REVIEW

Beyond mineralisation: metabolic functions for matrix mineralisation regulators

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Abstract

The physiological mineralisation of skeletal tissues, as well as the pathological mineralisation of soft tissues involves a fine balance between regulators that either promote or inhibit the process. In recent years, several studies have advocated a non-skeletal role for some of these mineralisation regulators in a range of human diseases, including diabetes, cardiovascular disease, obesity and neurodegenerative disease. This is an emerging area of interest and the functional roles and mechanisms of action of these various endocrine factors, phosphatases and phosphodiesterase's in important pathologies are the focus of this review. Mechanistic insight of the pathways through which these acknowledged regulators of skeletal mineralisation act beyond the skeleton has the potential to identify druggable targets for commonly experienced morbidities, notably those related to metabolism and metabolic syndrome.

Key Words

- ▶ endocrine
- ▶ metabolism
- ▶ mineralisation
- ▶ bone

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Introduction

Bone has numerous classical functions including organ protection, calcium and phosphate ion homeostasis, and facilitation of movement. The skeleton is structurally suited to function: strong and flexible enough to bear load without damage, yet light enough to allow locomotion. Bone is composed of organic constituents including collagenous proteins (principally type I collagen) and non-collagenous proteins (including but not limited to osteocalcin, osteonectin, osteopontin (OPN) and bone sialoprotein (BSP)) (Termine 1988, Roach 1994, Shekaran & García 2011, Dillon *et al.* 2019). Bone also contains non-organic poorly crystalline substituted hydroxyapatite (HA) mineral and water (Glimcher & Muir 1984, Clarke 2008, Stock 2015). Within bone, a large population of living cells (the osteocytes) are entombed in the mineralised matrix. Bone's material properties are a function of both the relative proportions of these elementary constituents and

its structure across length scales, conveying remarkable mechanical properties in a range of contexts from the ear ossicles to the load-bearing bones of the lower limb (Fyhrie 2010, Hart *et al.* 2017, Schwarcz *et al.* 2017).

The maintenance of bone quality is critical to facilitate its function (Clarke 2008). To ensure this, there is life-long remodelling of the bone matrix (Kenkre & Bassett 2018) which involves the coordinated function of two orthologous cell types – the osteoblast (which forms bone) and the osteoclast (which resorbs bone). During bone formation, polarised osteoblasts secrete an extracellular matrix (ECM) which contains a variety of collagenous and non-collagenous proteins, which subsequently mineralises over a period of time. The controlled mineralisation of this osteoid is critical in allowing the anatomically specialised functions of bone. The emergence of sophisticated technologies

in cell biology, imaging of genetically altered mouse models and mineral phase chemical characterisation has improved our understanding of the fine control of ECM mineralisation during skeletal development and the resultant pathological ramifications.

Both bone formation and bone resorption are energetically costly. This has led to a hypothesis, whereby energy metabolism and bone formation regulate one another in a feedback loop. Following recent work which led to the discovery of metabolically active osteocalcin, a hormone secreted specifically by the osteoblast to promote insulin sensitivity, further evidence has emerged for a metabolic role for bone (Fig. 1) (Lee *et al.* 2007 Ferron *et al.* 2010, Clemens & Karsenty 2011, Wei & Karsenty 2015). Contemporary research has revealed varied roles for key regulators of ECM mineralisation in a range of physiological and pathophysiological processes. This review will explore these emerging roles.

Classic functions of mineralisation regulators

Before elaborating on the function of mineralisation regulators beyond the skeleton, we will address the

critical role they play in ensuring that bone mineralises physiologically. The mineralisation of bone is dependent upon optimum concentrations of calcium (Ca^{2+}) and inorganic phosphate (P_i), and the precise mechanism(s) by which mineral is embedded within the collagen fibrils of the ECM is tightly regulated. The regulatory substances include magnesium ions, carbonate, proteoglycans, polyphosphates and poly-phosphorylated molecules and the small integrin-binding ligand, N-linked glycoprotein, non-collagenous proteins (including OPN, BSP, dentin matrix protein, dentin sialophosphoprotein and matrix extracellular phosphoglycoprotein) (George *et al.* 1993, Sodek *et al.* 1995, Macdougall *et al.* 1998, Ganss *et al.* 1999, Qin *et al.* 2002, 2004, Nampei *et al.* 2004, Margolis *et al.* 2014). The mechanisms responsible for the initiation of matrix mineralisation are not fully understood. It has been proposed that the pre-formed extracellular collagen I template mediates mineral nucleation (Christoffersen & Landis 1991, Landis & Silver 2009, Nudelman *et al.* 2013). Others have proposed that matrix vesicles (MVs) control localised accumulation of Ca^{2+} and P_i (Anderson, 1969, 1995, Ali *et al.* 1970). MVs are small (100–300 nm diameter) extracellular membrane-bound particles released by osteoblasts and chondrocytes. MVs are

Figure 1

Schematic representation of matrix mineralisation regulators. The hydroxyapatite (HA) crystals consist of P_i and Ca^{2+} . In matrix vesicles (MVs), which are secreted by osteoblasts, P_i accumulates via the actions of PIT1/2 phosphate transporters which shuttle in extracellular P_i (ePi) from TNAP hydrolysis of PP_i . P_i is also generated in MVs by (PHOSPHO1) which hydrolyses phosphomonoesters phosphoethanolamine (PEA) and phosphocholine (PChol). Annexin is responsible for transporting Ca^{2+} into the MV. ENPP1 generates extracellular PP_i (ePP_i) and this PP_i is further hydrolysed by TNAP to yield P_i . ANK transports intracellular PP_i (iPP_i) to the ECM. The iPP_i drives the expression of osteopontin, which inhibits HA formation. RANKL promotes osteoclastogenesis and causes the formation of acidic osteoclast resorption lacunae. The acidic pH drives the formation of metabolically active OCN via de-carboxylation. Metabolically active OCN acts systemically on the adipose tissue skeletal muscle and liver.

considered to provide a locally protected environment within which Ca^{2+} and P_i accumulate in an amorphous state, a process that is regulated by a complex network of pathways (Wuthier & Lipscomb 2011).

Systemic hormonal regulators of ECM mineralisation include 1,25-dihydroxyvitamin D3, fibroblast growth factor 23 (FGF-23) and parathyroid hormone (PTH). These regulators control Ca^{2+} and P_i balance via actions on the intestine and kidney to ensure Ca^{2+} and P_i availability for HA formation in bone (Parfitt 1976, Canalis & Delany 2002, Rowe 2012). Furthermore, the mineralisation process is also mediated by several mineralisation inhibitors. Of notable importance is inorganic pyrophosphate (PP_i) which has long been acknowledged as an inhibitor of skeletal mineralisation and regulator of physiological mineralisation (Fleisch *et al.* 1966). Critically, PP_i inhibits the growth, dissolution and formation of HA crystals (Fleisch & Bisaz 1962a,b, Fleisch *et al.* 1966). Therefore, it is not surprising that many proteins within bone exist to ensure that a P_i/PP_i ratio conducive to matrix mineralisation is maintained. This can be understood as a series of highly regulated pathways, which anastomose such that mineral homeostasis is intricately maintained to avoid pathology (Fig. 1).

Ectonucleotide pyrophosphatase phosphodiesterase-1 (ENPP1; EC 3.6.1.9) inhibits mineralisation by the extracellular generation of PP_i via the hydrolysis of ATP. Encoded by the ankylosis gene (*Ank*), ANK facilitates the transport of intracellular PP_i to the extracellular milieu (Ho *et al.* 2000, Orimo 2010). Deficiency in either ENPP1 or ANK leads to a disruption of the PP_i/P_i ratio, resulting in dysregulated pathological mineralisation in humans (Ferreira *et al.* 2014, Mitton-Fitzgerald *et al.* 2016). Mice which have a mutated *Ank* locus and subsequent non-functional ANK protein demonstrate a phenotype reflective of the *Enpp1*^{-/-} mice, with joint ankylosis and concomitantly decreased PP_i levels (Gurley *et al.* 2006). Conversely, tissue-nonspecific alkaline phosphatase (TNAP; EC 3.1.3.1), which is anchored within the cell membranes of osteoblasts, chondrocytes and MVs, hydrolyses PP_i to remove this inhibitor and to generate P_i and thus promote HA formation (Johnson *et al.* 2000, Hesse *et al.* 2002, Harmey *et al.* 2004, Ciancaglini *et al.* 2010). The importance of TNAP for skeletal mineralisation is demonstrated in conditions where inactivating mutations in the gene encoding TNAP are present. Hypophosphatasia is associated with decreased enzymatic function and increased PP_i levels resulting in rickets, osteomalacia and hypomineralised teeth (Whyte *et al.* 1979, 2015, Anderson *et al.* 1997b, Mornet 2000,

Mumm *et al.* 2001, van den Bos *et al.* 2005, Linglart & Biosse-Duplan, 2016). Global *Akp2* knockout mice present with elevated urinary PP_i levels and recapitulate the bone mineralisation abnormalities noted in patients with hypophosphatasia (Narisawa *et al.* 1997, Fedde *et al.* 1999). TNAP also regulates ectopic calcification (Narisawa *et al.* 1997, Fedde *et al.* 1999, Anderson *et al.* 2004). Increased TNAP activity in the vasculature is associated with medial vascular calcification, vascular stiffness and resultant heart failure (Sheen *et al.* 2015).

Orphan phosphatase 1 (PHOSPHO1; EC 3.1.3.75) is also responsible for generating P_i to facilitate skeletal mineralisation through its highly specific phosphatase activity (Roberts *et al.* 2007, Dillon *et al.* 2019). Present within the lumen of the MV, PHOSPHO1 exhibits high specificity for phosphocholine (PCho) and phosphoethanolamine (PEA), which it cleaves to generate intra-vesicular P_i (Houston *et al.* 1999, Stewart *et al.* 2006, Roberts *et al.* 2007). In addition, the sodium/phosphate co-transporter type III (Pit_1) is hypothesised to be responsible for the transport of P_i into the MV, while the Ca^{2+} transport ATPase (Annexin 5) is responsible for the transport of Ca^{2+} into the MV (Arispe *et al.* 1996, Kirsch *et al.* 1997, Nielsen *et al.* 2001, Yoshiko *et al.* 2007, Golub 2009, Bourguine *et al.* 2011).

Metabolic syndrome

Metabolic syndrome is a global epidemic, with rates increasing alarmingly across both developed and developing countries. Defined by the WHO as a 'pathological condition characterised by abdominal obesity, insulin resistance (IR), hypertension and hyperlipidaemia', the high incidence among the public necessitates action to identify risk factors, causation, intervention and treatment. Metabolic syndrome itself is a cluster of syndromes associated with disrupted metabolic homeostasis (e.g. in chronic caloric excess) (Fig. 2) and can be caused by both environmental and genetic factors (Cornier *et al.* 2008, Hutcheson & Rocic 2012, Khan *et al.* 2015). A seminal study in epigenetics and obesity in twins has revealed that the obesogenic environment is a 'trigger', but not the leading cause of excess weight gain (Bouchard *et al.* 1990). Indeed, a genetic susceptibility to adipose tissue gain must be present for an individual to develop obesity (Wardle *et al.* 2008, Silventoinen *et al.* 2010). Other studies have estimated that the heritability of obesity ranges from 40% to 70% (Rose *et al.* 1998). The incidence of monogenic causes of obesity has been documented

Figure 2

The risk factors and associated syndromes of metabolic syndrome. The risk of developing metabolic syndrome includes non-modifiable and modifiable risk factors. Metabolic syndrome is classically recognised as the concomitant incidence of abdominal obesity, insulin resistance, hypertension and hyperlipidaemia. Metabolic syndrome may include other aetiologies such as cancer, cardiovascular disease and high cholesterol. By its nature, metabolic syndrome is a cluster of various syndromes and so the aetiology is variable between individuals.

over the last 30 years, with over 20 single gene mutations resulting in obesity discovered to date (O'Rahilly 2009). Although the scientific community through the utilisation of contemporary genetic tools, including whole-genome linkage analysis, will undoubtedly identify additional genetic factors contributing to the risk of metabolic syndrome, there is now an emerging body of evidence documenting the ability of bone proteins historically associated with the mineralisation process to impact whole-body energy metabolism. This text will now review these emerging roles in previously unanticipated fields including cardiovascular health and metabolic syndrome.

Ectonucleotide pyrophosphatase/phosphodiesterase-1 and insulin resistance

The enzyme ENPP1 (NPP1 in mice) is recognised as a pathogenic factor for IR (Abate *et al.* 2003), a major feature for patients with type 2 diabetes mellitus (T2DM). The expression levels of ENPP1 are reported to be elevated in muscle and fat of insulin-resistant humans (Maddux *et al.* 1995). Furthermore, overexpression of ENPP1 is reported to negatively affect the downstream action of the insulin receptor in peripheral tissues such as liver, muscle and fat

resulting in a decreased responsiveness to insulin (Frittitta *et al.* 1998, Goldfine *et al.* 2008, Prudente *et al.* 2009).

Various mouse models have been generated to delineate the effect of elevated ENPP1 expression and include animals injected with an adenovirus encoding ENPP1 construct and ENPP1 transgenic mice (Dong *et al.* 2005, Maddux *et al.* 2006, Pan *et al.* 2011). In both models, animals exhibited worsening metabolic phenotypes and IR, while transgenic mice further demonstrated overt diabetic phenotypes (Dong *et al.* 2005, Maddux *et al.* 2006). Conversely, *Enpp1* ablation in mice (*Enpp1*^{-/-}) results in pronounced resistance to both obesity and IR following high-fat diet feeding (Huesa *et al.* 2014). As such, *Enpp1*^{-/-} mice are metabolically protected, adding further credence to the pathogenicity of the NPP1 phenotype (Huesa *et al.* 2014). Specific polymorphisms of ENPP1, including the ENPP1 K121Q mutant, inhibit signalling through the insulin receptor compared to WT mice and present with an increased associated risk of T2DM (Abate *et al.* 2003). The K121Q mutation is observed in the Asian Indian population at a frequency of 33% for at least one copy of K121Q (compared to 27% in the Caucasian population) and is strongly associated with IR (Abate *et al.* 2003).

The continued identification of individuals who are genetically susceptible to IR, the pre-requisite for T2DM,

will undeniably prove a critical tool in aiding intervention strategies. Further studies are required to understand the exact mechanisms of actions underpinning different polymorphisms of ENPP1, population incidence of polymorphisms and overall susceptibility of population groups to metabolic syndrome. As the liver is the primary source of circulating PP_i , it is plausible that the liver has a critical role in ENPP1's effect on IR (Fig. 3), although to date little is known about the precise mechanisms involved (Rafter 1960, Jansen *et al.* 2014, Roberts *et al.* 2019). Further transgenic mouse models will help us uncover the cell-specific contributions of genes that are associated with an increased risk of IR and T2DM.

Progressive ankylosis protein polymorphisms are a risk factor for obesity related traits

ANK (encoded by the *ANKH* gene (*Ank* in mice)) is a multiple-pass transmembrane PP_i channelling protein, which facilitates the movement of PP_i through the plasma membrane from the cytoplasm to the ECM (Ho *et al.* 2000). Having established ENPP1 as a candidate gene for obesity phenotypes, the scientific community have turned their attention towards the 'functional partners' of ENPP1 which include ANK. A seminal study by Malkin and colleagues who investigated the role of *ANKH* in the heritability of anthropomorphic traits demonstrated that *ANKH* is associated with both body weight and BMI (Malkin *et al.* 2006). This initial study of 212 ethnically homogenous nuclear European origin families prompted further investigation into the role of *ANKH* in the

heritability of obesity and adipose tissue metabolism. Genetic analysis of single nucleotide polymorphisms (SNP) revealed that polymorphisms in *ANKH* has associations with BMI, waist-to-hip circumference ratio and EGF receptor and leptin plasma levels, with an overall conclusion that *ANKH* may have a generalised effect on adipose tissue physiology (Korostishevsky *et al.* 2010). These data support the concept that ANK is a critical player in the complex network connecting bone, adipose tissue and P_i metabolism.

PHOSPHO1 is critical for lipid metabolism

While the vast majority of research investigating PHOSPHO1 function has focussed on its role in biomineralisation of cartilage, teeth and bone, there is evidence for physiological roles of PHOSPHO1 as a PCho/PEA phosphatase in other body systems (Dillon *et al.* 2019). During erythropoiesis, the immature erythroblasts terminally differentiate, undergoing a host of phenotypic and transcriptomic adaptations, and PHOSPHO1 was found to be upregulated during this process (Gautier *et al.* 2016). Following this, Huang and colleagues found that PHOSPHO1 was critical for proper erythroblast differentiation via its role in PCho metabolism (Huang *et al.* 2018).

Additional studies have found associations between PHOSPHO1 and disorders of lipid metabolism. Increased methylation at loci within the *PHOSPHO1* gene is associated with T2DM risk (Chambers *et al.* 2015, Dayeh *et al.* 2016); a phenomenon which was also suggested

Figure 3

ENPP1 contributes to insulin resistance and type 2 diabetes mellitus through its actions in the liver. ENPP1 is expressed in the liver and generates PP_i . The overexpression of ENPP1 leads to metabolically detrimental effects. The decreased expression of ENPP1 is metabolically protective. This has been demonstrated through observations in mouse models and in humans.

as a potential biomarker for the disease (Willmer *et al.* 2018). Furthermore, increased methylation of *PHOSPHO1* is related to increased high-density lipoprotein in serum (Dayeh *et al.* 2016, Sayols-Baixeras *et al.* 2016), while a SNP was found to be significantly associated with risk of clinical measures of obesity (Wu *et al.* 2018). Although the specific mechanisms at play remain unknown, much evidence suggests that *PHOSPHO1* is critical for lipid metabolism in health and disease. These studies indicate a promising avenue for future research to explore these functions in a host of physiological systems outside of bone.

Tissue-nonspecific alkaline phosphatase as a potential therapeutic target for inflammation and diabetes

The first published identification of ALP dates back to almost 100 years (Robison & Soames 1924). Continued probing of the non-skeletal functionality of TNAP will undoubtedly reveal previously unexpected roles of this enzyme; indeed, TNAP has been identified to regulate neurogenesis in adults suggesting a critical role for neuronal function (Diaz-Hernandez *et al.* 2015). These findings demonstrate that Alzheimer's patients have an increased hippocampal TNAP activity and associated increased neuronal death. Furthermore, TNAP is accepted as a regulator of purinergic signalling, hydrolysing ATP and ADP to generate AMP (Moss & Walli 1969). The role of purinergic signalling in bone was first identified in 1991, following the observation that extracellular ATP is important for osteoblast function and can increase intracellular calcium (Kumagai *et al.* 1991, Schöfl *et al.* 1992). Furthermore, purinergic signalling is important in the regulation of inflammatory signalling, whereby enzymatic conversion to AMP aids in the induction of anti-inflammatory responses via adenine receptor associations (Zimmermann 2006). As such, TNAP is proposed to protect against inflammation (Lallès 2014, Peters *et al.* 2015).

Given that ENPP1 – another critical regulator of purinergic signalling – exhibits notable roles in both bone and energy metabolism, it is plausible to speculate that TNAP will also play a regulatory role in global energy metabolism. The supportive literature on this, however, remains scarce. One large, multi-ethnic population study in non-diabetic patients with hyperinsulinemia and associated IR reported increased levels of TNAP activity in serum (Cheung *et al.* 2013).

Morphological and biochemical features of obesity have also been associated with polymorphisms in the *TNAP* gene (Korostishevsky *et al.* 2010). *In vitro* investigations reflect these associations, whereby TNAP expression was higher in cultured osteoblasts like cells in the presence of elevated glucose and insulin levels (Cunha *et al.* 2014). Given that the aetiology of diabetes includes retinopathy, the TNAP expression within the retina in animal models of diabetes has also been studied (Kantor *et al.* 2015). Rat models of diabetes demonstrate decreased brain-specific TNAP activity, indicating a neurological role of TNAP. This supports the notion that TNAP is important for brain (neuronal) functionality, and that TNAP plays multi-factorial roles in the body (Zhao *et al.* 2013). Further analysis, utilising cell-specific TNAP knockout rodent models, will likely identify pharmacological targets of this 'jack of all trades' alkaline phosphatase.

Receptor activator of nuclear factor- κ B ligand plays a role in regulating glucose homeostasis

Receptor activator of NF- κ B ligand (RANKL) is responsible for the formation and activation of osteoclasts by binding to its cognate receptor RANK, conversely, osteoprotegerin (OPG) inhibits osteoclastogenesis (Anderson *et al.* 1997a, Lacey *et al.* 1998, Suda *et al.* 1999, Tong *et al.* 2019). The administration of neutralising antibodies against RANKL (Denosumab) results in improved bone strength in postmenopausal osteoporotic patients due to an inhibition of osteoclast formation and activity.

RANK regulates glucose homeostasis. In mice with a liver-specific inhibition of RANK activity there is prevention of diet-induced glucose intolerance (Kiechl *et al.* 2013). On the other hand, the stimulation of NF- κ B pathways in liver cells exposed to RANKL resulted in the increased expression of pro-inflammatory genes – a phenomenon associated with hepatic IR and disturbed glucose homeostasis (Bonnet *et al.* 2019). In addition, transgenic mice with a high expression of RANKL in the soleus muscle present with muscular alterations including decreased glucose uptake (Bonnet *et al.* 2019). The work by Kiechl and colleagues revealed that RANKL may be used as a risk predictor of T2DM (Kiechl *et al.* 2013). They reported that increased expression of RANK likely results in increased activation of the NF- κ B and thus increased systemic and liver-specific inflammatory signalling pathways (Kiechl *et al.* 2013). The molecular mechanisms which govern this action are unclear, but such events are key to the development of hepatic IR and pancreatic

β -cell dysfunction. The increasing evidence of a role for RANKL signalling in the pathogenesis of T2DM may lead to the development of novel pharmacological strategies.

Fibroblast growth factor 23 regulates matrix mineralisation and is associated with important pathologies and mortality

The hormone FGF23 is one of the three endocrine FGF's, which also includes FGF19 and FGF21 (Angelin *et al.* 2012, Richter & Faul 2018). FGF23 is secreted by osteoblasts and osteocytes and plays a critical role in ion mineral homeostasis. The importance of FGF23 for bone health was initially recognised when assessing human genetic and acquired rachitic disease (Liu *et al.* 2008).

FGF23's is a phosphatonin and its classical role is to promote phosphaturia by increasing P_i excretion via the kidney (Shimada *et al.* 2004a,b, 2005, Ben-Dov *et al.* 2007, Olauson *et al.* 2013, Richter & Faul 2018, Vervloet 2019). FGF23 signals through the FGF receptor 1 (FGFR1) together with a scaffolding protein named Klotho, which can be membrane-bound or soluble/circulating (Matsumura *et al.* 1998, Chen *et al.* 2007, Hu *et al.* 2016, Richter & Faul 2018, Vervloet 2019). This action promotes Ras/mitogen-activated protein kinase (MAPK) signalling and subsequent changes in tissue-specific gene expression, which includes the downregulation of PTH and sodium-dependent phosphate transporter (NaPi-2a&c) expression (Shimada *et al.* 2004a,b, Ben-Dov *et al.* 2007, Olauson *et al.* 2013, Richter & Faul 2018).

There is growing knowledge of the role of FGF23 outside the regulation of mineral homeostasis and bone health with signal transduction observed in a variety of cell types including hepatocytes, immune cells (macrophages and neutrophils) and cardiac cells (myocytes and cardiac fibroblasts) (Richter & Faul 2018, Stohr *et al.* 2018, Vervloet 2019). Elevated FGF23 endocrine signalling in these cell types has predominantly been associated with pathology including left ventricular hypertrophy, heart failure, T2DM, increased risk of infection and chronic kidney disease (CKD) (Faul *et al.* 2011, Wojcik *et al.* 2012, Singh *et al.* 2016, Richter & Faul 2018, Stohr *et al.* 2018, Vervloet 2019). In these pathological scenarios, FGF23 signalling through the classical FGFR1 in a Klotho-dependent manner is a minor route. Instead Klotho-independent signalling primarily occurs through FGFR4 inducing a calcineurin/nuclear factor of activated T cells (NFAT) signalling cascade (Grabner *et al.* 2015, Richter & Faul 2018, Vervloet 2019). This pathological signalling is

likely due to increased serum FGF23 and a concomitant decrease of Klotho isoforms. Indeed, this is evident in CKD, where up to a 1000-fold increase in circulating FGF23 has been reported (Isakova *et al.* 2011, Richter & Faul 2018, Vervloet 2019).

While many studies have demonstrated an association of FGF23 with several pathologies, it is still poorly understood whether FGF23 plays a causative role in the progression of non-skeletal disease. Understanding the endocrine/molecular mechanisms through which FGF23 elicits its actions will be critical to direct potential future therapeutics. It has been shown in several *in vitro* studies the damaging effect of Klotho-independent signalling can have on cells, including the promotion of vascular dysfunction, oxidative stress and fibrosis in cardiac cells and induction of inflammatory pathways in hepatocytes (Richter *et al.* 2016, Singh *et al.* 2016, Leifheit-Nestler *et al.* 2018, Richter & Faul 2018, Millar *et al.* 2019). As these *in vitro* studies are unable to mimic the complexity of an organism with multiple tissues involved, it will be critical to know if such FGF23 actions are also observed in humans or animal models. Studies have shown that a reduction in circulating FGF23 levels is associated with better mortality outcomes in humans. However, since FGF23 affects P_i levels, it is unclear whether it is the reduction in FGF23 *per se* that causes these observed improvements (Wetmore *et al.* 2010, Moe *et al.* 2015, Sprague *et al.* 2018, Vervloet 2019).

Conclusion

The regulators of ECM mineralisation are intrinsic for skeletal formation and maintenance, yet their roles extend to crucial functions beyond mineralisation. Indeed, several studies have reported that mineralisation regulators are associated with a range of conditions including the regulation of metabolism, IR, cardiovascular health and T2DM. These findings suggest that the mechanisms through which bone mineralisation regulators exert their effects, throughout various tissues and their systemic effects, are more complicated and varied than first considered.

This review has provided a concise body of evidence to support the roles of mineralisation regulators beyond the classical functions of bone formation. It is evident that skeletal mineralisation regulators exert systemic effects and are involved in a range of physiological development and pathological conditions. It is likely that increased understanding of the role of mineralisation regulators

will allow for the development of novel therapeutics for individuals with bone disease and will glean insights into targets for cardiovascular and metabolic pathologies.

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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