in the brain.

Pathological relevance and Therapeutic Implications

Osteocalcin had been used traditionally as a diagnostic marker of the quality of bone formation. The realization that bone is an endocrine organ with a growing number of functions revolutionizes our view of the role of osteocalcin in various pathological conditions. In the recent few years, a burgeoning body of clinical studies has demonstrated that osteocalcin is negatively associated with the metabolic syndromes of Type 1 diabetes, Type 2 diabetes, obesity and cardiovascular disease [100–124]. However, due to their correlative nature, these clinical observations provide only limited information regarding whether or not osteocalcin plays a significant role in the pathogenesis of these diseases. Again mouse genetics provides a more powerful way to address this issue albeit in an animal model. Resembling human type 2 diabetes, mice fed a high-fat diet (HFD) develop insulin resistance and obesity that is associated with a marked decrease in the circulating levels of the undercarboxylated form of osteocalcin [125]. Molecular investigations revealed that this decline in osteocalcin activity resulted from a bone-specific insulin resistance triggered by excessive levels of saturated fatty acids. Those saturated fatty acids induce proteosomal degradation of the insulin receptor in osteoblasts [125]. If osteocalcin is a significant contributor to the systemic insulin resistance observed in mice fed a HFD, one could expect at least some degree of rescue of the metabolic phenotypes when the circulating osteocalcin levels are normalized in these mice. Indeed, restoration of osteocalcin activity through specific overexpression of the *Insulin receptor* in osteoblasts completely corrected the bone-specific insulin resistance and therefore partially protected mice from HFD-induced systemic insulin resistance and obesity. Conversely, haploinsufficiency of either *Osteocalcin* or the *Insulin receptor* specifically in osteoblasts worsened HFD-induced insulin resistance due to a significantly lower osteocalcin activity than that of control mice fed the same HFD [125].

Long-term glucocorticoid treatment often causes insulin resistance, diabetes, obesity and osteoporosis in humans [126]. Since osteocalcin synthesis is suppressed by glucocorticoid treatment, Brennan-Speranza et al. proposed that osteocalcin might be one of the contributing factors of the metabolic disorders associated with glucocorticoid therapy. In addressing this hypothesis, they showed that the ablation of the glucocorticoid signaling specifically in osteoblasts normalized osteocalcin synthesis and thereby prevented the development of insulin resistance, glucose intolerance, and obesity in corticosterone-treated mice. In agreement with this result, ectopic production of uncarboxylated osteocalcin in the liver significantly relieved the metabolic side effects of glucocorticoid treatment [127].

The potential of osteocalcin as a treatment of metabolic diseases is suggested by several pharmacological studies. Ferron *et al.* showed that daily intermittent intraperitoneal injections of recombinant osteocalcin in mice fed a HFD resulted in decreased insulin resistance, reduced weight gain, reversal of liver steatosis and increased energy expenditure [41]. Zhou *et al.* showed that the intermittent administration of osteocalcin can attenuate

autophagy and ER stress in adipose and vascular tissues of mice fed a high-fat diet [128, 129]. Gupte *et al.* demonstrated that osteocalcin treatment substantially reduced nonalcoholic steatohepatitis in mice fed a HFD or high-cholesterol diet [130]. Interestingly, osteocalcin can also be administered through oral route to improve glucose tolerance in mice by triggering GLP-1-mediated stimulation of insulin secretion, which also suggested another potential mechanism of action of osteocalcin in regulating glucose homeostasis [131, 132].

The observation that long term continuous infusion of osteocalcin at 0.3 or 3 ng/h but not at 30ng/h significantly increased insulin secretion and pancreatic β -cell proliferation, suggests chronic osteocalcin treatment could have a risk to develop resistance to this hormone [34]. This notion was supported by a study from the Clemens' group showing that mice harboring osteoblast specific ablation of *Tsc2* have improved glucose handling with increased levels of insulin and undercarboxylated osteocalcin at 1 week of age. However, with age, these mutant mice developed opposite metabolic phenotypes due to chronic elevations in undercarboxylated osteocalcin that down-regulating *Gprc6a* expression and therefore desensitizing osteocalcin signaling in pancreatic β -cells [133]. Taken together, these genetic and pharmacological studies reveal the pivotal role that osteocalcin plays in the development of metabolic diseases and suggests that harnessing osteocalcin signaling may be of therapeutic use for metabolic diseases.

Osteocalcin and beyond

The concept of bone being an endocrine organ has prompted a number of researchers in the field of skeletal biology to expand their investigations beyond skeleton, cell biology per se and to tackle problems of whole organism physiology. For instance, mice with osteoblast-specific deletion of Fra-2, a transcription factor favoring osteocalcin expression, are osteopenic and glucose intolerant associated with reduced serum levels of osteocalcin [134]. Others have also suggested that bone could regulate energy metabolism in an osteocalcin-independent manner. For example, ablation of osteoblasts in adult mice resulted in both *Osteocalcin*^{-/-} mice like metabolic phenotypes, such as hypoinsulinemia, hyperglycemia, glucose intolerance, and insulin resistance, and osteocalcin-independent abnormalities, such as increased energy expenditure and the expression of resistin [135]. Similarly, mice with osteoblast-specific knockout of HDAC4, a class II histone deacetylase favoring *Osteocalcin* expression by stabilizing the transcription factor ATF4, developed osteocalcin-deficient phenotypes, such as impaired spatial learning, memory, male fertility and insulin secretion, however, they also showed decreased appetite, a physiological function that is not regulated by osteocalcin [136]. Gillespie *et al.* showed that osteoblast-specific inactivation of *Gsk3b* gene, which encodes glycogen synthase kinase 3 β , resulted in the development of type 2 diabetes, kidney damage, and hydronephrosis in mice with no detectable changes in osteocalcin levels [137]. Lee *et al.* showed that mice lacking neuropeptide Y, Y1 receptors specifically in cells of the early osteoblastic lineage displayed reduced glucose tolerance while osteocalcin levels were normal [138]. Overall, these exploratory investigations not only further support the notion that osteoblasts are necessary for energy metabolism but also suggest that besides osteocalcin, other bone-derived hormones may contribute to the endocrine functions of skeleton in regulation of whole-body physiology.

Expert commentary & Five-year view

Based on the hypothesis that bone and other tissues or physiological processes should be subjected to coordinated regulations, the body of work presented here has demonstrated that bone, through osteocalcin, exerts multiple endocrine functions to regulate energy metabolism, reproduction and cognition. These findings open up new exciting directions in searching the endocrine functions of skeletal system.

Despite an increasing number of correlated clinical evidence, the critical importance of these novel findings needs to be further strengthened by exploring the functions of osteocalcin mechanistically. Several important questions regarding osteocalcin biology remain to be addressed. For instance: what are the functions of osteocalcin in various Gprc6a-expressing tissues or cell types and what is (are) the receptor(s) and signaling cascade of osteocalcin in the brain? Moreover, a number of studies had already suggested that besides osteocalcin, bone might synthesize other hormones to carry out its endocrine functions. The identity of these hormones and their functions will definitely worth further investigations. To date, human genetic evidence supporting the endocrine roles of bone is still very limited. With the rapid advancing of the DNA sequence technology and the new initiative on precision medicine, we can expect in the near future that more mutations or variations will be discovered in patients with diseases of metabolism, fertility or cognition. By addressing these questions in the next five years, we could uncover new endocrine functions of bone, reach a better understanding of the physiological interplays among organs and bring new insights into the development of therapeutic treatment for various systemic diseases such as skeletal diseases, diabetes, infertility and cognitive diseases.

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* of interest

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Key issues

- Bone through osteocalcin, a hormone specifically produced by osteoblasts, exerts multiple endocrine functions.
- Cell-specific gain-of and loss-of function mouse genetic studies revealed that osteocalcin, favors glucose homeostasis by promoting pancreatic β -cells proliferation, insulin synthesis, secretion and sensitivity.
- Osteocalcin is active in its undercarboxylated form. By promoting bone resorption, insulin signaling in osteoblasts favors decarboxylation of osteocalcin, i.e. activate this hormone.
- Osteocalcin favors male fertility by promoting Leydig cells maturation and testosterone production.
- Gprc6a, a GPCR, is a bona fide receptor of osteocalcin in mouse and in humans mediating osteocalcin functions in Leydig cells and pancreatic β -cells.
- Osteocalcin prevents anxiety and depression and promotes learning and memory by favoring neurogenesis prenatally and postnatally.
- Both clinical observations and mouse genetic studies suggest that osteocalcin is a significant contributor to the pathogenesis of diabetes and the metabolic side effects of chronic glucocorticoid therapy.
- Pharmacological studies had demonstrated that osteocalcin could be a potential treatment for metabolic diseases.
- Identification of additional functions of osteocalcin and novel bone-derived hormones will bring new insight in the development of therapeutic treatment of various systemic diseases such as bone disease, diabetes, infertility and cognitive diseases.

Figure 1. Bone is an endocrine organ favoring glucose homeostasis

The endocrine regulation of glucose homeostasis by the bone is mediated by osteocalcin, an osteoblast-specific hormone. Osteocalcin regulates glucose metabolism by increasing insulin secretion, favoring pancreatic- β -cell proliferation, and increasing insulin sensitivity in various insulin sensing tissues.

Figure 2. Insulin signaling in osteoblasts favors activation of osteocalcin

Insulin signaling in osteoblasts inhibits the expression of *Osteoprotegerin* and therefore enhances osteoclastic bone resorption. Osteoclastic bone resorption by providing a low pH environment in resorption lacuna favors decarboxylation i.e. activation of osteocalcin. Undercarboxylated osteocalcin, the active form of osteocalcin, promotes glucose metabolism by increasing insulin secretion, pancreatic-β-cell proliferation, and insulin sensitivity.

Figure 3. Mutual dependence between bone and glucose homeostasis

Schematic representation of Glut1, Runx2 and osteocalcin pathway by which bone formation, osteoblast differentiation and glucose metabolism are coordinated. Glucose is transported in osteoblasts through *Glut1*. Glucose uptake in osteoblasts inhibits AMPK activity and therefore on the one hand, favors mTORC1 pathway to promote protein synthesis and bone formation, on the other hand, suppresses Smurf1 activity to favor accumulation of Runx2. Runx2 promotes osteoblast differentiation and up-regulates the expression of *Osteocalcin* to favor glucose metabolism.

Figure 4. Osteocalcin favors male fertility and glucose homeostasis through Gprc6a

Osteocalcin signals through Gprc6a, a GPCR, in pancreatic β -cell and testis Leydig cell to carry out its endocrine functions. In Leydig cells, osteocalcin signaling stimulates the release of cAMP to activate CREB and thereby increases the expression of a set of key enzymes of testosterone biosynthetic pathway, such as StAR, Cyp11a, Cyp17, 3 β -HSD, Cyp19, and HSD-17, to facilitate testosterone synthesis and spermatogenesis. In pancreatic β -cells, osteocalcin signaling increases the expression of *Insulin* and *Cyclin D1* to promotes insulin synthesis and proliferation.

Figure 5. Osteocalcin favors cognition and brain development

The active form of osteocalcin crosses the blood brain barrier and functions at brainstem, midbrain and hippocampus to favor synthesis of serotonin and catecholamine, while inhibit GABA synthesis. The maternal osteocalcin crosses placenta and regulates fetal neurogenesis by favoring survival of neurons. Through these two levels of regulation, osteocalcin favors spatial learning and memory and reduces anxiety and depression.