Endocrine role of bone: recent and emerging perspectives beyond osteocalcin

K J Oldknow, V E MacRae and C Farquharson

Developmental Biology, The Roslin Institute, Edinburgh, UK

Correspondence should be addressed to K J Oldknow
Email
Karla.Oldknow@roslin.ed.ac.uk

Abstract

Recent developments in endocrinology, made possible by the combination of mouse genetics, integrative physiology and clinical observations have resulted in rapid and unanticipated advances in the field of skeletal biology. Indeed, the skeleton, classically viewed as a structural scaffold necessary for mobility, and regulator of calcium–phosphorus homoeostasis and maintenance of the haematopoietic niche has now been identified as an important regulator of male fertility and whole-body glucose metabolism, in addition to the classical insulin target tissues. These seminal findings confirm bone to be a true endocrine organ. This review is intended to detail the key events commencing from the elucidation of osteocalcin (OC) in bone metabolism to identification of new and emerging candidates that may regulate energy metabolism independently of OC.

Key Words
- osteoblast
- osteocalcin
- metabolism
- fertility
- osteoclast

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Evolution and bone

The vertebrate skeleton is one of the largest mammalian organs, providing the framework of the body, supporting the softer tissues and creating points of attachment for most skeletal muscles. In addition, the skeleton provides protection for vital organs and blood cells, assists in movement and acts as a storage system for minerals, namely calcium and phosphorus, in order to repair, micromanage and participate in fracture healing, thus maintaining a high bone quality adequate to fulfil its major functions. Uniquely, bone has the ability to renew itself through a process of remodelling. Bone remodelling is a biphasic process occurring throughout life in a constant and balanced manner, responsible for linear growth and bone maintenance during adulthood, thus demonstrating true homoeostatic functions. These processes are fully dependent upon two antagonistic cell populations: the osteoblasts and osteoclasts. The primary function of mesenchyme-derived osteoblasts is the deposition of bone matrix that is subsequently mineralised. Conversely, the haematopoietic-tissue-derived osteoclasts are a unique cell type possessing the capability to destroy the host tissue by reabsorbing mineralised bone matrix (Rodan & Martin 2000, Teitelbaum 2000, Harada & Rodan 2003, Teitelbaum & Ross 2003, Karsenty 2006). The misregulation of bone remodelling inevitably results in bone loss and disease and the most common, by far, is osteoporosis.

Considering the sheer size and dynamic homoeostatic nature of the skeleton, it is not implausible to postulate that the skeleton has a high energetic cost. Simple clinical observations add credence to the possible relationship between energy and bone, exemplified by patients with anorexia nervosa, who display decreased or arrested bone growth and low bone mass in adults (Legroux-Gerot *et al.* 2005). Converesly, obesity has traditionally been observed

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to have a positive effect on mechanical loading, thus providing protection from osteoporosis. Nevertheless, results from recent clinical studies have indicated that increased adiposity is associated with low total bone mineral density (BMD) and total bone mineral content (reviewed in Cau (2011)).

Insulin and the insulin receptor

For the survival of all species, the ability to precisely regulate energy production and expenditure is critical. In a once unstable environment, mammals evolved intricate paracrine, autocrine and endocrine signalling pathways that coordinate energy expenditure and storage in metabolically active tissues. Metabolic imbalance between energy intake and expenditure is detrimental, with a positive imbalance resulting in obesity and diabetes (diseases encompassed by the metabolic syndrome) or a negative energy imbalance resulting in anorexia nervosa (Fulzele *et al*. 2010, Fulzele & Clemens 2012). Insulin is a peptide hormone synthesised in the β-cells of the pancreatic islets of Langerhans as its precursor proinsulin and has pleiotropic roles within the body, regulating glucose homoeostasis, carbohydrate, lipid and protein metabolism, and promoting cell division and growth through its mitogenic effects (reviewed in Wilcox (2005)); thus, insulin regulates whole-body energy utilisation, mediating its downstream effects by binding to the insulin receptor (IR). First identified in 1971, the IR is a heterotetrameric membrane glycoprotein situated in the plasma membrane of target cells. The IR is composed of two α and two β subunits linked by disulphide bonds. Upon binding of insulin to the extracellular α subunit of the IR, a conformational change in the intracellular β subunit is elicited, thus allowing for the binding of ATP, triggering phosphorylation of the β subunit. Accordingly, it also confers tyrosine kinase activity, leading to phosphorylation of various effector molecules including IR substrate 1 (IRS1). IRS1 can subsequently bind to further signalling molecules, mediating the cellular effects of insulin (Hubbard *et al*. 1994, Hubbard 1997, Kido *et al*. 2001).

It is well established that bone possesses a functional IR (Pun *et al*. 1989). Results from *in vitro* studies, utilising osteoblast cultures, and *in vivo* studies have indicated that insulin increases bone anabolic markers, modulating collagen synthesis (Rosen & Luben 1983), alkaline phosphatase production (Canalis 1983, Kream *et al*. 1985, Yamaguchi *et al*. 1993), parathyroid hormone (PTH) responsiveness (Thomas *et al*. 1995) and glucose uptake (Ituarte *et al*. 1989). Importantly, the heterogeneous distribution of IR in neonatal rat calvaria was reported subsequently (Thomas *et al*. 1996). Results from complementary studies indicated that insulin-challenged primary and cultured osteoclast-like cells dose-dependently suppressed osteoclast function via inhibiting resorptive pit formation, supporting the anabolic role of insulin in bone (Thomas *et al*. 1998). Indeed, insulin deficiency in humans exemplified by patients with type 1 diabetes mellitus (T1DM) has in some, but not all, subjects been associated with decreased bone mass (Kemink *et al*. 2000) coupled with poor bone regeneration following injury (Loder 1988).

DM is a group of metabolic diseases resulting from defects in insulin secretion, insulin action or both. Patients with DM have an increased risk of bone fractures; however, T1DM and T2DM result in differing osteopathy (Leidig-Bruckner & Ziegler 2001). T1DM results in low BMD, increasing fracture risk by approximately six times, whereas the fracture risk is increased by approximately only two times in T2DM compared with the general population due to bone quality deterioration (Tuominen *et al*. 1999, Jackuliak & Payer 2014). Specifically, T1DM patients have an absolute deficiency of insulin-like growth factor 1 (IGF1), that results in impaired bone formation and lower peak bone mass. Conversely, T2DM patients may display increased BMD due to both increased mechanical loading and hyperinsulinaemia; however, both T1DM and T2DM patients have microarchitectural bone changes, resulting in bone which has an inferior quality compared with that of the general population (Brown 2004, Yamagishi *et al*. 2005, Melton *et al*. 2008, Milczarczyk 2008, Nyman *et al*. 2011). As DM is beyond the remit of this review, we direct the reader to the review by Jackuliak & Payer (2014).

Given these insights, it has been postulated that there is a bone–energy endocrine loop. The first supportive evidence originated from the initial realisation that leptin, an adipocyte-derived hormone, inhibits both appetite (Flier & Elmquist 1997, Friedman & Halaas 1998) and bone mass accrual through a hypothalamic relay (Ducy *et al*. 2000). Thereafter, a rapid expansion of evidence supporting this crosstalk has occurred, further elucidating the complex roles of leptin and identifying further adipocyte- (adiponectin) and gut-derived hormones (glucagon-like peptides 1 and 2 and serotonin) that regulate bone mass, remodelling and energy homoeostasis. The revelation that bone itself regulates energy metabolism in a reciprocal manner via a secreted hormone osteocalcin (OC) was finally uncovered several years ago (Lee *et al*. 2007). Thus, in the last few years, an explosion of avant-garde research has explored this concept,

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uncovering new and atypical roles of bone beyond its traditional functions. This aims of this review are to succinctly discuss the crosstalk between insulin and the osteoblast as well as introducing and considering new concepts beyond the current dogmas in an attempt to demonstrate the complexity of this field.

Osteocalcin

OC or bone Gla-protein was isolated from bone over three decades ago by two independent groups (Hauschka *et al.* 1975, Price *et al.* 1976) and is the most abundant osteoblast-specific non-collagenous protein (Hauschka *et al.* 1989). Named due to the presence of three vitamin K-dependent γ carboxyglutamic acid residues, OC is a small protein (46 and 49 amino acids long in mice and humans respectively) initially synthesised in the osteoblast as a pre–pro molecule. Vitamin K-dependent post-translational modifications occur causing three glutamic acid residues (GLU13, GLU17 and GLU20) to be γ carboxylated into Gla residues by a γ carboxylase. Final intracellular cleavages produce the mature OC, which is subsequently secreted. The presence of the three γ carboxyglutamic acid residues is critical for the structure and function of OC in the fully carboxylated state allowing the binding of OC to hydroxyapatite (HA) with a high affinity, regulating the maturation of bone mineral (Hauschka & Wians 1989, Hauschka *et al.* 1989). However, OC also 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, Ferron *et al.* 2010*a*). On the basis of results from human and rodent studies, serum OC concentrations have been correlated with bone formation and osteoblast number, thus being used as a serum marker of bone formation (Brown *et al.* 1984; reviewed in Gundberg *et al.* (2012)). To investigate the role of OC in bone health, OC-deficient mice were generated ($Oc^{-/-}$); however, surprisingly no major skeletal deformities were observed in these mice (Ducy *et al.* 1996). In 2007, further phenotypic evaluation of these mice resulted in an unanticipated finding. $Oc^{-/-}$ mice were hyperglycaemic, hypoinsulinaemic and had reduced insulin secretion and sensitivity compared with WT mice. Additionally, islet size, number, β-cell mass, pancreas insulin content and insulin immunoreactivity were all markedly decreased in $Oc^{-/-}$ mice. Moreover, $Oc^{-/-}$ mice had increased fat mass and adipocyte number, being insulin-resistant in the liver, muscle and white adipose tissue (Lee *et al.* 2007). This study also focused on the small number of genes encoding secreted or signalling molecules that are expressed exclusively by the osteoblast in the hope of identifying further osteoblast-enriched genes affecting energy metabolism. One gene was found to be of most interest, expressed in only two cell types: the osteoblast and Sertoli cells of the testis. This gene was *Ptprv* (*Esp*), encoding osteotesticular protein tyrosine phosphatase (OST–PTP; Mauro *et al.* 1994). *In vitro*, *Ptprv* coordinates the progression of the preosteoblast to a mature, mineralising cell, and *in vivo* it may be a critical regulator of the commitment of mesenchymal cells to the ossification of new bones during skeletogenesis (Mauro *et al.* 1994, Chengalvala *et al.* 2001, Yunker *et al.* 2004). It is well established that PTPs are key regulators of IR signalling in many cell types, dephosphorylating and inactivating the IR within minutes of stimulation to maintain glucose homoeostasis (Mauro *et al.* 1994, Hunter 1995, Schlessinger 2000, Dacquin *et al.* 2004, Tonks 2006, Lee *et al.* 2007). As a result, two mutant mice were created: a global knock out of *Ptprv* (Lee *et al.* 1996) and an osteoblast-specific knock out of the phosphatase domain of OST–PTP (Dacquin *et al.* 2004). Both mutants exhibited severe hypoglycaemia and hyperinsulinaemia, resulting in postnatal lethality in the first 2 weeks of life. Results from further analysis indicated that the pancreas of $Ptprv^{-/-}$ mice had greater islet content, number of islets, islet size and β-cell mass, resulting in increased insulin secretion. In addition, mutants were significantly more tolerant to glucose upon challenge, displaying an insulin-sensitive phenotype, thus mice were protected from induced obesity and diabetes (Lee *et al.* 2007, Ferron *et al.* 2008). In parallel, mice overexpressing full-length *Ptprv* cDNA selectively in osteoblasts exhibited hyperglycaemia, hypoinsulinaemia, glucose intolerance, insulin resistance, decreased β-cell proliferation, lower β-cell mass and impaired insulin secretion. Subsequently, it was noted that the phenotype of $Ptprv^{-/-}$ mice mirrored the $Oc^{-/-}$ mouse phenotype, while the *Ptprv* mice overexpressing full-length *Ptprv* cDNA selectively in osteoblasts were a phenocopy. Results from further genetic studies indicated that the metabolic phenotype of $Ptprv^{-/-}$ mice was fully corrected by removing one allele of *Oc*, implying that $Ptprv^{-/-}$ mice are a model for a gain of function of *Oc*, providing solid evidence that *Ptprv* and OC reside in the same regulatory pathway (Lee *et al.* 2007). Biochemical analysis revealed that $Ptprv^{-/-}$ mice have significantly higher serum undercarboxylated OC levels than WT controls; however, OC expression and serum levels were normal in $Ptprv^{-/-}$ mice, indicating that OST–PTP is involved in the decarboxylation of OC and the subsequent

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release of undercarboxylated OC into the systemic circulation (Lee *et al.* 2007, Ferron *et al.* 2010*a*).

Notwithstanding, it still remained unclear as to how OC carboxylation status could regulate whole-body energy metabolism. Clues came from several key studies concerning forkhead box protein O1 (*Foxo1*) and activating transcription factor 4 (*Atf4*) (Seo *et al.* 2009, Yoshizawa *et al.* 2009, Rached *et al.* 2010, Kode *et al.* 2012). *Foxo1* is a transcription factor targeted by insulin and regulates glucose homoeostasis in tissues involved in energy metabolism including adipocytes and hepatocytes; however, its function in osteoblasts has not been explored until recently. A *Foxo1* osteoblast conditional knockout mouse was generated, that displayed decreased fasting blood glucose levels and increased insulin sensitivity. The mice also displayed a 30% increase in serum OC levels, coupled with a 75% reduction in *Ptprv* expression, indicative of an association between *Ptprv* and carboxylation status of OC. In the same study, it was demonstrated, utilising various mouse models, that heterozygous mice lacking one allele of *Foxo1* in osteoblasts and one allele of *Ptprv* showed improved insulin sensitivity. Similarly, the metabolic phenotype was corrected in heterozygous mice lacking one allele of *Foxo1* in osteoblasts by the removal of one allele of OC. Utilising these models to investigate the mechanisms underlying the phenotype, it was established that *Foxo1* regulates the bioactivity of OC via OST–PTP through direct binding to its promoter, reducing serum OC (Rached *et al.* 2010, Kousteni 2011, 2012). In a separate study, the role of *Atf4* was also investigated. *Atf4* belongs to the subfamily of cAMP-response element-binding proteins/ATF basic leucine zipper proteins broadly expressed throughout the body; however, it predominantly accumulates in osteoblasts where it regulates virtually all functions of the osteoblast related to the control of bone mass including bone formation and matrix mineralisation (Yang & Karsenty 2004, Elefteriou *et al.* 2005, Yoshizawa *et al.* 2009). $Atf4^{-/-}$ mice primarily show phenotypic abnormalities in the skeleton; however, the global or osteoblast-specific ablation of $Atf4^{-/-}$ in mice results in favourable metabolic changes, including improved glucose tolerance and insulin sensitivity associated with decreased *Ptprv* expression. In contrast, the overexpression of *Atf4* in osteoblasts reflected this phenotype, resulting in glucose intolerance associated with increased *Ptprv* expression. This effect was due to the direct regulation of *Ptprv* expression in osteoblasts by *Atf4*, established by a ChIP array confirming that *Atf4* binds to the CRE element in the *Ptprv* promoter (Yoshizawa *et al.* 2009). Finally, it has been shown that *Foxo1* co-localises with *Atf4* in the osteoblast nucleus, promoting the transcriptional activity of *Atf4*, thus up-regulating the expression of *Ptprv* in osteoblasts, resulting in OC inactivation (Kode *et al.* 2012).

But how does *Ptprv* affect insulin signalling in osteoblasts? In the search for the OST–PTP substrate in osteoblasts, utilising multiple genetic and biochemical modalities, the IR was identified as a potential substrate. As a result, two studies conducted simultaneously by the laboratories of Professors Karsenty and Clemens to explore the role of insulin signalling in osteoblasts were initiated. They generated osteoblast-specific IR-deficient mice ($Insr_{osb}^{-/-}$) that presented with hyperglycaemia, increased peripheral adiposity, reduced insulin secretion, severe glucose intolerance and decreased levels of circulating undercarboxylated OC. These mice also displayed a skeletal phenotype with a reduction in bone acquisition due to reduced bone formation; however, the marker of bone resorption (CTx) was decreased. Upon infusion of exogenous undercarboxylated OC, the metabolic phenotype was fully corrected, indicating that insulin signalling in osteoblasts has the potential to regulate whole-body glucose homoeostasis via carboxylation status of OC (Ferron *et al.* 2010*b*, Fulzele *et al.* 2010). It was also suggested that insulin signalling in osteoblasts might favour bone resorption, due the observation that decreased CTx levels in $Insr_{osb}^{-/-}$ mice reflected the increase in CTx observed in $Ptprv^{-/-}$ mice. Utilising osteoblasts from $Insr_{osb}^{-/-}$ and $Ptprv^{-/-}$ mice, Ferron and colleagues established, using a co-culture system, that WT osteoclast precursor cells cultured with osteoblasts isolated from $Insr_{osb}^{-/-}$ mice decreased osteoclast resorption pit formation, while a 50% increase in osteoclast resorption pit formation was observed when $Ptprv^{-/-}$ primary osteoblasts were used in the co-culture system. Moreover, osteoprotegerin (*Opg* (*Tnfrsf11b*)), a negative regulator of osteoclast formation and function, encoding the decoy receptor for receptor activator of nuclear factor κ B ligand (RANKL), was increased by twofold in $Insr_{osb}^{-/-}$ and decreased by 50% in $Ptprv^{-/-}$ osteoblasts. Further unravelling of this complex pathway revealed that insulin signalling in osteoblasts inhibited *Foxo1* expression, favouring bone resorption via suppression of *Opg* and *Twist2* (RUNX2 inhibitor; Ferron *et al.* 2010*b*, Fulzele *et al.* 2010, Rached *et al.* 2010). It appeared that osteoclasts were pivotal for the connection between bone and energy metabolism; therefore, Ferron and colleagues investigated genes associated with *Opg*-dependent events in the osteoclast. It was found that *Tcirg1*, an essential part of the plasma membrane proton pump, responsible for the acidification of the bone before bone resorption by

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osteoclasts, was decreased in co-culture osteoclast/$Insr_{osb}^{-/-}$ osteoblast models (Teitelbaum 2000, Teitelbaum & Ross 2003, Bronckers *et al*. 2012). These results indicated that insulin signalling in osteoblasts induces osteoclast acidification and bone resorption via decreased *Opg* expression. Utilising biochemical and mass spectroscopy analysis, it was established that an acidic environment generated by osteoclasts situated in the resorption lacuna can decarboxylate OC present in the extracellular matrix (Engelke *et al*. 1991).

In addition to the classical osteoblast-specific PTP, *Ptprv*, which is defined by its specificity for phosphotyrosine (Alonso *et al*. 2004, Barr *et al*. 2009), 37 other mammalian classical PTPs exist. Of these, the only other identified PTP able to bind to the osteoblast IR and respond to isoproterenol treatment similarly to OST–PTP (Hinoi *et al*. 2008) is T-cell PTP. This finding further supports the notion that bone is involved in the regulation of glucose metabolism, increasing our understanding of the complex regulation of OC-mediated glucose homoeostasis (Zee *et al*. 2012) (for comprehensive and recent reviews, see Karsenty & Ferron (2012) and Ferron & Lacombe (2014)).

Even in light of this new concept of bone acting as an endocrine organ, it still remains unclear as to why osteoporotic or osteopenic mice all do not display metabolic imbalances. This is indicative of a far more complex regulation of energy by bone, and indeed supportive of the notion that additional osteoblast- or osteocyte-derived factors are likely to exist.

Male fertility and the discovery of the OC receptor

Diet-induced obesity in rodent models leads to a decrease in sperm motility and reduced hyperactivated progression, which is associated with a trend towards a reduction in fertility potential (Ghanayem *et al*. 2010, Fernandez *et al*. 2011). In humans, obesity is associated with infertility by reducing semen quality, changing sperm proteomes and contributing to erectile dysfunction (reviewed in Cabler *et al*. (2010) and Palmer *et al*. (2012)).

The discovery of the OC receptor (GPRC6A) occurred simultaneously with the elucidation of the role of OC in fertility. Briefly, male and female patients with gonadal failure possess low bone mass; furthermore, menopause favours bone loss (Riggs *et al*. 1982, 1998, Wishart *et al*. 1995). These clinical observations led to the investigation of the possible relationship between bone and fertility. Fortuitously, it was noted that $Oc^{-/-}$ mice were poor breeders, as a result of from decreased testes weight with a 50% reduction in sperm count associated with impaired Leydig cell maturation and decreased circulating testosterone. Reflecting this phenotype, $Ptprv^{-/-}$ mice had increased male reproductive organ weights with a 30% increase in sperm count and increased circulating testosterone (Oury *et al*. 2011). These results indicated a link between OC and testosterone production, which was relevant to males only, as no change in circulating oestrogen or the aromatase enzyme required to convert testosterone to oestrogen (Cyp19A1) was observed in the *Ptprv*- or *Oc*-deficient mice. In an effort to clarify the signalling mechanism underlying this pathway, several factors were taken into consideration, namely the target cells affected by OC (β-cells of the pancreas and the Leydig cells of the testis) and the sexually dimorphic aspects of OC. These clues led to the identification of GPRC6A, a G protein-coupled receptor linked to adenylate cyclase. *Gprc6a* is expressed in the Leydig cells, and its inactivation in mice leads to a metabolic phenotype very similar to that of $Oc^{-/-}$ mice characterised by glucose intolerance and decreased β-cell area and β-cell mass. In addition, these mice demonstrate defective bone mineralisation (Pi *et al*. 2008, 2010). Moreover, the compound heterozygous mice ($Oc^{-/+}$ $Gprc6a^{-/+}$) had a reproductive phenotype similar in all aspects to that observed in *Oc*- and *Gprc6a*-deficient mice models (Oury *et al*. 2011). These results indicated GPRC6A to be an OC receptor, demonstrating that OC mediates testosterone biosynthesis. Additionally, utilising the $Gprc6a^{-/-}$ mouse model, it was shown that i.p. injection of OC failed to markedly stimulate ERK activity, thus having minor effects on circulating serum insulin levels, which were increased in WT mice exposed to the same treatment. GPRC6A has been shown to be integral in the promotion of β-cell proliferation during development and adulthood via OC, thus highlighting GPRC6A as an important receptor for skeletal-tissue-mediated energy regulation via the pancreas (Pi *et al*. 2011, Wei *et al*. 2014*a*). Most recently, Oury *et al*. (2013) demonstrated that OC acts via a pancreas–bone–testis axis, such that OC-stimulated testosterone synthesis is positively regulated by insulin signalling in osteoblasts and is independent of luteinising hormone (LH). No connection between $Ptprv^{-/-}$ and $Oc^{-/-}$ mice in osteoblast-stimulated oestradiol production was identified, illustrating that the regulatory mechanisms of fertility of male and female mice are vastly distinct (Oury *et al*. 2011).

It was noted that the reproductive phenotype of $Oc^{-/-}$ and $Gprc6a^{-/-}$ male mice was very similar to that of $Lhb^{-/-}$ (LH-deficient) male mice, all displaying

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defective testosterone synthesis and testosterone-dependent events (Oury *et al*. 2011). LH is a key regulator of male fertility, favouring testosterone biosynthesis via the hypothalamo-pituitary axis (Kumar 2007). Surprisingly, further analysis of $Oc^{-/-}$ or $Gprc6a^{-/-}$ mice revealed increased circulating levels of LH, which is indicative of a dual regulation of male fertility, or of OC acting downstream, of LH (Themmen & Huhtaniemi 2000, Kumar 2007). By means of elaborate studies from Karsenty's groups have since demonstrated that OC regulates male fertility independently of the hypothalamo-pituitary axis. Indeed, the regulation of testosterone synthesis by OC is independent of a measurable influence of *Gprc6a* on *Lh* (*Lhb*) expression and there is no evidence that LH regulates OC expression (Ferron *et al*. 2010*a*,*b*, Oury *et al*. 2013; reviewed in Karsenty & Oury (2014)).

To emphasise the importance of the role of bone in energy metabolism, Wei *et al*. (2014*b*) evaluated the consequences of osteoblast-specific overexpression of or loss of IR in high-fat-diet (HFD)-fed mice. Results from these studies indicated that insulin resistance in bone affects whole-body glucose homoeostasis in mice fed on a HFD by decreasing OC activity; moreover, it was demonstrated that SMURF1-mediated IR ubiquitination contributes to the development of insulin resistance in osteoblasts. These results support the notion that bone is a highly important site for the regulation of global energy homoeostasis (Wei *et al*. 2014*b*).

What else controls OCN?

As discussed, results from a number of seminal studies have indicated that a feed-forward link exists between OC and insulin; however, leptin and glucocorticoids have been shown to negatively regulate OC activity. In brief, leptin secretion by adipocytes results in increased *Ptprv* expression via *Atf4*, occurring via a central pathway (Hinoi *et al*. 2008) and glucocorticoids decrease OC activity by suppressing osteoblast function and OC production (Brennan-Speranza *et al*. 2012; reviewed in Ferron & Lacombe (2014)).

Clinical evidence: OC and metabolism/fertility

One of the earliest studies to show an association between OC and glucose metabolism was published over a decade ago. OC levels were significantly lower in diabetic patients, although OC levels increased with improved glycaemic control (Rosato *et al*. 1998). In many human studies only total OC levels were quantified; however, the effects on glucose metabolism via bone are attributed to undercarboxylated OC. These studies yielded mixed results with several of them indicating a positive correlation between serum undercarboxylated OC levels and enhanced β-cell function (Hwang *et al*. 2009, Prats-Puig *et al*. 2010, Pollock *et al*. 2011). However, results from other studies indicate no association between lower circulating uncarboxylated OC levels and higher HOMA-IR (Shea *et al*. 2009). Results from one recent study have indicated that there is a sex-specific action of the bone–energy homoeostasis axis with OC being associated with improved metabolic state via adiponectin in females, and via testosterone in males (Buday *et al*. 2013). Direct clinical evidence has been reported for the role of OC in energy metabolism, via the removal of an OC-producing osteoid osteoma, which resulted in elevated serum glucose, potentially associated with decreased levels of undercarboxylated OC (Confavreux *et al*. 2012). This conflicting results may be attributable to the lack of a commercially available undercarboxylated assay, or differing methodologies (Ducy 2011). Similarly, it appears that the reproductive function of OC translates to humans, with the identification of a positive association between OC and testosterone serum levels in the general population, patients with bone disorders and patients with T2DM (Hannemann *et al*. 2013, Kanazawa *et al*. 2013). Furthermore, two subjects were identified from a cohort of patients displaying testicular failure who harboured a heterozygous missense variant in one of the transmembrane domains of GPRC6A, giving credence to a role of OC function in humans (Oury *et al*. 2013; reviewed in Karsenty & Oury (2014)).

Beyond OC

Intriguingly, recent evidence has indicated that other osteoblast-derived hormones may contribute to the emerging function of the skeleton as a regulator of energy metabolism. This was demonstrated by the partial ablation of osteoblasts in transgenic mice, which resulted in profound effects on glucose metabolism and gonadal fat mass, combined with increased energy expenditure. OC administration partially corrected the metabolic phenotype; however, it did not reverse the increased energy expenditure or decreased gonadal fat. This indicates that osteoblasts have the ability to affect glucose metabolism through both OC-dependent and -independent mechanisms (Yoshikawa *et al*. 2011). Herein, we will discuss novel candidates that influence energy metabolism, with a focus on emerging concepts (summarised Fig. 1).

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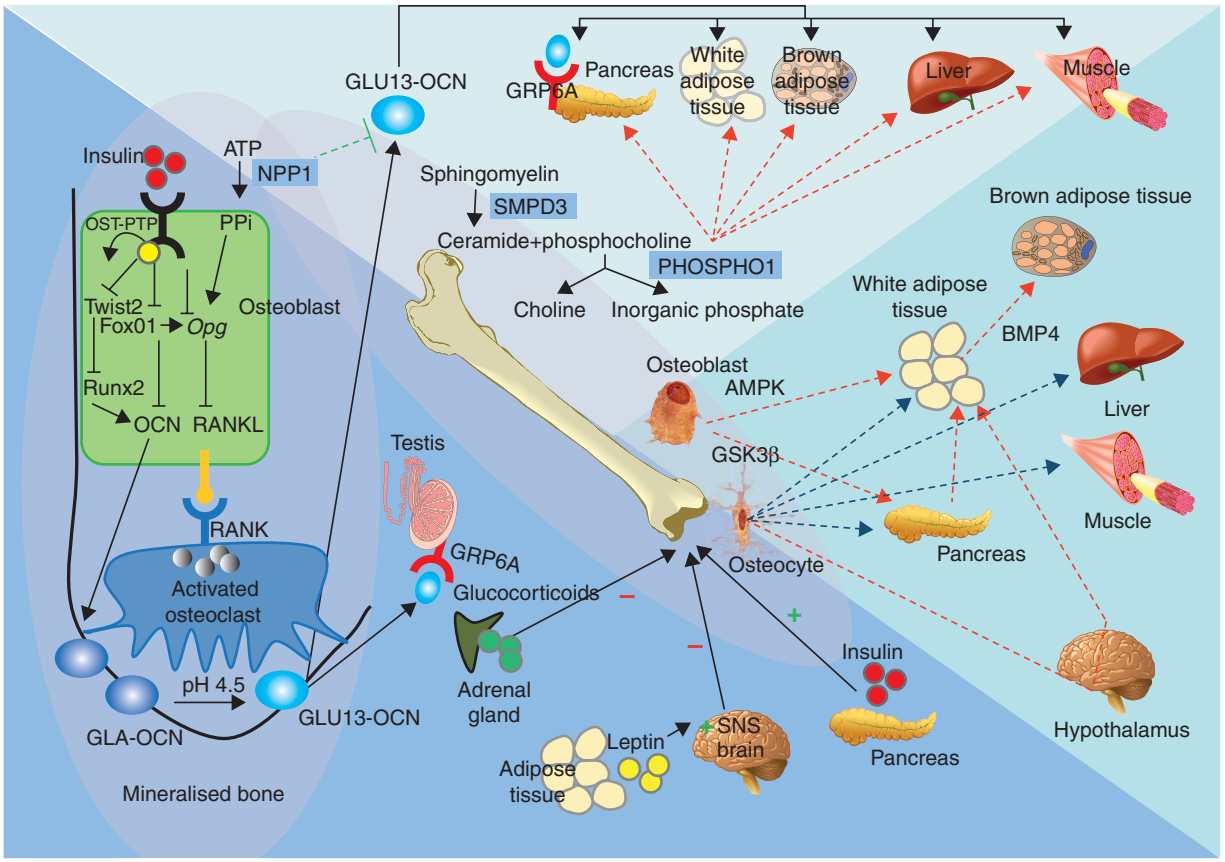

Figure 1
The endocrine role of bone: osteocalcin and beyond. Arrows: continuous, accepted; dashed, speculative; black, known interactions; green, indirect interactions; red, direct interactions; blue, osteokines. A feed-forward loop links insulin, bone resorption and osteocalcin activity. Insulin signalling in osteoblasts decreases the expression of *Opg* by decreasing the ratio of *Opg* (a RANKL decoy receptor) to RANKL, thus increasing bone resorption by osteoclasts. This osteoclastic bone resorption generates an acidic pH in the resorption lacunae necessary to decarboxylate osteocalcin stored in the bone extracellular matrix. Undercarboxylated osteocalcin (GLU13-OC) is released into the bloodstream, affecting glucose metabolism by binding to the osteocalcin receptor (GPRC6A), thus stimulating insulin secretion and β-cell proliferation in the pancreas and promoting insulin sensitivity in peripheral organs. In addition, GLU13-OC promotes male fertility by stimulating testosterone synthesis in Leydig cells of the testis through GPRC6A activation. OST-PTP acts as an inhibitor, dephosphorylating the IR and suppressing the levels of GLU13-OC. To complete this feed-forward loop, peripheral/central tissues (adrenal gland, adipose tissue and pancreas) can further indirectly regulate the release of GLU13-OCN into the peripheral circulation. New emerging evidence indicates that, in addition, NPP1 can indirectly inhibit GLU13-OCN release via OPG. Independently of OCN, osteoblast-specific proteins (PHOSPHO1, AMPK and GSK3β) can influence insulin secretion from β-cells, their functions and adiposity. Osteocyte-derived factors – osteokines – may also be implicated in the endocrine regulation of glucose metabolism (figure adapted from Rosen & Motyl (2010) and Ferron & Lacombe (2014)).

Glucose transporter and bone

Cellular uptake of glucose is mediated by either of the two families of membrane-associated carrier proteins, namely the sodium coupled glucose transporters (SGLTs) via active transport and glucose transporter (GLUT) facilitators via facilitated diffusion (Bell *et al.* 1990, Carruthers 1990). The SGLT family comprises 12 members including co-transporters for sugars, anions, vitamins and short-chain fatty acids (Wright & Turk 2004). Currently, the presence of SGLT in bone has not been reported; however, SGLT2 receptor inhibitors, acting as glucose-lowering agents in the management of T2DM, have been reported to have no significant effects on bone formation and resorption or BMD in humans (Ljunggren *et al.* 2012). In contrast, GLUT receptors have recently been reported to be expressed in bone. To date, the GLUT family consists of 14 members subclassified into three groups, according to sequence similarities and characteristic elements (Joost &

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Thorens 2001, Mueckler & Thorens 2013). GLUT receptors exhibit striking tissue-specific expression, each possessing differential sensitivities to stimuli such as insulin, thus allowing for complex and specific regulation of glucose uptake according to cellular requirements (Gould & Holman 1993). It was first suggested that insulin promotes increased glucose uptake via GLUT1 in the osteoblast, independently of IGF1 signalling to increase the metabolic activity of the osteoblast (Fulzele *et al.* 2007). Most recently, *Glut4* has been found to be expressed at similar levels to those in skeletal muscle in osteoblasts, osteocytes and chondrocytes, with the genetic ablation of *Glut4* in osteoblasts/osteocytes resulting in increased peripheral adiposity associated with mild hyperinsulinaemia. These mice also presented with insulin resistance. These metabolic changes were assumed to originate from osteoblasts/osteocytes as no altered gene expression was identified in the liver or adipose tissue, indicating that decreased GLUT4-mediated glucose uptake in bone is sufficient to influence whole-body metabolism (Zhu *et al.* 2013). Recent emerging results from two independent laboratories have indicated that, in addition to *Glut4, Glut1* is necessary for bone formation and whole-body glucose homoeostasis. Moreover, *Glut1* is modulated by high glucose levels (Virta *et al.* 2014, Wei *et al.* 2014*a*,*b*,*c*). Collectively, these results provide a deeper understanding of the role of bone in the regulation of glucose metabolism (summarised in Fig. 2).

AMP-activated protein kinase and energy metabolism

It has recently been suggested that AMP-activated protein kinase (AMPK) is a key enzyme in the relationship between bone and fat. AMPK is a downstream component of a kinase cascade composed of differing subunits (α1, α2, β1, β2, γ1, γ2 and γ3). AMPK forms heterotrimers that exhibit differences in subcellular localisation and regulation (Hardie 2007), playing a key role in the orchestration of cellular energy homoeostasis (Hardie *et al.* 2006, Lage *et al.* 2008). In response to physiological/pathological stimuli, AMPK acts to restore cellular energy balance (AMP:ATP ratio). During cellular energy deprivation, AMPK increases the potential for ATP production via ATP-generating pathways such as fatty acid oxidation, while concurrently decreasing cellular energy-consuming anabolic processes (Corton *et al.* 1994, Kahn *et al.* 2005). Impairment of AMPK is associated with the metabolic syndrome, demonstrating its physiological requirement, reflected by the improvement of energy metabolism, namely insulin sensitivity in the presence of AMPK (Steinberg & Kemp 2009, O'Neill *et al.* 2011). It has recently been suggested

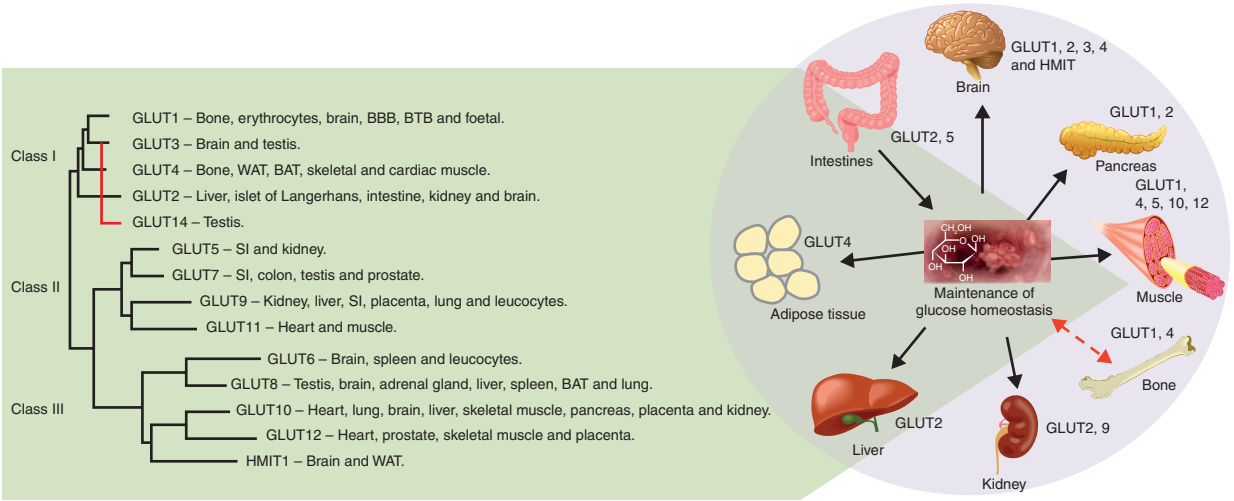

Figure 2
GLUT transporter family. Dendrogram of the extended GLUT family highlighting GLUT receptor tissue distribution and cellular/subcellular expression. Additionally, the role of GLUT proteins in the maintenance of glucose homoeostasis is summarised, outlining the relevant glucose-responsive tissues and the associated GLUT receptors (newly reported GLUT receptors in bone are also highlighted; figure adapted from Joost & Thorens (2001) and Mueckler & Thorens (2013)). Potential similarities between GLUT3 and GLUT14 are indicated by a red line. The role of bone GLUT1 and GLUT4 in glucose homoeostasis is speculative and indicated by a red double-headed arrow. BBB, blood–brain barrier; BTB, blood–testis barrier; WAT, white adipose tissue; BAT, brown adipose tissue; SI, small intestine.

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that AMPK is central to the regulation of skeletal metabolism. The α1 subunit is the dominant catalytic isoform expressed in bone, and, when removed in mice, cortical and trabecular bone compartments were shown to be smaller compared with those of the WT controls (Shah *et al*. 2010). Moreover, the administration of metformin, a drug used widely in the control of T2DM, ameliorates hyperglycaemia and is known to activate AMPK (Stumvoll *et al*. 1995, Zhou *et al*. 2001). AMPK has been reported to enhance differentiation and mineralisation of osteoblastic MC3T3-E1 cells and dose dependently increase trabecular bone nodule formation *in vitro*, supporting the hypothesis of a role of AMPK in the regulation of bone formation and bone mass (Kanazawa *et al*. 2008, Shah *et al*. 2010). Recently, Jeyabalan *et al*. (2012) have elegantly reviewed AMPK and bone metabolism and suggested that AMPK activation may be involved in the relationship between bone and fat. Indeed, the activation of AMPK may enable the skeleton to sense energy status, initiating either adipogenesis or osteoblastogenesis depending on energy needs. This hypothesis is corroborated by the observation that AMPK reduced adipogenesis *in vitro*, by phosphorylating β-catenin, suppressing and directly phosphorylating PPARγ coactivators (Leff 2003, Zhao *et al*. 2010, Jeyabalan *et al*. 2012). Supporting this notion, AMPK has been shown to regulate thyroid-hormone-stimulated OC synthesis in osteoblasts, potentially indicating a direct link between AMPK and the regulation of energy metabolism via the skeleton (Kondo *et al*. 2013).

Bone morphogenetic proteins

Bone morphogenetic proteins (BMPs) are multi-functional growth factors that are members of the transforming growth factor β superfamily. BMPs have a critical role in embryogenesis and are important in bone and cartilage formation and function. BMPs have been the subject of other recent and extensive reviews (Chen *et al*. 2012). Genetic manipulation of mice has allowed a wealth of knowledge to be obtained regarding the complexity of BMPs that may have clinical relevance, such as in the treatment and clinical management of bone grafting and non-unions (reviewed in Carreira *et al*. (2014)). Roles of BMPs in adipogenesis and energy metabolism have recently been described, including in adipocyte development, adipose cell fate determination, differentiation of committed preadipocytes and function of mature adipocyte (Tang *et al*. 2004, Taha *et al*. 2006, Huang *et al*. 2009). More recent results have indicated that BMPs play a role in the 'browning' of white adipocytes. Moreover, the genetic ablation of *Bmp4* results in enlarged white adipocyte morphology and impaired insulin sensitivity, whereas overexpression of *Bmp4* in white adipocytes results in reduced adipocyte tissue mass and size coupled with an increased number of white adipocyte cell types with brown adipocyte characteristics, indicating that BMP4 can regulate the induction of brown-adipocyte-like cells and insulin sensitivity by affecting white adipocyte development (Qian *et al*. 2013). These characteristics of BMPs appear to be conserved in human tissue, where BMP4 and BMP7 have been shown to induce the white-to-brown transition in primary human adipose stem cells (Elsen *et al*. 2014, Obregon 2014).

Glycogen synthase kinase

Glycogen synthase kinase 3 (GSK3) is composed of two mammalian isoforms, GSK3α and GSK3β, playing largely overlapping roles. Explaining simply, GSK acts mainly as a brake in many anabolic pathways including the Wnt/β-catenin and insulin pathways. Moreover, GSK has been implicated in a range of human pathologies including cancer, Alzheimer's disease, non-insulin-dependent DM and bipolar disorder (reviewed in Patel *et al*. (2004) and Forde & Dale (2007)). Recent evidence has indicated that, in addition to the outlined pathologies, GSK3β functions in bone to regulate skeletal development and whole-body metabolism. It has been reported previously that germline loss of GSK3β in mice results in skeletal abnormalities; however, these abnormalities were not present in cartilage-specific GSK3β (*GSK3B*)-deficient mice, possibly due to a compensatory increase in GSK3α (GSK3A) protein levels (Hoeflich *et al*. 2000, Kugimiya *et al*. 2007, Liu *et al*. 2007, Gillespie *et al*. 2011). Subsequently, mice were created in which GSK3β was inactivated in early differentiating skeletal cells and osteoblasts only (Gillespie *et al*. 2013). These mice displayed delayed skeletal development and ossification and increased trabecular bone. However, most relevant to this review, *Col1a1–Gsk3β*$^{-/-}$ mice displayed decreased fat content, smaller adipocytes, pronounced hypoglycaemia and hypoinsulinaemia. Interestingly, female *Col1a1–Gsk3β*$^{-/-}$ mice were significantly more insulin-sensitive. These metabolic changes were independent of food consumption and undercarboxylated or total OC. The mechanisms underlying this connection still remain unclear; however, the authors suggested that these metabolic changes may be due to the hyperactivation of the insulin pathway, resulting in the uptake of glucose, or due to the presence of an unknown factor other

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than OC that contributes to increased insulin sensitivity in*Col1a1–Gsk3β*$^{-/-}$ mice (Gillespie *et al*. 2013).

Osteocyte and energy

In addition to the discussed specialised bone cells (osteoblasts and osteoclasts) osteocytes have also recently been suggested to be involved in energy metabolism. Osteocytes are the most abundant bone cells, formed from differentiated mature osteoblasts, thus becoming terminally differentiated osteocytes. Osteocytes become entrenched within the mineralised bone matrix, forming canalicular networks with other osteocytes and bone-surface osteoblasts, acting as important mediators for intracellular communication and potentially orchestrating bone remodelling. Additionally, osteocytes are able to detect gravitational forces and are thought to play a role in matrix mineralisation and phosphate homoeostasis; however, the precise functions of osteocytes still remain unclear (Karsenty & Wagner 2002, Bonewald 2007, 2011). Intriguingly, Sato and colleagues have recently suggested that osteocytes may play a role in the regulation of the control of fat mass in association with the hypothalamus. Mice were generated in which the receptor for diphtheria toxin (DT) was under the control of the dentin matrix protein 1 promoter (*Dmp1*). Mice then received injections of DT at 15 weeks to render them osteocyte-less mice (OL mice). Following injection, mice lost weight and white adipose tissue mass, with a drastic reduction in mesenteric and subcutaneous fat; however, these mice were not diabetic. These effects were reversed when osteocytes were replenished within the bone. The mechanism underlying this phenotype remains unknown; however, total OC was decreased in the OL mice (Sato *et al*. 2013). However, the *DMP1* promoter also targets the osteoblast and, therefore, the assumption that the phenotype is entirely OC-driven is open to interpretation (Moverare-Skrtic *et al*. 2014). Moreover, Ferron & Lacombe have recently suggested the potential presence of 'osteokines', osteocyte-derived factors that may be implicated in the endocrine regulation of glucose metabolism; however, these factors are yet to be discovered (Sato *et al*. 2013, Ferron & Lacombe 2014).

Excitingly, results from other recent studies have indicated that osteocyte-derived fibroblast growth factor 23 (FGF23) functions in an endocrine manner. Since its identification in 2000, FGF23 has been shown be most highly expressed in bone (osteocyte), acting as an important hormone in regulating serum phosphate levels primarily via actions on the kidney (Shimada *et al*. 2004) (reviewed in Bonewald & Wacker (2013)). In addition to the role of FGF23 in phosphate homoeostasis and bone mineralisation, the PHEX, DMP1, FGF23, KLOTHO and the MEPE/ASARM peptide axis has been demonstrated to be involved in the regulation of energy metabolism via the bone (David *et al*. 2009*a*,*b*). Briefly, mouse models either overexpressing MEPE, ASARM peptides or infused ASARM peptides display increased adiposity, are hyperglycaemic and have increased OC, whereas FGF23-null mice are hypoglycaemic (ASARM peptide modulates PHEX–DMP1-mediated FGF23 expression; Rowe *et al*. 1996, David *et al*. 2009*a*,*b*, 2011). Intriguingly, patients subjected to a 4-h euglycaemic–hyperinsulinaemic clamp show increased FGF23 that correlates positively with insulin infusion (Winther *et al*. 2011). These combined data are indicative of key roles for FGF23 in energy metabolism (reviewed in Rowe (2012)).

Fracture burden and global energy metabolism

It seems plausible that fracture may be associated with a large metabolic expense, thus directly affecting global energy metabolism. Reviewing the literature, we found no clear link between fracture burden and energy metabolism. However, Hamann and colleagues have recently assessed the effects of intermittent PTH on metabolic function in both diabetic and non-diabetic rats, with internally stabilised induced subcritical femoral defects. PTH had no effect on body weight, glucose tolerance or pancreatic islet morphology in both groups, despite PTH therapy resulting in bone anabolic effects and bone defect repair. Unfortunately, the authors were unable to detect undercarboxylated OC; however, they reported no change in carboxylated OC between vehicle and PTH-treated non-diabetic and diabetic rats (Hamann *et al*. 2014). These results are surprising as intermittent therapy is known to increase serum levels of OC (Neer *et al*. 2001, Greenspan *et al*. 2007). These insights indicate that improved fracture repair may not have a global effect on energy metabolism. Paradoxically, it has been shown that vitamin K-dependent γ carboxylation of OC positively enhances the efficacy of PTH following a closed fracture osteotomy. After osteomy, carboxylated OC increased by 18% from baseline and uncarboxylated OC was increased by 100% after surgery; however, insulin sensitivity was not assessed (Shimizu *et al*. 2014).

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Sphingolipids and PHOSPHO1

Sphingolipids are a large class of lipid molecules containing a sphingoid backbone, derived from the condensation of an amino acid and fatty acid; modifications of this basic structure result in a large sphingolipid family (Hannun & Obeid 2011, Mullen *et al*. 2012). Sphingolipids are primarily synthesised *de novo* in the endoplasmic reticulum and Golgi apparatus, before transportation to the plasma membrane and endosomes; however, sphingomyelinases also play vital roles in sphingolipid biosynthesis. Categorised as acidic, alkaline or neutral, sphingomyelinases cleave sphingomyelin, thus generating ceramide and phosphocholine (Merrill *et al*. 1997, Marchesini & Hannun 2004, Futerman & Riezman 2005). Until recently, sphingolipids were considered structurally inert; however, they are now accepted to be fundamental signalling molecules, responsible for eliciting a wide range of signalling properties and cellular functions, encompassing roles in the regulation of cell growth, proliferation, differentiation, programmed death, death, senescence, adhesion, migration, inflammation, angiogenesis and intracellular trafficking. Current efforts are focused on deciphering the mechanisms underlying these varied roles, enabling a greater understanding of sphingolipid metabolism and lipid generation and action (Hannun & Obeid 2008, Merrill 2011, Airola & Hannun 2013; reviewed in Gault *et al*. (2010)).

Recent *in vitro* results have indicated that sphingolipids are implicated in osteoblast and chondrocyte apoptosis and in the regulation of osteoclastogenesis (Takeda *et al*. 1998, MacRae *et al*. 2006; reviewed by Khavandgar & Murshed (2014)). *In vivo*, sphingolipid metabolism plays a critical role in skeletogenesis; mouse models lacking the ceramide-generating neutral sphingomyelinase 2 enzyme (nSMase2/SMPD3 – gene-targeted $Smpd3^{-/-}$ and *fro/fro* mice) display gross skeletal abnormalities, including deformed long bones, short-limb dwarfism, hypomineralisation, delayed dentin mineralisation and enamel formation (Aubin *et al*. 2005, Stoffel *et al*. 2005, Alebrahim *et al*. 2014). Conversely, the overexpression of SMPD3 in osteoblasts only (fro/fro;Col1a1–Smpd3 mice) corrects embryonic bone abnormalities, demonstrating a direct role of SMPD3 in skeletal mineralisation (Khavandgar *et al*. 2011, 2013). However, the mechanisms underlying this role, while remaining unclear, are now becoming a little more evident.

As highlighted, SMPD3 hydrolyses sphingomyelin to phosphocholine (Stoffel *et al*. 2005), which is subsequently hydrolysed into choline and phosphate by the bone-specific phosphatase PHOSPHO1 (Houston *et al*. 2004, Stewart *et al*. 2006, Roberts *et al*. 2007). Complete ablation of *Phospho1* in mice results in a similar phenotype to that of *fro/fro* mice, with $Phospho1^{-/-}$ mice having significant skeletal pathology, spontaneous fractures, bowed long bones, osteomalacia and scoliosis in early life (Huesa *et al*. 2011, Yadav *et al*. 2011, 2014, Rodriguez-Florez *et al*. 2014). These results indicate that PHOSPHO1 and SMPD3 are within the same metabolic pathway required for skeletal mineralisation in the mouse (Khavandgar Z, Oldknow KJ, Murshed M & Farquharson C, unpublished observations).

Interestingly, both *Phospho1*- and *Smpd3*-deficient models exhibit decreased body size, indicating that, in addition to the *de novo* pathway, the sphingomyelinase pathway may have the potential to regulate energy metabolism (Stoffel *et al*. 2005, Oldknow *et al*. 2013). Supporting this notion, results from metabolic studies conducted in our laboratory have highlighted the finding that *Phospho1* ablation confers remarkable protection against obesity and diabetes in mice, independent of serum levels of uncarboxylated and undercarboxylated OC (Oldknow *et al*. 2013). The mechanisms underlying this metabolic protection in both *Phospho1*- and *Smpd3*-deficient models remain unclear; therefore, it is important to determine whether concentrations of either circulating or bone-derived choline/ceramide are decreased in these models. Choline supplementation by others results in hepatic insulin resistance (Wu *et al*. 2013). Moreover, the impairment of *de novo* synthesis of choline via phosphatidylethanolamine *N*-methyltransferase, which catalyses the methylation of phosphatidylethanolamine in the liver, protects mice from diet-induced obesity (Jacobs *et al*. 2010). However, in contradiction to the results of these studies, it has recently been reported that choline can promote liver health by maintaining cholesterol homoeostasis (Al Rajabi *et al*. 2014). Furthermore, *de novo* ceramide accumulation results in an alteration in metabolism (Summers *et al*. 1998, Merrill 2002, Yang *et al*. 2009, Ussher *et al*. 2010). Pharmacological inhibition of dihydroceramide desaturase 1 (DES1), an enzyme involved in the *de novo* pathway of sphingolipid metabolism (responsible for the insertion of a double bond into the sphingosine backbone of prevalent sphingolipids, e.g. conversion of dihydroceramide into ceramide), improves insulin sensitivity (Bikman *et al*. 2012). Such $Des1^{-/-}$ mice have alterations in energy expenditure, and haploinsufficiency of DES1 in the mouse model protects against lipid- and glucocorticoid-induced insulin resistance. (Holland *et al*. 2007, Siddique *et al*. 2013).

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Taken together, these findings strongly support a role of sphingolipids in the endocrine function of bone; however, the importance of ceramide and choline in energy regulation by the skeleton has not yet been fully investigated.

Ectonucleotide pyrophosphatase/phosphodiesterase 1

Ectonucleotide pyrophosphatase/phosphodiesterase 1 (NPP1) is the founding member of the NPP family. These glycoproteins have pleiotropic roles in hydrolysing phosphodiester or pyrophosphate bonds in various substrates, including nucleoside triphosphates, lysophospholipids and choline phosphate esters (Bollen *et al.* 2000, Stefan *et al.* 2005, Zimmermann *et al.* 2012). Specifically, NPP1 forms disulphide-bonded homodimers and is highly expressed in the plasma membrane and mineral-depositing matrix vesicles of osteoblasts (Johnson *et al.* 1999, 2001, Vaingankar *et al.* 2004, Terkeltaub 2006). Thus, NPP1 has been identified as a critical regulator of tissue mineralisation, hydrolysing nucleotides into extracellular inorganic pyrophosphate (PPi), a potent inhibitor of HA crystal formation in mineralisation-competent tissues (Terkeltaub 2001). Mice lacking NPP1 (*Enpp1*$^{-/-}$) have severe mineralisation defects in long bones and calvariae, with pathological perispinal soft tissue and medial arterial mineralisation associated with abnormally low PPi levels (Sali *et al.* 1999, Johnson *et al.* 2003, Anderson *et al.* 2005, Mackenzie *et al.* 2012*a*,*b*). In addition to its recognised roles in mineralisation, increased NPP1 expression has been associated with insulin resistance in both *in vitro* and *in vivo* models by negatively modulating IR signalling. (Maddux *et al.* 1995, Belfiore *et al.* 1996, Costanzo *et al.* 2001, Goldfine *et al.* 2008, Prudente *et al.* 2009, Huesa *et al.* 2014). Additionally, insulin-resistant subjects have been found to have NPP1 overexpression in skeletal muscle, adipose tissue, fibroblasts and lymphocytes (Frittitta *et al.* 1997, 1998, Teno *et al.* 1999, Stentz & Kitabchi 2007, Goldfine *et al.* 2008). Combing the necessity of NPP1 for mineralisation and the known role of NPP1 in insulin resistance led ourselves and our colleagues to investigate whether NPP1 has a functional role in bone as a novel regulator of energy metabolism. Genetic ablation of *Enpp1* resulted in insulin sensitisation and mildly improved glucose homoeostasis. Upon challenge with a chronic HFD, *Enpp1*$^{-/-}$ mice displayed improved insulin tolerance and resistance to obesity. Unlike the *Phospho1*$^{-/-}$ mice, *Enpp1*$^{-/-}$ mice displayed increased levels of undercarboxylated OC and the bone resorption marker CTX, which is indicative of increased insulin signalling in osteoblasts favouring resorption by osteoclasts (Huesa *et al.* 2014). However, the results of *in vitro* studies did not reveal a role for NPP1 as a modulator of insulin signalling, indicating a more complex underlying pathway. Taken together, results from our laboratory indicate a far more complex story underlying the reciprocal regulation of bone and energy metabolism.

Perspective

The concept of the whole-body study of physiology has established the skeleton as a *bona fide* endocrine organ, considerably expanding the classical view of bone towards it being a more complex organ. These provocative results have challenged and fascinated researchers, resulting in an increased number of laboratories working in this field. Further exploration of the endocrine role of the skeleton is necessary in the search for additional candidates for molecules involved in the skeletal control of whole-body energy metabolism. The potential therapeutic implications of these recent findings have not yet been fully exploited. Whether the use of OC is efficacious in the treatment of DM remains to be determined. Indeed, many unanswered questions remain and some have been highlighted previously by others, including the following: does OC regulate insulin secretion over the short/long term? How does the osteoblast or osteocyte sense and use glucose or other fuels? Do bone cells utilise glucose or amino acids? Does bone fracture increase whole-body energy expenditure? Do osteocytes truly have an effect on energy metabolism? (Martin 2007, Fulzele & Clemens 2012). The answers to these challenging questions are unquestionably attainable, and should ultimately result in better diagnosis, clinical management and treatment of patients with metabolic diseases.

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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