Skeletal energy homeostasis: a paradigm of endocrine discovery

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Abstract

Throughout the last decade, significant developments in cellular, molecular and mouse models have revealed major endocrine functions of the skeleton. More recent studies have evolved the interplay between bone-specific hormones, the skeleton, marrow adipose tissue, muscle and the brain. This review focuses on literature from the last decade, addressing the endocrine regulation of global energy metabolism via the skeleton. In addition, we will highlight several recent studies that further our knowledge of new endocrine functions of some organs; explore remaining unanswered questions; and, finally, we will discuss future directions for this more complex era of bone biology research.

Key Words
- marrow adipose tissue
- metabolism
- bone
- osteoblast
- pancreas

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Introduction

Bone has long been regarded as an organised collection of inert calcified structures that facilitate the motility of land animals. The skeleton's mass and composition provides vital organ protection, a niche for haematopoiesis and allows for weight-bearing motion (Guntur & Rosen 2012, Oldknow *et al*. 2015). To facilitate these classical functional roles, and to maintain bone integrity, there is a continuous homeostatic adjustment of the skeletal architecture and composition. Central to this adjustment is the highly regulated interplay of two distinct bone cell types, the osteoblast and the osteoclast, which have opposing functions (Crockett *et al*. 2011).

Osteoblasts comprise 5% of all bone cells and facilitate the formation of bone (Florencio-Silva *et al*. 2015). Mature osteoblasts synthesise and release type 1 collagen, which forms the majority (85–90%) of the organic matrix of the bone (Karsenty *et al*. 2009). Osteoblasts that become embedded in the bone matrix undergo terminal differentiation, giving rise to osteocytes – the most abundant skeletal cell type (90% of total bone cells) (Dallas & Bonewald 2010). These immobilised cells are ideally suited to perform the function of translating mechanical strain into biochemical signals in order to regulate bone composition (Sugiyama *et al*. 2010) (Fig. 1). The bone itself is a dynamic organ that is constantly being remodelled. This is possible due to the unique function of osteoclasts, which mediate destruction (resorption) of the bone tissue in which they reside (Holtrop & King 1977). The biphasic action of osteoblasts and osteoclasts enables bone modelling and remodelling. Bone modelling occurs throughout the lifespan, allowing the bone to adapt altered stresses and strains put on it (e.g. the tennis players serving arm), whereas bone remodelling (maintenance) occurs when the resorbed bone is completely replaced by new bone (Hadjidakis & Androulakis 2006). The regenerative process of a structure that contributes to such a large proportion of the body mass (approximately 15% in men and 10% in women) requires an abundance of proteins to be synthesised and secreted. It is therefore plausible that a high energetic cost is associated with

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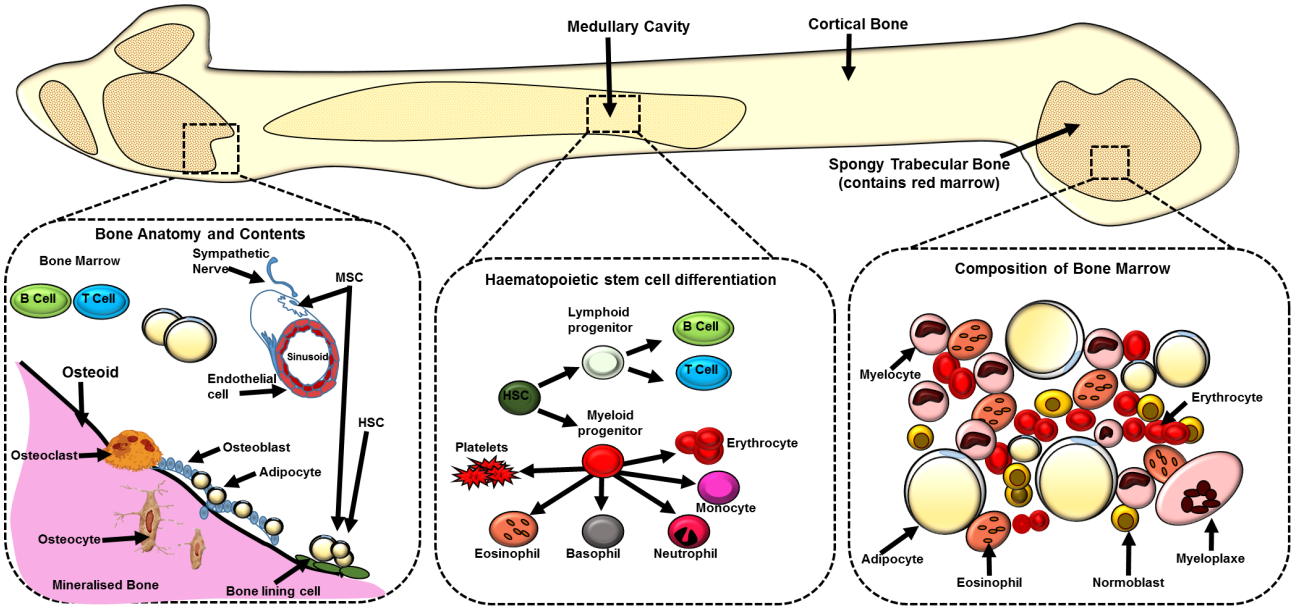

Figure 1

Bone anatomy and composition. Bone is organised into two distinct structures, cortical and trabecular. Cortical bone accounts for 80% of the skeletal mass and is highly organised, consisting of concentric lamellae arranged in *Haversian* systems. Trabecular, or 'spongy' bone, possesses ten times the surface area of cortical bone, accounting for 20% of the bone mass and enabling bone to withstand compressive and tensile forces. The bone contains osteoblasts, osteocytes and osteoclasts. Osteoblasts constitute approximately 5% of all bone cells and are the specialised 'bone-building' cells, originating from pluripotent mesenchymal stem cells (MSCs). Following matrix deposition and mineralisation, osteoblasts either remain on the surface of the bone as inactive lining cells; undergo apoptosis or become entombed by their secreted matrix and differentiate into osteocytes. Osteocytes reside within the mineralised bone matrix and are organised in functional syncytia collectively referred to as the *osteocytic* lacunar–*canalicular system*. Osteoclasts are derived from the haematopoietic lineage and are responsible for the resorption of mineralised bone and, in partnership with osteoblasts, regulate remodelling of bone tissue. The bone marrow further provides the haematopoietic niche, which supports the survival, self-renewal and differentiation of the haematopoietic stem cell (HSC). HSCs are capable of differentiation into two cell types: firstly, the common myeloid progenitor, which further differentiates to give rise to a number of blood cells including platelets, eosinophils, basophils, neutrophils, monocytes and erythrocytes; and secondly, the common lymphoid progenitor, which further differentiates to form B- and T-cells of the immune system. Within the bone marrow cavity, maintenance of the haematopoietic niche is orchestrated through vascular niches, which balance quiescence of HSC, proliferation and also regeneration following injury to the bone marrow. This regulation of HSC homeostasis involves intrinsic and extrinsic signals from the niche, including bound or secreted molecules, contractile force or even temperature. Haematological malignancies, or chemotherapy/radiation as a treatment for the disease, cause a limit to the regenerative and differentiation potentials of HCSs, causing a functional deficit (further discussed within text – see 'Disease and bone' section).

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these diverse skeletal functions (Vaananen *et al*. 2000, Karsenty & Ferron 2012).

From an evolutionary perspective, bones likely represent a strongly selected survival factor that permitted enhanced movement to allow scavenging, survive injury and therefore the survival of the organism. However, it is now clear that part of the selection process for bones involves its integral role in the endocrine control of whole-body energy metabolism (Guntur & Rosen 2013). One example of the poorly understood metabolic functions of the skeleton is the presence of adipose tissue within the bone marrow – referred to as marrow adipose tissue (MAT). Accounting for approximately 10% of the total fat mass in healthy humans, the function of MAT and its association with bone-specific cells, namely osteoblasts, osteocytes and osteoclasts, remains unknown. Here, we focus on recent discoveries that explain the endocrine functions and molecular mechanisms linking bone (inclusive of MAT and muscle) and energy expenditure.

Bone as an endocrine organ

In addition to its structural role, bone is a well-recognised target for endocrine function. This is exemplified by the orchestrated inter-organ regulation of phosphate, which involves the parathyroid glands, kidneys and intestines facilitating homeostatic maintenance of phosphate, in the mineralisation of bone extracellular matrix (Karsenty & Olson 2016). Implicit to the theory of homeostatic control is reciprocal crosstalk between the bone and these organs (Ramsay & Woods 2014). Indeed, the skeleton acts not only as an endocrine target but also as an endocrine

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organ with possible roles in the hormonal modulation of systemic energy homeostasis.

Osteocalcin

Also known as BGP (bone Gla protein), osteocalcin (OCN) is the most abundant osteoblast-specific non-collagenous protein. OCN is initially synthesised by the osteoblast as a pre–pro-molecule and is commonly used as a serum marker of bone formation (Brown *et al*. 1984). OCN exists in the general circulation in fully carboxylated, partially carboxylated and completely uncarboxylated forms (Plantalech *et al*. 1991, Cairns & Price 1994, Vergnaud *et al*. 1997, Schilling *et al*. 2005). Uncarboxylated OCN is formed when carboxylated OCN in the bone extracellular matrix is decarboxylated by the acidic pH (4.5) in osteoclastic resorption lacunae. Uncarboxylated OCN promotes β-cell proliferation, insulin secretion, peripheral insulin sensitivity and energy expenditure and impacts memory and male fertility (Lee *et al*. 2007, Oury *et al*. 2011, 2013). Recently a role for OCN in muscle function has been demonstrated. OCN levels doubled during endurance exercise in young adult wild-type (WT) mice, decreased significantly prior to or at mid-life, and OCN failed to increase during exercise in older mice. Importantly equivalent decreases in circulating OCN levels were observed in female rhesus monkeys and humans (Mera *et al*. 2016*a*). OCN administration was sufficient to reverse the age-induced decrease in exercise capacity in mice. Specifically, in 15-month-old mice, injections of OCN raised circulating OCN levels more than 4-fold and allowed these mice to run for the same time and distance as 3-month-old mice. Moreover, undercarboxylated OCN promoted uptake and subsequent catabolism of glucose and fatty acids in myofibres (Mera *et al*. 2016*a*,*b*). These nutrients, in turn, facilitate physical adaptation to exercise, whilst concurrently promoting the exercise-induced release of interleukin-6 (IL-6) from skeletal muscle. IL-6 further drives the production of bioactive OCN, supporting the hypothesis of a bone–muscle feed-forward axis. Thus, in addition to its postulated role in glucose and weight homeostasis (Oldknow *et al*. 2015), OCN further contributes to the regulation of energy metabolism, through effects on skeletal muscle. This supports the hypothesis that insulin signalling mediates the link between bone remodelling, and whole-body energy expenditure, and points towards a key role for the osteoblast in this relationship (Huesa *et al*. 2014).

NPP1 and PHOSPHO1

In order to further increase our knowledge of the skeletons' endocrine links with energy expenditure, the role of bone mineralisation factors such as phosphoethanolamine/phosphocholine phosphatase 1 (PHOSPHO1) and ectonucleotide pyrophosphatase/phosphodiesterase 1 (NPP1) have been addressed. NPP1, encoded by the *Enpp1* gene in mice, is highly abundant in the plasma membrane (external side) and mineral-depositing matrix vesicles of the osteoblast (Mackenzie *et al*. 2012). NPP1 generates inorganic pyrophosphate (PPi) through the hydrolysis of nucleotides (ATP). PPi potently inhibits hydroxyapatite crystal formation in tissues capable of mineralisation (bone and soft tissue) and acts as a precursor for inorganic phosphate (Pi) (Buckley *et al*. 1990, Mackenzie *et al*. 2012). NPP1 regulates glucose homeostasis via suppression of insulin receptor signalling in various tissues, including adipose, bone and muscle (Maddux *et al*. 1995, Mackenzie *et al*. 2012). NPP1 binds to and inhibits insulin-induced receptor conformational changes and is a potential pathogenic contributor to insulin resistance (Huesa *et al*. 2014). This concept is supported by the phenotype of *Enpp1* ablated mice, which display improved glucose homeostasis and resist obesity-associated dysfunction in response to high-fat diet feeding (Huesa *et al*. 2014). Thus, NPP1 plays multifaceted roles in normal physiology, including the regulation of calcium and phosphate homeostasis, inhibition of soft tissue mineralisation, maintenance of skeletal function and structure regulation of insulin signalling and energy homeostasis.

The bone-specific phosphatase PHOSPHO1 is a member of the large haloacid dehalogenase (HAD) superfamily of Mg^{2+}-dependent hydrolases (Roberts *et al*. 2004). PHOSPHO1 is active inside the osteoblast-derived matrix vesicle, where it scavenges Pi from matrix vesicle membrane phospholipids to promote intravascular hydroxyapatite deposition. Recent studies have identified novel roles of this bone-derived factor in energy homeostasis. Mice with *Phospho1* ablation exhibit a decreased body size and protection against both obesity and diabetes, regardless of carboxylation status of OCN (Oldknow *et al*. 2013, Chambers *et al*. 2015, Dayeh *et al*. 2016, Sayols-Baixeras *et al*. 2016); however, the mechanisms conferring this metabolic-protective phenotype remain to be determined.

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PPARγ

The transcription factor PPARγ is critical for differentiation of adipocytes and maintenance of the adipogenic phenotype. This is achieved via directing lineage commitment of marrow mesenchymal stem cells from an osteoblast-fate and towards that of adipocytes (Lecka-Czernik 2010). PPARγ insufficiency in mice results in decreased adipose tissue and increased bone mass via inhibition of osteoclastogenesis and bone resorption (Akune *et al*. 2004). It remains unclear whether increased bone mass is a result of altered lineage commitment of bone marrow stem cells or an indirect effect through the modified function of adipose tissue. Alternatively, both direct and indirect mechanisms could account for the bone mass phenotype: PPARγ disruption in adipose tissue (i.e. lipodystrophic disease) resulted in increased osteoblast activity and concomitant increased bone formation. The mechanisms by which PPARγ regulates bone is not clear as mouse models of bone-specific PPARγ conditional knockouts have not been investigated to date (Cao *et al*. 2015). To add further complexity, PPARγ deletion in other tissues causes profound effects on bone, further complicating investigative efforts. Osteoblast-selective PPARγ deletion in mice (using PPAR(fl/fl):Col3.6-Cre) completely abolished adipogenesis, with the bone phenotype of increased osteoblastogenesis reflected in primary bone marrow culture and in isolated bone marrow stem cells. PPARγ is situated at the bifurcation of lineage commitment of bone and adipocytes, suggesting that therapeutic manipulation may help to manage obesity-related conditions and orthopaedic health (Lecka-Czernik 2010). For example, rosiglitazone (an insulin-sensitising thiazolidinedione) activates PPARγ and effectively treats T2DM by promoting insulin sensitivity. However, rosiglitazone use comes at a cost of increased fracture risk consistent with increased adipogenesis and reduced osteoblastogenesis. With a promise for the effective management of T2DM, further work must continue to determine and thus avoid, any negative bone phenotype associated with thiazolidinedione use (Fukunaga *et al*. 2015).

Unexplored candidates

In light of the newly identified function of bone in energy metabolism, it is of interest to review the evidence for substrate utilisation in bone cells types. Overexpression of the glucose transporter Glut1 in osteoblasts enhances osteoblast differentiation and bone formation (Wei *et al*. 2015). Assessment of glucose utilisation by the skeleton *in vivo*, using uptake of positron-emitting ^{18}F-fluorodeoxyglucose ([^{18}F]-FDG), revealed greater glucose uptake in bone than that in classical glucose storage and utilisation organs such as the liver, muscle and white adipose tissue (WAT) (Zoch *et al*. 2016). Furthermore, skeletal [^{18}F]-FDG uptake was greater in young than in older mice, which may be due to the rapid bone formation in young mice. Intriguingly, insulin administration significantly increased skeletal accumulation of [^{18}F]-FDG, whilst insulin receptor-deficient and obese mice had reduced uptake (Zoch *et al*. 2016). These findings suggest that the skeleton is a preferential and significant site of glucose uptake that is regulated by insulin and global metabolism.

Bone and adipose tissue

In times of a positive energy balance (i.e. energy intake > energy expenditure), WAT stores excess energy as triacylglycerol (TAG) and releases fatty acids (FA) and glycerol to be used for β-oxidation or gluconeogenesis during negative energy balance, respectively (Cahill 2006, Rosen & Spiegelman 2014). In addition to the role of adipose tissue in energy storage and release, adipose tissue also provides vital structural/mechanical protection for organs (e.g. the eye fat, pad, toes and heel) (Rosen & Spiegelman 2014) and offers a critical thermoprotective layer against low ambient temperatures.

Discovery of adipose-derived circulating factors such as adipsin, TNF-α, leptin and adiponectin (Badman & Flier 2007, Rosen & Spiegelman 2014) defined adipose tissue as a bona fide endocrine organ. Through the release of these 'adipokines', WAT can exert diverse systemic effects, not only on energy homeostasis but also on other aspects of physiology such as blood pressure, immune function and fertility (Michalakis *et al*. 2013). Thus, despite its association with metabolic diseases, WAT performs many essential physiological functions. Indeed, in the absence, and/or the redistribution of adipose tissue (lipodystrophy), patients develop insulin resistance, hyperglycemia, hypertriglyceridemia, hepatic steatosis and polycystic ovary syndrome underscoring the importance of adipose formation for normal physiological function (Cortes & Fernandez-Galilea 2015).

In contrast to white adipocytes, brown adipose tissue (BAT) is specialised for heat generation by non-shivering thermogenesis. Brown adipocytes, unlike white adipocytes, have an enrichment of mitochondria

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that express uncoupling protein-1 (UCP-1). This protein uncouples the respiratory chain, allowing protons to pass from the inner membrane space to the mitochondrial matrix without passing through ATP synthase. This causes a futile cycle: oxygen is consumed to pump protons, but the resulting chemiosmotic gradient generates no ATP and instead results in the dissipation of energy as heat (Nubel & Ricquier 2006). BAT is developmentally distinct to WAT, deriving from a distinct lineage that is shared with skeletal muscle (Rosen & Spiegelman 2014). BAT activity is relatively high in small mammals and in newborn humans, whereas BAT in adult humans is less active and is situated deep within the neck and supraclavicular region. Nevertheless, BAT in adult humans remains cold responsive, as exemplified in Scandinavian workers exposed to chronic cold (Huttunen *et al*. 1981). Similarly, prolonged cold exposure in rodents has shown to alter WAT cells by developing a brown fat-like morphology. These cells have been named 'beige' adipocytes, with a gene expression pattern overlapping but distinct to that of classical BAT (Wu *et al*. 2012, Rosen & Spiegelman 2014).

Whilst these adipose subtypes have received extensive research focus, the MAT within the marrow cavity of the skeleton has been largely ignored. Concurrent with the emergence of the field of skeletal energy homeostasis, the research into the form and function of MAT has begun to expand. Postnatally, MAT forms at distal skeletal sites, including the tailbone, hands and feet in mice and humans (Scheller & Rosen 2014). Throughout life, MAT (yellow marrow) continues to form in areas of the haematopoietic marrow (red marrow) until almost the entirety of the appendicular skeleton is converted into yellow marrow by the age of 20 years in humans (Moerman *et al*. 2004); however, red marrow persists in the axial skeleton, only declining with advanced age (Justesen *et al*. 2001). Marrow adipocytes are derived from a distinctive progenitor cell that expresses osterix, Prrx1, LepR and Gremlin1 (Chen *et al*. 2014). Thus, marrow adipocytes may be highly related to osteoblast precursors and play a role in bone maintenance and skeletal energy (Liu *et al*. 2013, Mizoguchi *et al*. 2014).

MAT consists of two subtypes: constitutive MAT (cMAT) and regulated MAT (rMAT). cMAT is found predominantly in the distal skeleton, giving the bone marrow a yellow appearance (Scheller *et al*. 2015). In contrast, rMAT develops much later than cMAT, in the proximal skeleton, hip, ribs and lumbar/thoracic vertebrae postnatally and consists of adipocytes interspersed with red marrow. rMAT is not necessarily formed in a normal developmental/physiological manner, instead, rMAT seems to reflect adverse stimuli or disease states (Pichardo *et al*. 2007, Rosen & Spiegelman 2014).

Many questions remain regarding the formation and function of MAT. In animal models, MAT increases in response to the contrasting interventions of calorie restriction (CR) and high-fat diet feeding (Devlin *et al*. 2010, Cawthorn *et al*. 2014, Doucette *et al*. 2015). Similarly, humans with anorexia nervosa show MAT expansion (Misra & Klibanski 2013). Thus, does MAT, like WAT, play a role in regulating systemic energy homeostasis? Consistent with this possibility, is the suggestion that MAT may function as an energy reservoir for ectopic lipid, protecting skeletal osteoblasts from lipotoxicity (Gunaratnam *et al*. 2014), as well as secreting FA, cytokines (IL-6/1β and TNF-α) (Caers *et al*. 2007) and adipokines (leptin and adiponectin) (Rosen *et al*. 2009, Cawthorn *et al*. 2014). Moreover, there is often a relationship between bone loss and MAT expansion, which can coincide during ageing, osteoporosis, elevated glucocorticoids and cancer treatments. This further suggests a close relationship between bone-specific cells and marrow adipocytes (Moerman *et al*. 2004, Georgiou *et al*. 2012).

The diseased state

The skeleton and associated bone-secreted factors provide a complex endocrine system that is finely orchestrated with other organs including the gut, brain, liver and kidney to ensure homeostatic balance and health. Indeed, bone-associated proteins act as a bridge to link complex pathways that bring together bone turnover, mineralisation, mineral and metabolic homeostasis. When these pathways become dysregulated, affected individuals may suffer from bone, muscle and adipose pathology (Fig. 2).

Multiple myeloma and myeloma bone disease

In the instance of multiple myeloma, affected individuals with myeloma bone disease (MBD) may experience altered bone metabolism, as a consequence of myeloma cells colonising the bone marrow (Walker *et al*. 2014, Xi *et al*. 2016). The pathophysiology of MBD is characterised by an imbalance in osteoblast and osteoclast activity. The resultant disruption of bone turnover is due to two distinct mechanisms. Firstly, engrafted myeloma cells are capable of secreting osteoclast-activating factors including, but not limited to, IL-6, IL-β, TNFα and parathyroid hormone-related protein. Secondly, these engrafted cells can also

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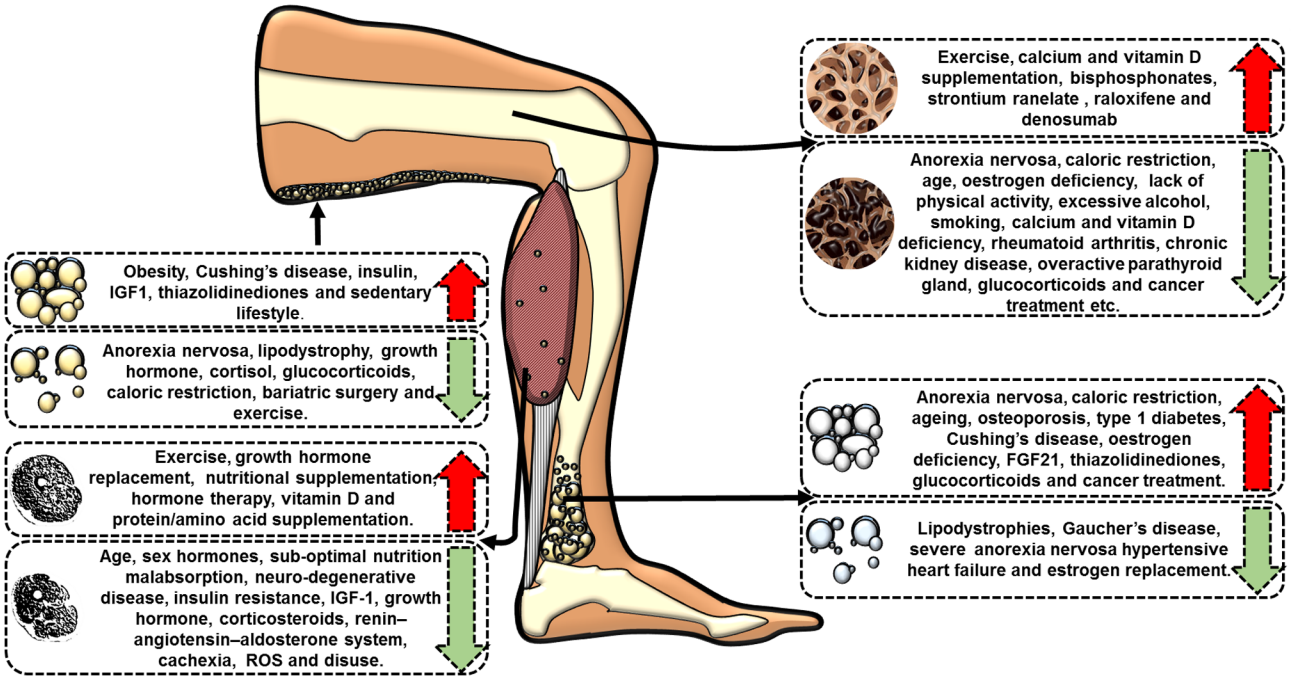

Figure 2
Regulators of bone volume, muscle mass, subcutaneous and marrow adipose tissue. (Arrow key: Red solid – increased; green solid – decreased.) Schematic representation of the key regulators of bone volume, muscle mass, subcutaneous and marrow adipose tissue. It is interesting to highlight that both calorie restriction and glucocorticoids result in the loss of adipose tissue, muscle and bone (discussed further in the text).

interact with bone marrow microenvironment-regulating cells to further encourage secretion of osteoclast-activating factors (Roodman 2010, Terpos *et al.* 2014). By orchestrating this two-pronged 'attack', myeloma cells increase osteoclastic bone resorption. Further, several molecular mechanisms have been attributed to promoting osteoblastic reduction within MBD: Wnt-antagonists Dickkopf-1 (DKK1), runt-related transcription factor 2 (RUNX2), secreted frizzled related protein-2 (sFRP-2), transforming growth factor-beta (TGF-β), heparanase and hepatocyte growth factor (HGF) (Xi *et al.* 2016).

Such mechanisms, which compromise the normal physiological bone environment, are likely linked to energetic costs and wider metabolic consequences to the individual. Indeed, energy is expended upon the synthesis and secretion of an abundance of proteins required in the bone destruction process orchestrated by osteoclasts. Furthermore, in advanced disease states, lytic regions co-localise with elevated osteoclast activity and depressed osteoblastic activity. In accordance, the greater degree of bone acidification in osteoclastic resorption lacunae provides the conditions required to liberate the hormonally active form of OCN from the bone matrix via decarboxylation. An inverse correlation of serum decarboxylated OCN levels and the severity of MBD are reported in the literature (Bataille *et al.* 1990). Furthermore, hypercalcaemia is present at the site of bone lesions due to increased osteoclastic activity. This increased bone endocrine function represents changes to normal bone homeostasis and wider systemic and metabolic effects associated with the previously discussed roles of decarboxylated circulating OCN (i.e. increased insulin sensitivity, increased pancreatic β-cell proliferation, enhanced adipocyte secretion and reduced insulin resistance; Fig. 3).

Improved understanding of the pathogenesis of MBD has led to the identification of novel therapeutic targets. DKK1 is a key regulatory factor in the normal development of bone in adulthood, acting to inhibit osteoblastogenesis and promote differentiation of mesenchymal stem cells towards adipocytes by suppressing Wnt/beta-catenin signalling. It can be hypothesised that the associated endocrine function of the increased MAT serves to propagate myelomagenesis and tumour growth, with elevated adipocyte numbers giving secretion of free fatty acids, signalling molecules (e.g. leptin, adiponectin) and myeloma-supportive adipokines (e.g. IL-6, TNFα). A recent study revealed that blocking of DKK1 activity (or, alternatively, the addition of DKK1 antibody) resulted in a decrease of osteolytic bone disease, with a restoration

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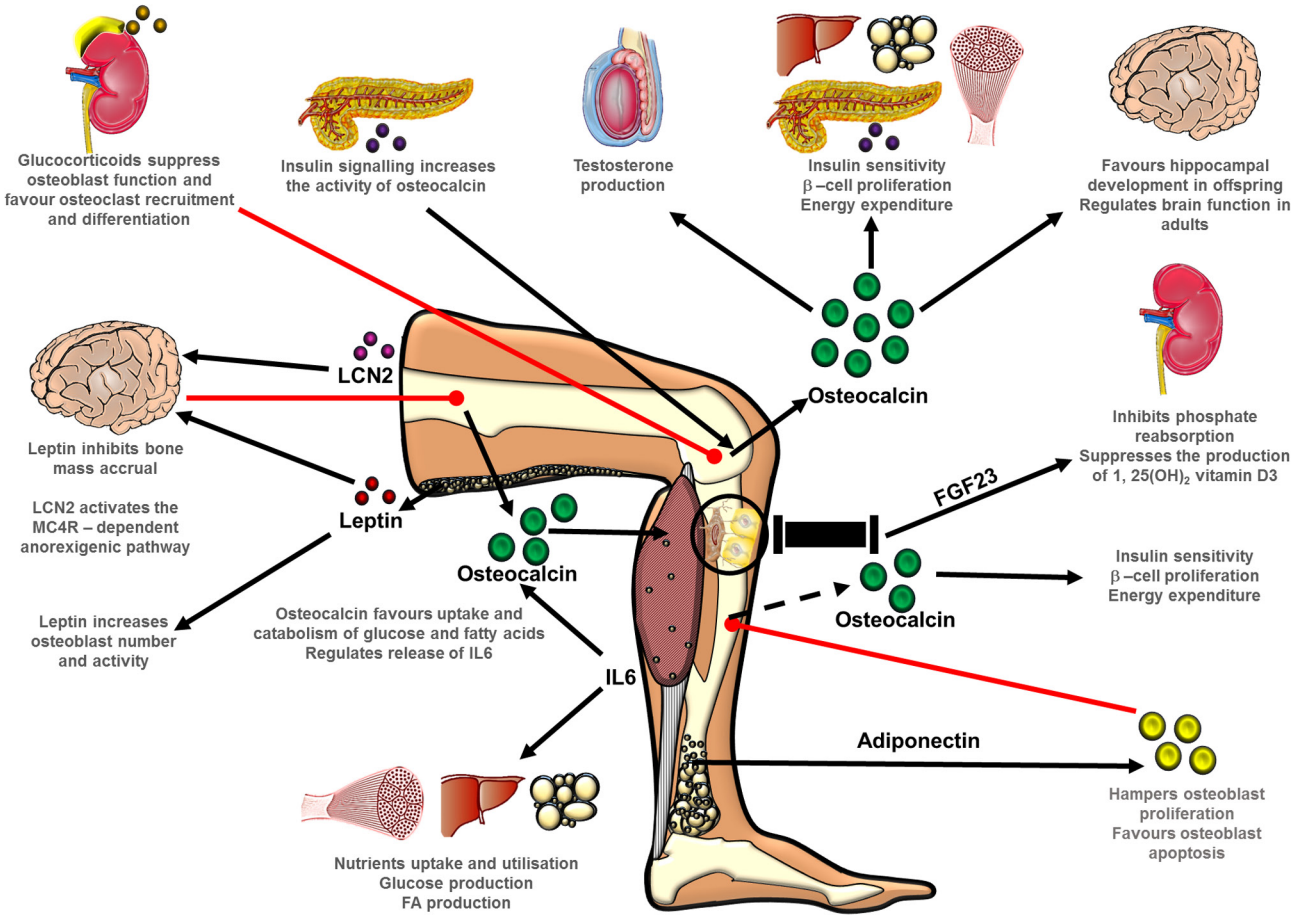

Figure 3

Integrative model of the regulation of the new endocrine functions bone, muscle and marrow adipose tissue. (Arrow key: black solid – accepted; black dashed – speculative; red – inhibitory.) Insulin secretion from the pancreas acts upon the insulin receptor on the osteoblast, which subsequently inhibits Forkhead box protein O 1 (*FoxO1*) expression and suppresses twist basic helix-loop-helix transcription factor 2 (*Twist2*), favouring bone resorption via osteoclast activation. The adipocyte-derived hormone leptin has been shown to have two opposing roles, acting centrally to inhibit bone mass accrual and peripherally, increasing osteoblast number and activity. The acidic pH generated in the resorption lacunae decarboxylates OCN on its three glutamic acid residues (GLU13, GLU17 and GLU20), which enable it to be released from the bone matrix into the general circulation. Once circulating, OCN can regulate global energy metabolism via the stimulation of insulin secretion and β-cell proliferation in the pancreas; energy expenditure by muscle and insulin sensitivity in adipose tissue, muscle and liver. Furthermore, OCN favours hippocampal development in offspring; brain function in adults and male fertility by stimulating testosterone synthesis in Leydig cells of the testis. A bone–muscle feed-forward axis exists where systemic undercarboxylated OCN signals to myofibres favouring uptake and subsequent catabolism of glucose and fatty acids, facilitating physical adaptation to exercise and release of exercise-induced IL-6. The latter drives the production of bioactive OCN. Adiponectin release from bone marrow adipose tissue may act to indirectly increase bioactive OCN by suppressing osteoblast proliferation, potentially favouring osteoclast activity. Another possibility is that excess local OCN production is responsible, at least in part, for elevated adiponectin production from MAT; however, this remains unclear.

of increased osteoblast activity and decreased myeloma tumour burden (Qiang *et al.* 2008). The bi-directional signalling of myeloma cells and bone cells requires further investigation to determine the impacts of these interactions on bone homeostasis and tumour growth. Despite accelerated interest in the field, to date MBD (and multiple myeloma) remain incurable: it is imperative that future work is conducted to further elucidate the molecular mechanisms underlying the disruption of the bone marrow microenvironment within the framework of this complex and multifactorial disease such that novel drugs may become a feasible reality for targeted therapy for the MBD patient.

Diabetes

Globally, 642 million adults are predicted to have diabetes by 2040 (Atlas 2016). The diabetic complication of fragility fractures is of increasing importance, representing an undeniably large burden for health care systems of the world. The burden of diabetic fracture can also be considered at the individual level: fracture healing

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necessitates three energetically costly processes including inflammation, repair and remodelling (Regard *et al*. 2012). It is conceivable that the associated energetic cost of this exerts a direct effect on global energy metabolism of the affected individual, although to date, no established link of fracture burden and energy metabolism has been acknowledged.

In type 1 diabetes mellitus (T1DM), bone mineral density (BMD) – the gold standard measure for the determination of fracture risk – is decreased, a product of decreased osteoblastogenesis and increased osteoblast death (McCabe 2007, Coe *et al*. 2011). Conversely, BMD is increased in type 2 DM (T2DM); yet, both T1DM and T2DM patients have a significantly higher fracture risk as a complication of diabetic bone disease, compared to the general public (Janghorbani *et al*. 2006). This indicates a wider role of under-appreciated and undefined pathophysiological mechanisms responsible for diabetes-associated bone fragility and highlights the shortcomings of our modern day fracture risk assessment techniques. It is likely that many T2DM patients of high fracture risk go unidentified prior to fracture incidence, owing to the higher BMD associated with this class of diabetes. It remains possible that the physiological paradox of elevated BMD coinciding with increased fracture risk could well be explained by the higher prevalence of fall-associated trauma amongst diabetic patients (Gregg *et al*. 2002, Schwartz *et al*. 2002). However, it is likely that the pathophysiological mechanisms that underlie bone fragility in diabetic patients are of greater complexity than initially anticipated: even when studies include falls and associated risk factors, the association between diabetes and increased fractures remains inconclusively explained (Schwartz *et al*. 2002).

Suggested mechanisms of diabetic fractures include complications with hyperglycaemia, oxidative stress and glycation end-product accumulation, which compromis the properties of collagen – the most abundant of the bone proteins (Napoli *et al*. 2016). Furthermore, diabetes is associated with declining renal function, associated with lower BMD, and microvascular complications, which limit blood flow to the bone. Consequently, bones have decreased exposure to circulating bioactive hormones, including OCN, which may further contribute to skeletal fragility. These factors indicate there is a poorer quality of the bone such that there is increased fracture risk for both T1DM and T2DM, despite differences in BMD between these cohorts.

Obesity and anorexia

Our knowledge of the pathogenicity of T2DM and bone disease is further complicated by the frequent overlap of T2DM with obesity. Indeed, a long-held concept is that obesity protects against fracture risk by increasing loading of the skeleton. The increased mechanical strain in obesity is sensed and translated by osteocytes, increased BMD. However, whilst seemingly logical, this concept has recently been debunked: obesity itself is an independent risk for fracture owing to compromised quality of bones, despite non-compromised BMD (Johansson *et al*. 2014, Palermo *et al*. 2016). This confounds our attempts to understand diabetes-specific endocrine mechanisms underlying diabetic-associated skeletal fragility.

Obesity further manifests bone disease through mechanisms affecting metabolism. As both marrow adipocytes and osteoblasts likely derive from a common progenitor within the BM stroma (Chen *et al*. 2014) and that obesity promotes the differentiation of adipocytes in WAT, it is possible that obesity may also stimulate marrow adipogenesis at the cost of osteoblast differentiation. This would result in the altered quality of the obese patient's bones, even if elevated mechanical strain may be giving rise to increased BMD.

In addition, obesity is often associated with chronic inflammation. Obese individuals have an altered hormonal milieu and higher circulating levels of pro-inflammatory cytokines. Such cytokines may serve to modify the activity of the osteoclast receptor activator of NF-κB (RANK)/RANK-Ligand (RANKL), thereby increasing osteoclastogenesis and bone resorption. In addition, the bioavailable 25 hydroxyvitamin D3 is decreased in obese individuals, likely due to storage within the excess adipose tissue, which compromises bone mineral content (Cândido & Bressan 2014). Amongst the obese population, there is also an increase in circulating bone-anabolic hormones. This includes higher levels of pancreatic hormones (insulin, amylin and preptin) and adipose-derived factors including aromatase, leptin and resistin (Karra & Batterham 2010).

On the other end of the weight spectrum, anorexia patients also exhibit a disease-bone phenotype, with greater fracture propensity. This serious psychiatric disorder manifests in emaciation of the self-starved individual (Dede *et al*. 2014). Alongside serious weight deficit, the anorexic patient further suffers from bone structural deficits, such that the skeletal mechanical capability is impaired. These individuals

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experience decreased cortical radius thickness and wider endocortical diameters (Dede *et al*. 2014). Such microarchitectural alterations increase susceptibility to bone fragility, regardless of documented BMD values. These structural defects persist even after recovery from the disease (Dede *et al*. 2014). In a similar fashion to the long-suffering anorexia patient, low-calorie intake during early stages of life (i.e. during skeletal development) results in decreased bone mass, increased fracture risk and osteoporosis in adulthood (Devlin *et al*. 2010). These defects are most harmful during adolescence when bone accrual is paramount for the development of peak bone mass. As previously discussed, anorexia (and caloric restriction) is associated with increased MAT (Fazeli *et al*. 2013, Scheller & Rosen 2014). To date, over 10 distinct animal studies have found increased MAT during states of CR or starvation, such that MAT significantly increases in the proximal femur and tibia of CR mice in comparison to the control mice (Devlin *et al*. 2010, Cawthorn *et al*. 2014). Furthermore, CR in young mice decreased serum leptin and IGF1 levels. Despite elevated bone resorption and decreased bone formation and percentage body fat, MAT was significantly increased in CR mice (Devlin *et al*. 2010), suggesting that increased MAT is associated with impaired skeletal maturity; however, CR in rabbits causes bone loss without MAT expansion, suggesting that the latter is not necessary for the former (Cawthorn *et al*. 2016). In addition to decreased circulating levels of leptin and IGF1 during CR, decreased circulating oestradiol and increased circulating FGF21, ghrelin and cortisol/corticosterone levels have also been linked to elevated BM adiposity; thus, each of these factors has been suggested as mediators of MAT expansion during CR (Thompson *et al*. 2004, Syed *et al*. 2008, Devlin *et al*. 2010, Shen *et al*. 2012, Cawthorn *et al*. 2014, Suchacki *et al*. 2016, Sulston & Cawthorn 2016). These studies highlight the possibility that MAT may be responsible for endocrine signalling such that the propensity of fracture for the anorexic sufferer is increased. One key question is whether the highly energetic cost of fracture repair, coupled with emaciated status of the anorexic individual, promotes the differentiation of skeletal stem/stromal cells towards MAT to act as an 'emergency storage' of adipocytes, and thus energy, to facilitate survival during self-starvation? If so, this likely comes at the expense of osteoblasts derived from the same skeletal progenitor, thereby further potentiating bone fragility in anorexic patients.

Pancreatic disease

Given the recently acknowledged bone-pancreas loop in the regulation of glucose metabolism by insulin (Faienza *et al*. 2015), it is possible that pancreatic diseases such as pancreatitis or pancreatic cancer may result in altered bone homeostasis and/or endocrine function. Studies both *in vitro* and *in vivo* have revealed the osteogenic nature of insulin, promoting cell proliferation, collagen synthesis and uptake of glucose. Insulin acts on bone by binding to the insulin receptor situated on the osteoblast. Recent studies (Ferron *et al*. 2010, Fulzele *et al*. 2010) have revealed that osteoblast-specific insulin receptor knockout results in decreased osteoblast numbers and bone formation, coupled with reduced OCN activity. Patients with pancreatitis suffer from the loss of exocrine and endocrine functions via inflammatory processes that cause the destruction of the pancreas. Concomitantly, a loss of islet cells (α and β cells) results in a decrease in the release of glucoregulatory hormones (glucagon, insulin and pancreatic polypeptides). This compromised insulin release is likely to also compromise osteoblast–endocrine signalling to the insulin receptor. Indeed, a study by Moran and coworkers (Moran *et al*. 1997) revealed that patients with pancreatic insufficiency, a product of chronic pancreatitis, exhibited osteopenia and osteoporosis, although they were unable to determine the pathological mechanisms underpinning this relationship. Furthermore, preptin, a peptide hormone cosecreted by pancreatic β cells with insulin and amylin has been shown to be anabolic to bone *in vitro* and *in vivo* (Cornish *et al*. 2007). During osteoporosis, preptin levels are diminished, positively correlating with BMD. It is understood that preptin is involved in the pathogenesis of osteoporosis through bone formation rather than resorption. However, further studies are required to clarify whether preptin can be a new target for treating osteoporosis by promoting bone formation (Li *et al*. 2013).

Liver disease

The prevalence of patients with chronic liver disease experiencing fracture is estimated at 40% (Nakchbandi 2014). As the liver coordinates many key metabolic pathways, it is unsurprising that the experience of disease within this organ results in atypical metabolism: liver disease itself is the secondary leading cause of osteoporosis. However, there is a lack of epidemiological data to support the true extent of osteoporosis amongst chronic liver

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disease sufferers (Nakchbandi 2014). The liver is central to the maintenance of health processes in the individual. For example, the liver secretes bone-health-associated factors, including IGFI and fibronectin. In health, liver-secreted fibronectin circulates prior to infiltrating the bone matrix: upon infiltration, matrix mineralisation and subsequent microarchitectural properties of bone are favourably promoted. In addition, the liver is capable of acting as a target molecule for bone-active hormones, responding with the production of various endocrine molecules including IL-6. IL-6 can act directly to activate osteoclasts or can serve to stimulate RANKL production via osteoblasts, such that osteoclasts are indirectly activated. Further, the liver is capable of metabolising bone-active molecules, including OCN, such that the period of bioavailability is reduced. Yet, in disease states, such as non-alcoholic fatty liver disease, IL-6 is upregulated as a by-product of liver injury and attempted consequential liver regeneration: this increase, in turn, promotes bone resorption by active osteoclasts. Furthermore, in chronic liver disease states, a reported 92% of patients have vitamin D deficiency: as such, calcium is liberated from the bone via osteoclastic resorption to retain homeostasis within the blood. The net result of this is the loss of bone (Nakchbandi 2014).

Perspective

The last decade has witnessed growing understanding of the skeleton's ability to act as an endocrine organ. Significant developments in cellular systems and mouse models have revealed increasingly convincing evidence in favour of the skeleton's endocrine function (Fig. 3). This adds further credence in reinforcing the importance of the skeleton for survival beyond its mechanical roles. It makes sense, from an evolutionary perspective, that the skeleton produces hormones that regulate skeletal mineralisation, cooperating with other endocrine organs to control the metabolism of phosphate and calcium.

Despite the significant advances in comprehending skeletal energy homeostasis, many questions remain unanswered. Putative investigations of other bone-secreted factors (such as NPP1 and PHOSHPO1) have revealed further candidates for links in metabolic health, including significant roles in diabetes and obesity pathology. Yet, much remains to be identified about the specific mechanisms of action and novel pathways of these new candidates with regard to skeletal and metabolic homeostasis. Continued identification of bone-secreted factors and their function will aid in answering the questions of how and why bone-specific regulation of energy metabolism arose. Most recently, lipocalin (LCN2), an adipokine once thought to be exclusively secreted by adipose tissue has been shown to be an osteoblast-rich, secreted protein. LCN2 crosses the blood–brain barrier to activate the melanocortin 4 receptor, resulting in appetite suppression. Murine loss- and gain-of-function experiments demonstrated that LCN2 maintains glucose homeostasis, improve glucose tolerance and insulin sensitivity; however, more compelling human data are required to fully establish the role of LCN2 (Mosialou *et al.* 2017) (Fig. 3).

Indeed, little is also known about the role of formation and function of MAT – does MAT contribute to the global regulation of energy metabolism by the skeleton? Does MAT provide a local reservoir of energy for bone-specific cells during bone remodelling or in pathological situations? Further understanding of the mechanisms involved in this bone-metabolic axis will have many diverse implications for the management of T2DM, metabolic syndrome and other diseases of bone and adipose physiology. Such knowledge will reveal unidentified mechanisms that regulate energy homeostasis, thereby allowing development of novel pharmacological approaches for managing and treating skeletal and metabolic diseases, underscoring the need for continued research into the endocrine and metabolic functions of the skeleton.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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