The skeleton as an endocrine organ

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Abstract | Surprising new discoveries in the field of skeletal biology show that bone cells produce endocrine hormones that regulate phosphate and glucose homeostasis. In this Review, we examine the features of these new endocrine pathways and discuss their physiological importance in the context of our current understanding of energy metabolism and mineral homeostasis. Consideration of evolutionary and comparative biology provides clues that a key driving force for the emergence of these hormonal pathways was the development of a large, energy-expensive musculoskeletal system. Specialized bone cells also evolved and produced endocrine hormones to integrate the skeleton in global mineral and nutrient homeostasis. The recognition of bone as a true endocrine organ represents a fertile area for further research and should improve the diagnosis and treatment of metabolic diseases such as osteoporosis and diabetes mellitus.

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Introduction

Several breakthroughs in bone science in the past few years have shown a true endocrine role of the skeleton, by demonstrating the presence of novel hormones produced by bone cells that control energy balance and mineral ion homeostasis. One breakthrough uncovered a new hormone called fibroblast growth factor 23 (FGF23), produced in bone by osteocytes, that regulates serum phosphate levels by altering levels of active vitamin D and the activity of specific phosphate transporters in the kidney.[1] Another advance has linked osteocalcin, another bone-specific protein, to a new pathway regulating glucose metabolism in mice.[2] Both FGF23 and osteocalcin function in a classic endocrine fashion; that is, they are produced exclusively in bone and act on distant target organs through regulatory loops subject to both feedforward and feedback control. These findings raise intriguing questions regarding the evolutionary origin of these pathways and the advantage of an endocrine role for the skeleton. The purpose of this Review is to summarize the latest discoveries from experimental models and attempt to rationalize their existence in mammals (including humans) through the lens of evolution and comparative biology.

Evolution of skeletal endocrine networks

One of the most dramatic episodes in the evolution of life on earth occurred during the late Devonian period when the first creatures colonized land.[3,4] To survive in this new environment, terrestrial animals evolved major changes in their anatomy and physiology, ultimately developing a much larger, energy-expensive musculoskeletal system to facilitate ambulation. These animals also needed new strategies to regulate extracellular mineral ion levels and energy homeostasis on land. Minerals could no longer be freely absorbed from the surrounding aqueous environment, but instead had to be consumed in the diet and stored. These evolutionary pressures favoured the development of a durable, mineralized skeleton and new endocrine organs such as the parathyroid gland,[5] which enabled minute-to-minute control of extracellular calcium, now stored in its bony repository.

The endoskeleton

Osteichthyes (bony fish) evolved ~420 million years ago[6] and were the first organisms to have a fully mineralized endoskeleton composed of the same four types of mineralized tissues found in modern mammals (bone, cartilage, enamel and dentin). Subsequent evolution in terrestrial animals gave rise to a more complex skeletal structure composed of endochondral bone, in which mineralized bone consisting of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is formed from an intermediate cartilage template.[7] This cartilage template is invaded by blood vessels that deliver bone-forming cells (osteoblasts) into what will ultimately become the marrow space. Osteoblasts use the cartilage template to form lattice-like spicules of trabecular bone, or 'spongy' bone, which is remodelled by osteoclasts, specialized bone cells that resorb bone matrix (Figure 1). Osteoblasts also differentiate in the fibrous tissue that surrounds the developing bone (the perichondrium), and form a dense cortical shell.[7] This newly evolved skeleton, engineered through endochondral ossification, was substantially stronger than a cartilaginous skeleton and replete with minerals, providing a clear evolutionary advantage for life on land.

The skeleton of early tetrapods also evolved gross anatomical and physiological alterations that favoured movement on land. Ultimately, tetrapods developed a large appendicular skeleton that served two, seemingly opposing, purposes: a stable structure to support

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ambulation and a dynamic repository of mineral ions to supply the extracellular fluid. However, to sufficiently mineralize their skeletons to meet mechanical demands, land animals faced a constant danger of extracellular calcium loss that would be detrimental to muscle and nerve function. To accomplish these opposing tasks, new endocrine systems arose to precisely regulate extracellular mineral ion concentrations using both dietary sources and dissolution from skeletal stores via osteoclastic resorption. In this regard, the structure of the newly evolved endochondral bone was well suited for the duality of its mission (Figure 1). The trabeculae of the 'spongy' bone provide a large surface area:bone volume ratio (trabecular bone accounts for 60% of the bone surface in the human skeleton despite constituting only 25% of its total volume[8]), allowing osteoclasts to resorb and rapidly release the requisite calcium into the circulation. The dense cortical shell bears most of the mechanical force experienced by bones (although trabecular bone also experiences some degree of strain) and its lower surface:volume ratio enables it to maintain mechanical integrity despite also being subject to resorption at the endocortical surface (a comprehensive review of bone biomechanics can be found elsewhere[9]).

Osteocytes

Coincident with the evolution of new endocrine systems was the emergence of osteocytes.[10] Osteocytes differentiate from a subset of mature osteoblasts as they produce and mineralize the bone matrix and become entombed within lacunae. These cells are interconnected to other osteocytes and osteoblasts by an extensive network of neuron-like cell projections that form the canalicular network[10] (Figure 1). The precise function of osteocytes is still unclear, but they are widely assumed to have a role in mineralization and transduction of mechanical signals into anabolic events.[11] Osteocytes are the most abundant bone cell, with roughly 10,000 cells per mm^3 in humans and an estimated life span of 10–20 years.[12] In addition to their homotypic interactions through the canalicular network, osteocytes also contact blood vessels, nerves, and other surface-lining cells on trabecular and cortical bone.[10] The vast cellular mass, long lifespan and extensive interconnected nature of the osteocyte network make it uniquely suited to sense changes in circulating minerals, energy status and the general 'health' of the skeleton (that is, microdamage accumulated during normal movement that requires remodelling and repair to maintain favourable mechanical properties in the skeleton[13,14]). In turn, osteocytes can directly modulate bone remodelling through local factors[15–17] to release mineral, repair damage and functionally adapt. Osteocytes also possess receptors for endocrine factors (for example, parathyroid hormone [PTH]), which also regulate mineral homeostasis and bone turnover.[10] Furthermore, as discussed later, osteocytes produce hormones to coordinate their response globally, working in concert with other organ systems involved in mineral and energy homeostasis through newly described endocrine networks.

Key points

- The endochondral skeleton evolved specialized bone cells (osteocytes), which produce endocrine hormones that integrate skeletal metabolism with global mineral and nutrient homeostasis
- FGF23, made by osteocytes, regulates phosphate disposal from the body, providing an additional layer of control to aid parathyroid hormone in the maintenance of phosphate levels during bone resorption
- Osteocalcin, produced by osteoblasts and osteocytes under the control of insulin, increases the efficiency of glucose utilization through its actions on the pancreas and adipocytes
- Understanding the endocrine roles of the skeleton should improve the ability to diagnose and manage patients with a broad range of metabolic diseases, including osteoporosis and diabetes mellitus

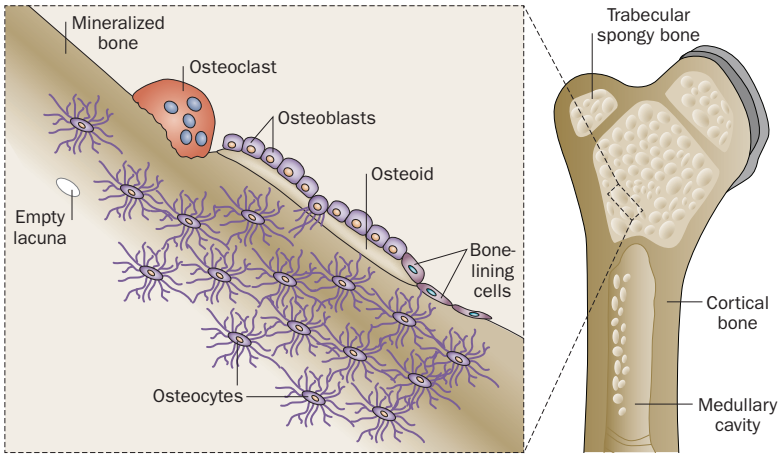

Figure 1 | Microstructure and macrostructure of mammalian bone. Microstructure (left) of an actively remodelling trabecular bone surface. The osteoclast initiates the remodelling cycle by resorbing an area of bone matrix, immediately followed by osteoblast differentiation and osteoid (unmineralized bone matrix) production to replace the resorbed bone. During this process, a small fraction of osteoblasts differentiate further to become osteocytes, encasing themselves within the mineralizing bone matrix and joining the osteocyte network. Mature bone surfaces are populated with bone-lining cells, whose origin and function remain unclear. Macrostructure (right) of the proximal femur illustrating the dense cortical shell and inner trabecular, or 'spongy', bone.

Neuroendocrine networks

The first animals to colonize land evolved new neuroendocrine systems[18,19] to coordinate the increasingly complex physiological processes needed to regulate growth, metamorphosis, sexual maturation and bioenergetics. Endocrine networks have enjoyed tremendous evolutionary success because they provide the means to sense internal changes (for example, energy status) and external changes (for example, day length, temperature and salinity) and to integrate this information into coordinated, tissue-specific responses in the organism. The ability of endocrine systems to elicit such complex, coordinated responses is the result of two key features: hormonal homeostasis and hormonal pleiotropy.[20] Hormonal homeostasis is achieved by multiple loops (feedback and feedforward) between hormones and the neurohormonal regulators that control their secretion, enabling the maintenance of hormone levels in the blood within a narrow range. In addition, the action of a particular hormone to elicit a

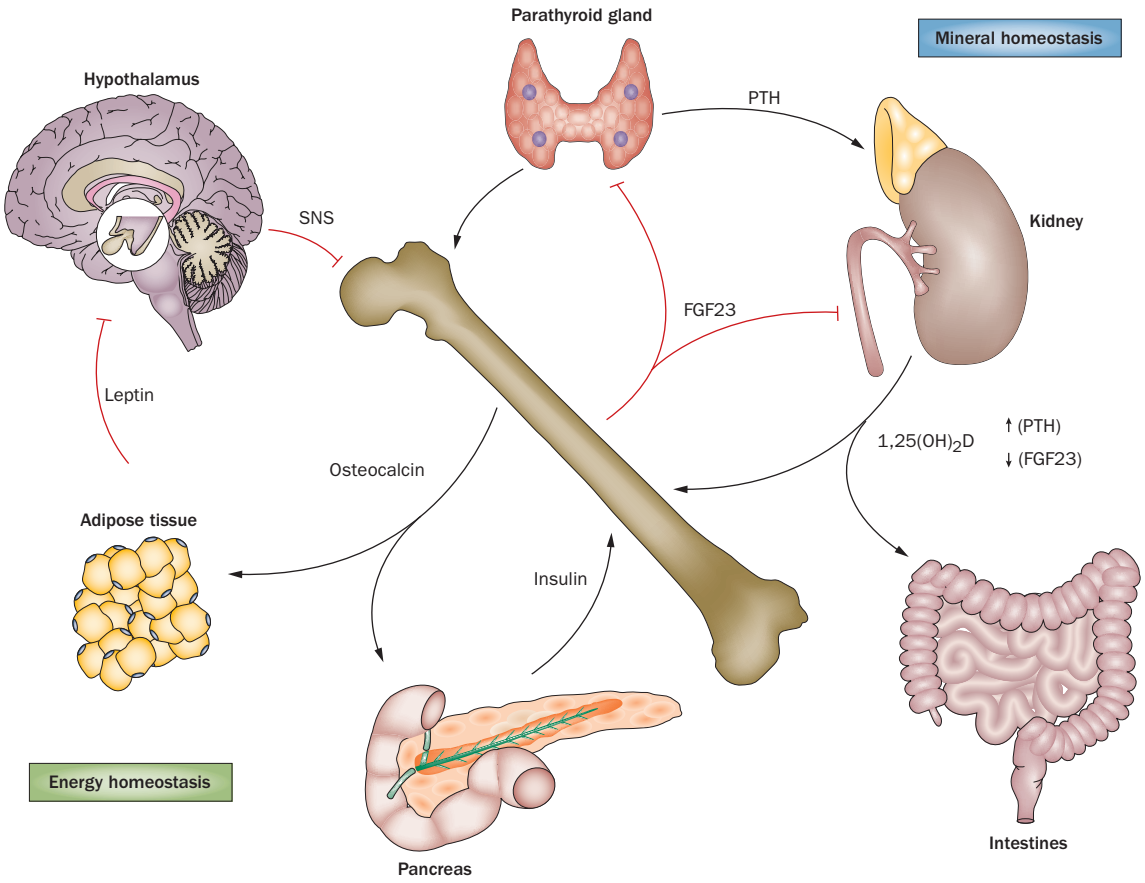

Figure 2 | Integration of the skeleton in mineral and energy homeostasis. In mineral homeostasis, a decrease in circulating calcium stimulates the parathyroid gland to release PTH, which then causes an increase in blood calcium levels by stimulating osteoclastic bone resorption, renal calcium reabsorption and renal production of $1,25(OH)_2D$ to increase intestinal calcium absorption. Increased serum phosphate and $1,25(OH)_2D$ stimulate FGF23 production in bone, which subsequently inhibits PTH production from the parathyroid gland, inhibits $1,25(OH)_2D$ production in the kidney (thereby inhibiting intestinal absorption) and promotes renal phosphate excretion. Endocrine regulation of energy homeostasis by the skeleton is comprised of two mini loops: a negative bone–hypothalamic loop and a positive bone–pancreas loop. Leptin inhibits bone formation and the homeostatic function of the skeleton indirectly through the hypothalamus by suppressing SNS tone. However, SNS signalling also increases the production of osteocalcin from bone, which feeds into the positive loop. Osteocalcin acts on pancreatic β-cells to increase insulin production, which feeds positively back to bone, stimulating osteoblasts and driving further production of osteocalcin. Osteocalcin also acts on fat to increase the production of adiponectin, an insulin-sensitizing hormone. Abbreviations: $1,25(OH)_2D$, active vitamin D; 25(OH)D, 25-hydroxyvitamin D; FGF23, fibroblast growth factor 23; PTH, parathyroid hormone; SNS, sympathetic nervous system.

response typically requires the priming action of another hormone.[20] Additional flexibility of the endocrine system occurs through hormonal pleiotropy—the ability of one hormone to influence multiple phenotypes (for example, testosterone drives, among others, the formation and maturation of male genitalia, voice deepening and growth of facial and axillary hair). The multivariate interaction of endocrine pathways as they control the complete phenotype of the organism is known as phenotypic integration.

The example of such endocrine pleiotropy most familiar to bone biologists is the emergence of the parathyroid–calcium axis. The parathyroid gland evolved to use PTH to access internal calcium stores through bone resorption; it detects changes in calcium levels in the blood by means of the calcium-sensing receptor, which then modulates the secretion of PTH.[21] A decrease in circulating calcium stimulates the parathyroid gland to produce and release PTH. Circulating PTH then works in a rapid, pleiotropic fashion to increase blood calcium levels by stimulating osteoclastic bone resorption, calcium reabsorption in the renal distal convoluted tubule, and production of active vitamin D ($1,25(OH)_2D$) by the kidney, which, in turn, increases intestinal calcium absorption[21] (Figure 2 and Figure 3, left).

A second major challenge faced by terrestrial animals was nutrient availability; a problem compounded by the fact that foraging for food on land was energy-expensive and that some necessary minerals could not be readily absorbed from the environment. Such environmental pressures required an increased ability to inventory the nutritional status of the organism and integrate this information between tissues to maintain energy homeostasis, coordinate growth and regulate reproduction. Of particular relevance are the insulin–insulin-like growth factor (IGF) pathways, which evolved greater complexity

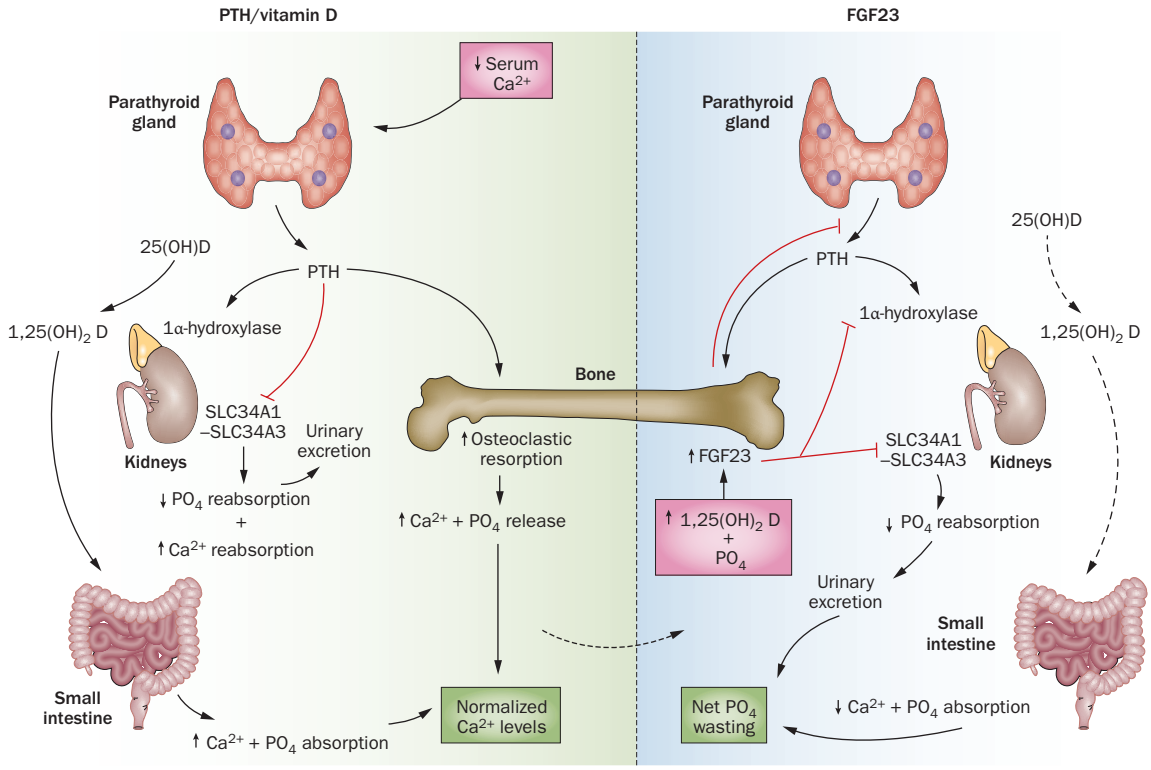

Figure 3 | The regulation of calcium and phosphate homeostasis by PTH, vitamin D and FGF23. The parathyroid gland detects changes in the level of calcium in blood by means of the calcium-sensing receptor, which then modulates the secretion of PTH. A decrease in circulating calcium stimulates the parathyroid gland to produce and release PTH. Circulating PTH then works in a rapid, pleiotropic fashion to increase blood calcium levels by stimulating osteoclastic bone resorption to release calcium and phosphate, calcium reabsorption and phosphate excretion in the renal distal convoluted tubule by downregulating the sodium–phosphate co-transporters SLC34A1–SLC34A3, and production of $1,25(OH)_2D$ by 1α-hydroxylase in the kidney, which, in turn, increases intestinal calcium and phosphate absorption. The kidney is the principle physiological target, where FGF23 signalling acts to promote phosphate excretion by downregulating SLC34A1–SLC34A3 and inhibiting $1,25(OH)_2D$ production, thus preventing vitamin-D-mediated phosphate absorption in the gut. Serum levels of FGF23 increase in response to increased serum phosphate and $1,25(OH)_2D$, and furthermore, FGF23 inhibits the production of PTH. Boxes in pink show input into the system, boxes in green show the output. Abbreviations: $1,25(OH)_2D$, active vitamin D; 25(OH)D, 25-hydroxyvitamin D; Ca^{2+}, calcium; FGF23, fibroblast growth factor 23; FGFR, fibroblast growth factor receptor; PO_4, phosphate; PTH, parathyroid hormone; SLC34A1, sodium-dependent phosphate transport protein 2A (also known as NPT2a); SLC34A3, sodium-dependent phosphate transport protein 2C (also known as NPT2c).

to assume a central role in growth and metabolism in higher organisms.[22–24] In higher animals, the primitive insulin–IGF-1 pathway diverged to perform a more circumscribed set of functions.[22] In mammals, insulin serves primarily metabolic functions, whereas IGF-1 functions predominantly to regulate growth, development and, perhaps, longevity.[22] A paucity of nutrients and/or the increased complexity of new terrestrial organisms might have provided substantial evolutionary pressure to further separate the actions of insulin and IGF-1. The requisite demands for growth in large, complex organisms would far exceed the nutritional and energy content derived from a single feeding episode. Ultimately, through the expansion and modification of primitive pathways, successful tetrapods reorganized their metabolic regulatory machinery to accommodate the storage and distribution of fuel among metabolically active tissues, and neuroendocrine pathways provided the means to regulate metabolic homeostasis. With these evolutionary considerations in mind, let us now turn to two recent discoveries that, we argue, establish the skeleton as a true endocrine organ.

Phosphate homeostasis and the skeleton

The first discovery that implicated bone as an endocrine organ concerns the role of bone in regulating phosphate homeostasis through the hormone FGF23.[25] As discussed earlier, the mammalian skeleton represents the largest reservoir in the body of both calcium and phosphate in the form of hydroxyapatite crystals, the main inorganic component of the mineralized bone matrix.[26] Phosphate ingested through the diet is absorbed by the small intestine through sodium–phosphate co-transporters (sodium dependent), as well as by diffusional absorption across intercellular spaces in the lumen (sodium independent).[25] However, the major control point for phosphate homeostasis is the kidney, which expresses numerous hormone-regulated proteins to modulate phosphate filtration. In particular, a sodium–phosphate co-transporter (SLC34A1, also known as NPT2) is

expressed in the renal proximal convoluted tubule and is central to renal phosphate reabsorption.[27] As serum phosphate concentration decreases, its levels are regulated by the activity of PTH and vitamin D in a manner analogous to that of calcium: phosphate is released from bone through resorption, intestinal absorption is increased and renal reabsorption is increased.[28]

Phosphate-handling disorders

Despite the above insights, it was clear to most in the field that PTH and vitamin D alone were not representative of the full complement of factors controlling phosphate levels. Patients with phosphate-wasting syndromes such as X-linked hypophosphataemia (XLH) provided strong evidence of an additional player in phosphate homeostasis, as patients with XLH continue to have reduced levels of circulating phosphate and poorly mineralized bone (rickets), even when PTH and vitamin D levels are fairly normal.[29] Further examination of the physiological defect in patients with XLH and in experimental mouse models revealed impaired proximal renal tubular reabsorption of phosphate caused by reduced expression of sodium–phosphate co-transporters, further suggesting a missing phosphate-regulating hormone.[30–32]

In the mid-1990s, positional cloning determined that a single gene, *PHEX*, was responsible for most cases of XLH.[33] Surprisingly, *PHEX* did not encode the phosphate-regulating hormone that had been anticipated, but instead encoded a protein with homology to endopeptidases: phosphate-regulating neutral endopeptidase. Subsequent studies in patients with another form of phosphate wasting, autosomal dominant hypophosphataemic rickets (ADHR), ultimately led to the identification of a phosphaturic hormone, which turned out to be FGF23.[34] Patients with ADHR carry mutations that render FGF23 resistant to enzymatic cleavage and inactivation.[35] Consequently, FGF23 levels accumulate in the circulation of patients with ADHR and cause phosphate wasting.[35] Interestingly, subsequent studies of patients with XLH also revealed high serum levels of FGF23, but despite extensive study, FGF23 does not seem to be a physiological substrate for PHEX.[36] Thus, the exact role of PHEX in normal and abnormal production of FGF23 remains unclear.

FGF23 has also been implicated in other rare phosphate-handling disorders. Patients with autosomal recessive hypophosphataemic rickets (ARHR) have increased circulating FGF23 levels, apparently caused by a loss-of-function mutation in the gene encoding dentin matrix acidic phosphoprotein 1 (*DMP1*).[37–39] A *Dmp1*-null mouse model recapitulates the ARHR phenotype and can be rescued by the addition of FGF23-neutralizing antibodies.[40] However, as was the case for XLH and PHEX, no evidence exists to demonstrate a direct link between DMP1 and FGF23 production.[36] Finally, elevated levels of FGF23 occur in a rare, paraneoplastic syndrome called tumour-induced osteomalacia. In this condition, a small, benign tumour aberrantly produces FGF23 and causes biochemical and skeletal abnormalities similar to that seen in patients with ADHR or XLH.[41] Once located, the tumour can be resected and serum biochemistry will normalize within hours, although recovery of the skeleton can take months to years.

FGF23 signalling

In each of the cases described above, FGF23 elicits its biological effects by binding to a signalling complex composed of an FGF receptor (FGFR) and a co-receptor, Klotho.[42] Whereas FGFR is widely expressed, Klotho expression is restricted to the kidney, parathyroid gland, testis, ovary, brain, pituitary and choroid plexus, conferring tissue specificity for FGF23 action.[42,43] Of these organs, the kidney is the principle physiological target, where FGF23 signalling acts to promote phosphate excretion by downregulating SLC34A1–SLC34A3 (NPT2a/c) and inhibiting $1,25(OH)_2D$ production, thus preventing vitamin-D-mediated phosphate absorption in the gut.[44] Serum levels of FGF23 increase in response to increased serum levels of phosphate and $1,25(OH)_2D$,[45] and FGF23 inhibits the production of PTH[46,47] (Figure 2 and Figure 3). In the context of basic calcium and phosphate metabolism, the need for such an additional regulatory control of phosphate levels becomes apparent. Although the average Western diet contains similar daily amounts of these minerals (1,000 mg of calcium and 1,000–1,500 mg of phosphorus), only ~30% of calcium is absorbed compared with ~70% of phosphorus.[48,49] Without a mechanism separate from the actions of PTH to remove phosphate, the excess phosphate released during resorption could lead to hyperphosphataemia. Thus, FGF23 seems to function as a counter-regulatory phosphaturic hormone to remove excess phosphate that accompanies PTH-stimulated bone resorption during calcium mobilization.

A most interesting aspect of the FGF23 story, in the context of this discussion, is that it is produced almost exclusively by osteocytes.[50] In light of the phosphaturic role of FGF23, however, the osteocyte is ideally situated to participate in the PTH–$1,25(OH)_2D$ endocrine loop. The vast, long-lived osteocyte network is intimately connected to blood vessels and presumably able to sense levels of phosphate to respond through FGF23 and provide negative feedback to the PTH–$1,25(OH)_2D$ axis. It can be anticipated that subsequent work will identify these putative phosphate sensors.

Glucose homeostasis and the skeleton

A second major discovery that implicated bone as an endocrine organ occurred in parallel with that of FGF23 and first materialized in a paper reporting that leptin altered bone mass through a hypothalamic–osteoblastic endocrine loop in mice.[51] To most of the bone field, these findings seemed surprising; however, in light of the evolutionary considerations discussed earlier, it is perhaps not surprising that a pathway regulating feeding and energy balance would also impinge upon the energy-demanding skeleton.

Leptin–serotonin network

As the leptin story emerged, it became clear from experimental studies in mice that this adipokine functioned

indirectly through the sympathetic nervous system (SNS),[52] and suggested that, as with other organs, the skeleton also falls under the purview of this homeostatic regulatory system (Figure 2). The realization that leptin regulates bone mass through the SNS also revealed a novel mode and location of leptin signalling in the brain. This new target of leptin in the brain was identified when tryptophan 5-hydroxylase 2 (Tph2), the rate-limiting enzyme of brain serotonin synthesis,[53] was inactivated in mice. Brain-derived serotonin is made only in neurons of the raphe nuclei and does not cross the blood–brain barrier. *Tph2*$^{-/-}$ mice were not only osteoporotic, but also anorectic, demonstrating that brain-derived serotonin favours bone mass accrual and appetite.[54] Subsequently, the molecular connections of a leptin–serotonin–hypothalamus axis were deciphered in experimental models in mice. In this network, leptin utilizes axonal connections between serotonin-producing neurons and hypothalamic neurons to inhibit *Tph2* expression and serotonin release from brainstem neurons.[54] Genetic studies in mice lacking either leptin (*ob/ob*) or its receptor (*db/db*) in neurons show that they develop a high-bone-mass phenotype due to a massive increase in bone formation.[51] This phenotype is evident despite the fact that these mice are hypogonadic, a condition known to increase bone resorption and decrease bone mass.[55] Thus, leptin delivers a doubly compromising signal to the skeleton by both suppressing bone formation and increasing bone resorption via centrally derived signals on osteoblasts.

Osteocalcin

The hallmark of all endocrine pathways, as reviewed earlier, is reciprocity between tissues to ensure homeostasis. The discovery that a pathway that regulates feeding and energy balance in the brain could also affect bone raised the question of whether the skeleton might produce a hormone that could participate in controlling energy homeostasis. The answer to this question would come from a study examining the function of the bone-specific protein osteocalcin, which is expressed by osteoblasts and osteocytes.[56] Mice that lack osteocalcin have moderately increased bone mass,[57] profoundly increased peripheral fat, hyperglycaemia, hypoinsulinaemia, insulin resistance and few pancreatic β cells;[58] a phenotype remarkably similar to that observed in type 2 diabetes mellitus (and potentially also in metabolic syndrome). These phenotypic abnormalities implicated osteocalcin in global metabolism and suggested that osteocalcin might be the reciprocal signal from bone to complement the leptin–SNS arm of this endocrine loop.

Energy metabolism

The role of osteocalcin in energy metabolism was ultimately revealed in the study of another gene, embryonic stem-cell phosphatase (*Esp*, also known as receptor-type tyrosine-protein phosphatase V, *Ptprv*). *Esp* encodes a protein tyrosine phosphatase called osteotesticular protein tyrosine phosphatase (OST-PTP), which, as the name would imply, is expressed in embryonic stem cells, the Sertoli cells of the testes and osteoblasts.[58] Genetic ablation of *Esp* in mice, either globally or selectively in osteoblasts, resulted in a metabolic phenotype precisely opposite that seen in mice lacking osteocalcin[58]: hypoglycaemia; hyperinsulinaemia; improved insulin sensitivity in liver, muscle and white adipose tissue; and increased numbers of pancreatic β cells. Eventually, a mechanistic connection was established between OST-PTP and osteocalcin whereby OST-PTP—through a then unknown mechanism—suppresses osteocalcin activity by promoting its carboxylation. Osteocalcin possesses three glutamic acid residues susceptible to carboxylation, a modification that confers high affinity for the hydroxyapatite crystals present in the mineralized bone matrix.[59] However, a small but measurable proportion of undercarboxylated osteocalcin is found in the serum.[60] This undercarboxylated form of osteocalcin (in which Glu13 is not carboxylated) acts as a hormone to promote β-cell proliferation and insulin expression.[58] Undercarboxylated osteocalcin also increases insulin sensitivity (via effects on adiponectin expression in adipocytes) and modulates a number of genes involved in energy expenditure (for example, *Ucp2*, a member of a family of mitochondrial uncoupling proteins that separate oxidative phosphorylation from ATP synthesis and dissipate the energy as heat). Thus, by fully carboxylating osteocalcin, OST-PTP can restrict the positive metabolic activities of osteocalcin.

As is the case in scientific research, a number of unknowingly related studies investigating insulin signalling in osteoblasts were also taking place around the same time and would ultimately result in the full elucidation of a bone–pancreas endocrine loop.[58,61–63] In this endocrine loop (discussed in full elsewhere[2]), insulin signalling in the osteoblast controls osteocalcin bioactivity and circulating concentrations (via a pathway involving OST-PTP and the carboxylation events described above) and thereby modulates glucose homeostasis in the whole organism. Indeed, mice lacking the insulin receptor (InsR) specifically in osteoblasts demonstrated decreased levels of undercarboxylated osteocalcin and were glucose intolerant and insulin insensitive.[61,62] Additional genetic mouse models confirmed the interaction of osteoblast InsR signalling and osteocalcin in this metabolic pathway. Compound heterozygote mice lacking one allele of the InsR gene specifically in osteoblasts, on an osteocalcin heterozygote background, demonstrated a metabolic phenotype similar to that observed in both the osteoblast-specific InsR-knockout and osteocalcin-knockout mice.[61]

Control of osteocalcin

A stunning example of evolutionary conservation, the **molecular mechanisms by which insulin regu**lates the bioavailability and activity of osteocalcin in osteoblasts—and ultimately, glucose homeostasis in the whole animal—were determined to be highly conserved pathways that control energy balance in other glucose-regulating organs. Forkhead box protein O1 (FOXO1) and cyclic AMP-dependent transcription factor ATF-4 (ATF4), two transcription factors firmly implicated

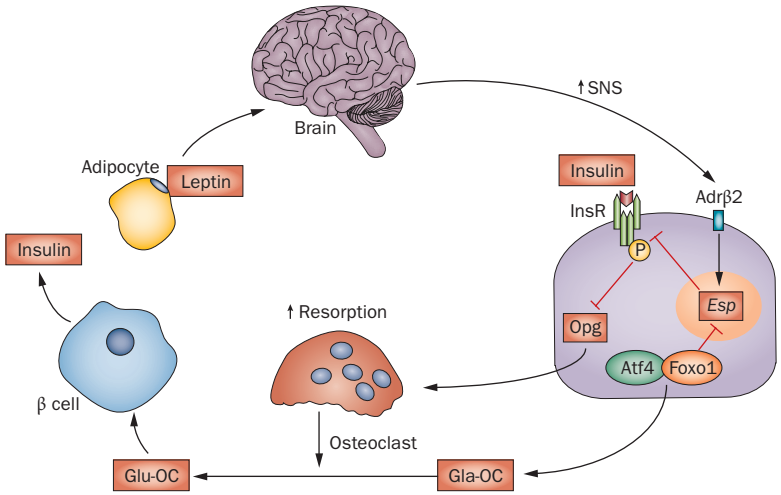

Figure 4 | The osteocalcin axis in the regulation of energy metabolism by the skeleton. Insulin signals in osteoblasts through InsR to suppress expression of the antiosteoclastogenic cytokine osteoprotegerin. This step promotes osteoclast activity, leading to increased acidity in the environment of the resorption lacunae. The decline in pH promotes the conversion of Gla-OC to Glu-OC, which, in turn, acts on β cells to induce β-cell proliferation and insulin production, as well as on other insulin-regulating tissues (for example, fat) to favour insulin sensitivity. OST-PTP (encoded by *Esp*) negatively regulates osteocalcin decarboxylation (and consequently osteocalcin activity) by inhibiting phosphorylation of InsR. *Esp* expression is controlled by two transcription factors, Foxo1 and Atf4, which synergize to promote *Esp* expression. The feedforward insulin loop from β-cells to osteoblasts is opposed by the actions of leptin through the SNS. Leptin (secreted by adipocytes) acts on the brain to increase sympathetic tone. Increased SNS signalling increases *Esp* expression in osteoblasts through Adrβ2 and thus, inhibits osteocalcin activity. Abbreviations: Adrβ2, β2 adrenergic receptor; ATF4, cyclic AMP-dependent transcription factor Atf4; Esp, embryonic stem cell phosphatase (also known as PTPRV); Foxo1, Forkhead box protein O1; Gla-OC, carboxylated osteocalcin; Glu-OC, undercarboxylated osteocalcin; InsR, insulin receptor; OST-PTP, osteotesticular protein tyrosine phosphatase; SNS, sympathetic nervous system.

in the regulation of metabolism, have been identified as controlling osteocalcin production and activity in osteoblasts, downstream of the InsR.[64]

FOXO1 is a well-established modulator of metabolism in all glucose-regulating organs, as well as insulin production by the pancreas.[64] Mice in which *Foxo1* was knocked out specifically in osteoblasts were hypoglycaemic and had markedly improved glucose tolerance.[63] The cause of hypoglycaemia was twofold: increased β-cell proliferation and mass that resulted in hyperinsulinaemia; as well as increased insulin sensitivity. Despite this hyperinsulinaemia and enhanced insulin sensitivity, mice with osteoblast-specific deletion of *Foxo1* had reduced gonadal fat pad weight, probably owing to increased energy expenditure and mitochondrial activity in the muscle.[63] The observations from osteoblast-specific deletion of *Foxo1* raised the possibility that it might be a transcriptional modulator of osteocalcin. In fact, studies in compound heterozygote mice described below demonstrated that Foxo1 regulates the bioactivity of osteocalcin via OST-PTP. Heterozygous mice lacking one allele of *Foxo1* specifically from osteoblasts and one allele of *Esp* (the OST-PTP gene) showed improved insulin sensitivity and glucose tolerance.[63] Likewise, removal of one allele of *Bglap* (the gene that encodes osteocalcin) from mice with osteoblast-specific deletion of *Foxo1* corrected the metabolic phenotype observed in osteoblast-specific *Foxo1*-knockout mice. Examination at the molecular level revealed that these phenotypes arose due to the presence of a cognate Foxo1 binding site in the *Esp* promoter, to which Foxo1 could bind and stimulate the expression of OST-PTP, thus suppressing osteocalcin bioactivity through the ability of OST-PTP to promote osteocalcin carboxylation.[63] Thus, in the grand scheme of the pathway, insulin would stimulate osteocalcin via its ability to phosphorylate Foxo1 and exclude it from the nucleus, rendering it unable to bind and stimulate *Esp* transcription (Figure 4).

Subsequent studies also identified a binding site for ATF4, a key osteoblast transcription factor in the *Esp* promoter. ATF4 has previously been demonstrated to suppress fat mass in mice and is involved in the regulation of glucose homeostasis in humans.[65–69] In a fashion quite similar to that of Foxo1 described above, Atf4 acts upon the *Esp* promoter in the osteoblast to suppress osteocalcin activity, thereby suppressing insulin secretion and insulin sensitivity to increase blood glucose levels in mouse models.[70] These two transcription factors, one ubiquitously expressed (Foxo1) and the second expressed mainly in osteoblasts (Atf4), synergize to regulate energy homeostasis by binding their cognate sites in the *Esp* promoter and stimulating its expression.[71] However, the fact that both transcription factors regulate osteocalcin activity, rather than its expression, suggested the possibility that osteocalcin might not be the principal target for the regulation of this novel endocrine loop.

Insulin signalling

Insulin signalling in osteoblasts also promotes bone resorption, in part, by decreasing the expression of the antiosteoclastogenic cytokine osteoprotegerin, which acts as a decoy receptor for pro-osteoclastogenic RANKL (RANK ligand, also known as TNF ligand superfamily member 11).[61] The reduction of osteoprotegerin allows RANKL to bind RANK (receptor activator of nuclear factor-κB; also known as TNF receptor superfamily member 11A) on osteoclast precursors and stimulate osteoclastogenesis. The acidic pH created in the resorptive environment of the osteoclast, in addition to demineralizing the matrix, is also capable of decarboxylating, and thus activating, the carboxylated osteocalcin bound to hydroxyapatite[61] (Figure 4).

If we return to our earlier discussion of why the skeleton might have evolved mechanisms to participate in global energy homeostasis, the role of the osteoclast becomes quite evident. Maintaining the skeleton was (and is) a highly energy-expensive process because it requires constant remodelling to maintain favourable mechanical properties. A large amount of evidence exists to suggest that these remodelling events begin with the resorption of old or damaged bone by osteoclasts, prior to its replacement by osteoblasts, although the precise signals initiating such events remains unclear. One can imagine then, that if the osteoclast initiates such an

energy-demanding process as remodelling, the activation of osteocalcin could be its means for promoting metabolic functions and creating a constant source of energy to support the remodelling cycle. Indeed, osteocalcin can be found in the vicinity of the osteoclast resorption lacuna, and is released by osteoclasts during bone resorption.[72] Moreover, incubating osteocalcin at 37 °C and pH 4.5 was sufficient to reduce its carboxylation status and to confer the ability to stimulate insulin secretion.[61] In turn, undercarboxylated osteocalcin increases pancreatic β-cell numbers and insulin production, as well as production of adiponectin (the insulin-sensitizing hormone) from adipocytes, to regulate global metabolism. Thus, in a feedforward loop, insulin signalling in osteoblasts promotes insulin secretion from β cells by activating osteocalcin.

The complete model

The latest chapter in this story tied together the 'forward' bone–pancreas endocrine loop and the 'reverse' hypothalamic–bone circuit regulated by leptin. In the complete model, leptin acts as the feedback suppressor that inhibits continuous activation of the insulin–osteocalcin axis that would otherwise lead to hypoglycaemia (Figure 4). Similar to its effects in the control of bone mass,[51,52] the function of leptin in this endocrine loop is mediated via upregulation of sympathetic tone.[73] The SNS then acts through β2-adrenergic receptors (*Adrb2*) in osteoblasts to increase the expression of *Esp* and subsequently decrease osteocalcin bioactivity, resulting in decreased insulin expression and secretion by the pancreas (Figure 2 and Figure 4). Indeed, leptin-deficient *ob*/*ob* mice have more undercarboxylated (active) osteocalcin in their circulation, and when the osteocalcin gene is genetically removed, they demonstrate normal insulin levels for a markedly longer period than *ob*/*ob* mice.[73] Consistent with these observations, mice lacking *Adrb2* specifically in osteoblasts are hypoglycaemic and hyperinsulinaemic, but become hyperglycaemic and hypoinsulinaemic when one copy of the leptin gene is inactivated.

The hormonal functions of osteocalcin in energy homeostasis, and its regulation by leptin and insulin, reveal a remarkable interdependence between energy metabolism and bone remodelling. The SNS regulation of bone mass described in experimental models seems to also be conserved in humans, as multiple studies have demonstrated that patients on β-blockers have increased bone mineral density (BMD) and reduced fracture risk.[74–77] Unlike this hypothalamic–bone circuit, there was initially concern that the bone–pancreas loop might not be conserved in humans, as the gene encoding OST-PTP (*Esp*) is a pseudogene in humans. However, a functional homologue, tyrosine-protein phosphatase non-receptor type 1 (PTPN1, also known as protein-tyrosine phosphatase IB or PTP1B),[78] is expressed in human osteoblasts. In addition, a growing number of clinical studies support the notion that osteocalcin is a marker of glucose tolerance,[58,62,79–82] and humans with genetically impaired bone resorption show decreased undercarboxylated osteocalcin, concomitant with glucose intolerance.[61]

Conclusions

The new endocrine pathways reviewed earlier suggest that the modern mammalian skeleton evolved a much broader array of physiological functions than we first imagined. Viewed through the lens of evolution, it seems reasonable to propose that the extraordinary physiological challenges encountered by early terrestrial creatures shaped the design of the skeleton and linked it to endocrine pathways controlling mineral and energy homeostasis. The mineralized skeleton possessed the requisite strength to ambulate on land, and the parathyroid axis provided a means for precisely controlling extracellular calcium using the skeleton as a reserve. However, phosphate was more readily absorbed in the gut than calcium (or simply present in greater abundance), and an additional bone hormone, FGF23, emerged to control the disposal of the excess phosphate released from hydroxyapatite during calcium mobilization. However, this new skeleton and its elaborate internal mineral homeostasis scheme carried a high price tag in terms of fuel demands, which were in short supply on land. Thus, skeletal muscle and fat evolved into robust storehouses of energy, regulated through elaborate endocrine networks that could inventory, report and distribute fuel amongst tissues. It seems logical, therefore, that the energy-expensive skeleton would be intimately involved in this exchange. In mineral and energy homeostasis, the osteocyte takes centre stage as the key endocrine player in bone. In fact, the latest evidence shows that the osteocyte itself can control osteoclastogenesis[16,17] by producing RANKL.

These exciting discoveries have invigorated the field of bone biology by raising important new questions for future study. For example, what is the nature of the phosphate-sensing mechanism in osteoblasts or osteocytes to detect the prevailing phosphate levels? What is the primary fuel that is used by bone? Do bone cells simply burn glucose, or can bone cells store glucose or even use it as a signalling mediator? Do drugs that alter bone resorption influence blood sugar levels? Answers to such questions will certainly expand our understanding of the biology of the skeleton, and should aid in the diagnosis and management of patients with a broad range of metabolic diseases, including osteoporosis and diabetes mellitus.

Review criteria

A search for original articles published between 2000 and 2012, focusing on skeletal evolution and endocrine pathways of the skeleton was performed in MEDLINE and PubMed. The search terms used were "bone", "osteoblast", "osteoclast", "osteocyte", "evolution", "endocrine", "FGF23", "osteocalcin", and "leptin", alone and in various combinations. All articles identified were English-language, full-text papers. We also searched the reference lists of identified articles for further relevant papers.

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Author contributions

All authors contributed equally to all aspects of this manuscript.