Hormonal regulation of biomineralization

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Abstract | Biomineralization is the process by which organisms produce mineralized tissues. This crucial process makes possible the rigidity and flexibility that the skeleton needs for ambulation and protection of vital organs, and the hardness that teeth require to tear and grind food. The skeleton also serves as a source of mineral in times of short supply, and the intestines absorb and the kidneys reclaim or excrete minerals as needed. This Review focuses on physiological and pathological aspects of the hormonal regulation of biomineralization. We discuss the roles of calcium and inorganic phosphate, dietary intake of minerals and the delicate balance between activators and inhibitors of mineralization. We also highlight the importance of tight regulation of serum concentrations of calcium and phosphate, and the major regulators of biomineralization: parathyroid hormone (PTH), the vitamin D system, vitamin K, fibroblast growth factor 23 (FGF23) and phosphatase enzymes. Finally, we summarize how developmental stresses in the fetus and neonate, and in the mother during pregnancy and lactation, invoke alternative hormonal regulatory pathways to control mineral delivery, skeletal metabolism and biomineralization.

Biomineralization, is the process by which organisms produce mineralized tissues and is critical in animals for enabling the rigidity and flexibility of the skeleton. These skeletal characteristics are required for ambulation, to anchor organs, tendons and muscles, and to protect vital organs. Furthermore, biomineralization is essential to produce hard teeth, which are required to tear and grind food. In contrast to fish, which swim in abundant minerals, humans and other terrestrial organisms also use the skeleton as a source of minerals in times of short supply, and the intestines absorb minerals and the kidneys reclaim or excrete minerals as needed.

This Review focuses on physiological and pathological aspects of the hormonal regulation of biomineralization. We address the interplay between multiple component systems, including the roles of calcium (Ca^{2+}) and inorganic phosphate (PO_4^{3-}, alternatively referred to as 'phosphate'), and dietary intake and renal excretion of minerals, which has implications for bone growth, maintenance and nutritional health. We cover the delicate balance between activators and inhibitors of mineralization, such as phosphate, ionized calcium and pyrophosphate. Furthermore, we summarize the fundamental importance of tight regulation of serum concentrations of calcium and phosphate in multiple biological processes. We also highlight the major regulators of mineral metabolism and biomineralization: parathyroid hormone (PTH), vitamin D, vitamin K, fibroblast growth factor 23 (FGF23) and phosphatase enzymes. Importantly, we discuss the ways in which these key systems represent vulnerabilities for pathological disruption. Such disruption can result in disorders with wide-ranging manifestations including rickets, osteoporosis, osteomalacia, nephrolithiasis, nephrocalcinosis and extraskeletal calcification. We also review how fetal and neonatal development, as well as pregnancy and lactation, provoke alternative regulatory pathways to ensure adequate mineral delivery and adjust skeletal metabolism and biomineralization. Finally, we highlight areas of uncertainty for which we expect future investigations to make important discoveries, in both pathophysiological understanding and advancement of human health.

Calcium and skeletal mineralization

Calcium is an essential element that has numerous biological functions in the body, including skeletal mineralization[1]. It is the major component of bone and provides strength and structure to the skeleton. Bone is a metabolic reservoir that maintains the intracellular and extracellular systemic calcium pools. Specifically, both osteoblasts and osteoclasts use calcium signals as regulators of differentiation and activity.

The most notable metabolic function of bone is mineral storage, particularly of calcium and phosphate, as 99% of calcium in the body is found in the skeleton[1]. By weight, ~70% of bone is mineral and 30% is organic. The composition of the mineral phase is about 95%

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Key points

- Biomineralization is the process by which organisms produce mineralized tissues, such as tooth enamel and bone.
- In land-based vertebrates, the skeleton also serves as a source of mineral in times of short supply, and the intestines absorb and the kidneys reclaim or excrete minerals as needed.
- Tight regulation of serum concentrations of calcium and inorganic phosphate are required for appropriate biomineralization.
- The major regulators of biomineralization are parathyroid hormone, the vitamin D system, vitamin K, fibroblast growth factor 23 and phosphatase enzymes.
- Pregnancy and development cause unique stresses to the fetus, neonate and mother; these conditions invoke alternative hormonal regulatory pathways to control mineral delivery, skeletal metabolism and biomineralization.

hydroxyapatite, a highly organized crystal of calcium and phosphate, and other ions (such as sodium, magnesium, fluoride and strontium). By contrast, the organic phase (also known as osteoid) is made up of 98% collagen fibres, as well as by the most abundant noncollagenous elements of the bone matrix, glycoproteins and proteoglycans[1].

Skeletal mineralization. Skeletal mineralization is a well-regulated biological process, which occurs in two steps. The first rapid step, called primary mineralization, involves the deposition of amorphous calcium–phosphate salts. A second slow step, called secondary mineralization, contributes to ~50% of the final mineral content of bone matrix and is characterized by progressive mineral maturation towards hydroxyapatite[2]. Although the precise mechanism of bone mineralization is not yet fully understood, it has been suggested that the process might be initiated by matrix vesicles, the small extracellular vesicles derived from osteoblasts and chondrocytes[3,4]. Inorganic phosphate is transported into matrix vesicles via sodium-dependent and sodium-independent pathways. The sodium-dependent pathways are probably mediated by sodium-dependent phosphate transporter 1 (PiT1, encoded by *SLC20A1*) and sodium-dependent phosphate transporter 2 (PiT2, encoded by *SLC20A2*)[5]. Calcium and phosphate ions taken up by matrix vesicles form hydroxyapatite crystals, which can propagate on collagen fibrils in the extracellular matrix. Tissue-nonspecific alkaline phosphatase (TNAP) is an enzyme found on the outer surface of matrix vesicles that promotes skeletal mineralization by breaking down pyrophosphate (PPi, which inhibits hydroxyapatite formation), ATP and the protein-bound form of phosphate, to produce inorganic phosphate[3,4]. Although studies carried out in mice have implicated another phosphatase, PHOSPHO1 (REFS[6,7]), its role in humans is not yet fully clear.

In growing children, new bone is formed at two sites. Bone modelling occurs owing to apposition of bone tissue, for example, at the outer ridge of long bones to allow growth in thickness. By contrast, bone remodelling occurs in cavities that form inside the mineralized matrix, where tissue is replaced. A 2020 study demonstrated that mineral crystal shape and composition are not the same between these two sites, which is a consequence of differences in mineralization precursors. This finding could reflect a longer mineral transport distance to sites of new bone formation, compared with remodelling, where mineral can be locally recycled[8].

Bone is classified into two types: cortical bone, which forms a dense layer on the outer surface of most bone and on the shafts of the long bones; and cancellous or trabecular bone, which is spongy in nature and found at the end of long bones and within flat bones and vertebrae. The calcification of these two types is different. Cortical bone has a predominantly structural function and 80–90% of its volume is calcified, whereas trabecular bone serves a metabolic function and is only 15–25% calcified[9].

Regulation of blood concentrations of calcium. The regulation of blood calcium levels is controlled by PTH, which is secreted from the parathyroid glands. Calcium in the extracellular fluid binds to and activates the calcium-sensing receptor (CaSR) on parathyroid cells, leading to an increase in intracellular calcium concentration and a rapid reduction in PTH secretion. By contrast, hypocalcaemia leads to reduced activation of the CaSR and a decrease in intracellular calcium levels that results in increased PTH secretion. PTH acts by increasing renal calcium reabsorption and over a more extended period of hours to days increases osteoclastic bone resorption and FGF23 release from mature osteoblasts and osteocytes (FIG. 1). PTH also stimulates the renal conversion of 25-hydroxyvitamin D (25(OH)D) into 1,25(OH)2D (known as calcitriol), probably over several hours, which in turn results in increased intestinal calcium and phosphate absorption[1].

Calcium intake. Genetic factors have a critical role in the growth and development of peak bone mass; however, an adequate intake of nutrients essential for bone is crucial for the full expression of a given genetic potential and for bone maintenance during adulthood. Among the various nutrients, an adequate supply of calcium and vitamin D is essential for normal bone growth and development in children and adolescents, and their supplementation can slow bone mineral loss in post-menopausal women. An optimal calcium intake is necessary for bone health at all stages of life[10–12]. Dietary requirements for calcium are determined by the processes of bone development

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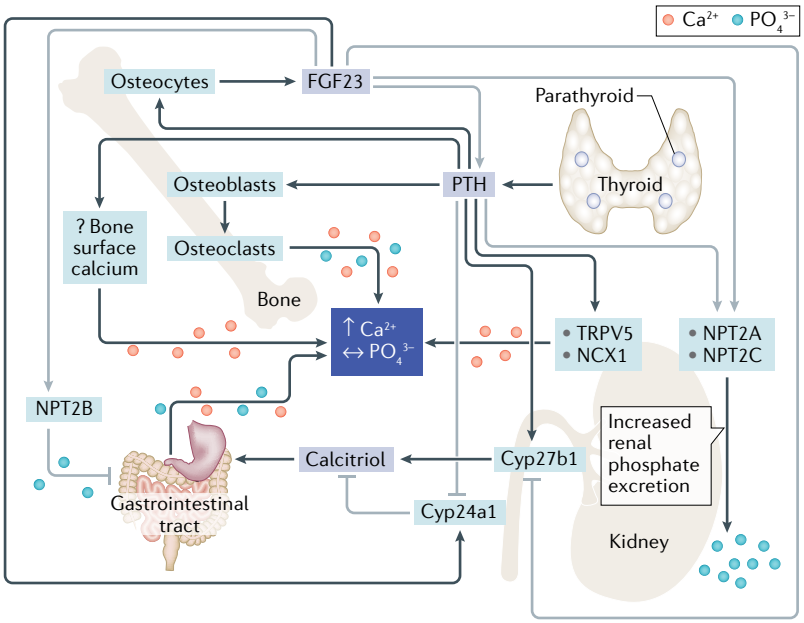

Fig. 1 | **Regulation of calcium and phosphate economy and bone mass.** The secretion of parathyroid hormone (PTH) from the parathyroid glands increases in response to low serum concentrations of calcium (Ca^{2+}). Within bone cells, PTH stimulates osteoblasts to form bone, indirectly stimulates osteoclasts to resorb bone and bring calcium and phosphate (PO_4^{3-}) into the circulation, and acts on osteocytes to stimulate the release of fibroblast growth factor 23 (FGF23). Within kidney tubules, PTH increases reabsorption of calcium and renal excretion of phosphate, and FGF23 also stimulates renal phosphate excretion. FGF23 acts indirectly on the intestines by controlling the level of calcitriol, but might also have direct actions to regulate the expression of the sodium-dependent phosphate transport protein 2B (NPT2B). Within intestines, calcitriol increases intestinal calcium and phosphate absorption. The integration of these regulators includes that PTH increases the release of FGF23 and increases calcitriol levels, whereas FGF23 has opposing effects to inhibit PTH and decrease calcitriol. The central, dark-blue box indicates the circulating or blood concentrations of calcium and phosphate. The question mark indicates an unclear process. Black arrows indicate pathways with net stimulatory actions whereas grey arrows are pathways with net inhibitory action. Flat arrowheads indicate inhibitory actions. Adapted from REF.[172], Springer Nature Limited.

and bone maintenance, which occur at varying levels throughout life, being highest during childhood and adolescence.

Babies that are born prematurely are particularly vulnerable to bone mineralization defects, as their intestines are not capable of absorbing adequate amounts of mineral, which would normally be provided by the placenta. Thanks to advances in neonatal care, many of these infants now survive. The fetus has a higher rate of skeletal growth than a newborn infant, especially during the last trimester. On average, 30 g of calcium[13–22] and 20 g of phosphate[13,15,20] are deposited in the fetal skeleton by term, with 80% of that accretion occurring during the third trimester. This accumulation corresponds to an overall calcium accretion rate of 100–150 mg/kg per day and a phosphate accretion rate of 50–65 mg/kg per day[13,14,16–19,23,24]. For an average-sized fetus, calcium accretion increases from 60 mg per day at week 24 to 300–350 mg of calcium per day between weeks 35 and 40 of gestation[13]. Similarly, phosphate accretion increases from 40 mg per day at week 24 to 200 mg per day over the final 5 weeks of gestation[13]. Bone volume increases very notably with advanced gestational age owing to bone remodelling and increased bone formation; the rate of trabecular thickening is 240 times greater in the fetus than in children, with the activity of osteoblasts increasing exponentially (involving 80% of mineral accretion) during weeks 24–37 of gestation[25–29].

Calcium can be obtained from the diet, with examples of calcium-rich foods including dairy produce and green leafy vegetables, and also through supplementation, which is often recommended together with vitamin D for patients with osteoporosis[10]. Despite the acknowledged importance of calcium in bone health, studies examining the effects of calcium supplementation on bone mineral density (BMD) have shown inconsistent findings[12]. Although some have shown positive effects of calcium supplementation on areal BMD (aBMD), a meta-analysis of calcium supplementation in healthy children showed differing effects depending on pubertal stage and site examined[11]. It has been suggested that calcium supplementation could be more beneficial in those children and adolescents who have low baseline levels of calcium intake, although this hypothesis has not been confirmed. Furthermore, it is uncertain whether any gains in bone mass remain once calcium supplementation ceases, with evidence, particularly in children, suggesting a lack of sustained effect. Later in life, the benefits of calcium supplementation without adjuvant vitamin D supplementation on fracture risk are not well demonstrated in older adults. Furthermore, some concerns exist regarding a possible cardiovascular risk associated with calcium supplementation, although this link remains uncertain and no such risk has been demonstrated with dietary supplementation[10,12]. Recommendations for dietary calcium intake are shown in TABLE 1.

Further studies are required to establish whether calcium supplementation might be more beneficial in those who have lower baseline levels of dietary calcium intake. In addition, clear evidence is needed regarding whether any gains in bone mass remain once calcium supplementation ceases, particularly in children.

Phosphate and pyrophosphate

The restriction of mineralization to the skeleton and teeth and the absence of mineralization in soft tissues (for example, blood vessels, tendons, skin) is essential for proper organ function in many species. About 60 years ago, Herbert Fleisch and Melvin Glimcher, both early pioneers in biomineralization research, found that native collagen, supersaturated calcium solution and phosphate were all that was needed to induce mineralization (that is, hydroxyapatite formation) in vitro. This finding was true even when collagen from soft tissues was used[30,31]. The addition of plasma inhibited this process, but the inhibition was lost upon treatment with alkaline phosphatase. The specific inhibitor was later identified as PPi[31]. Further insights have been provided by modern molecular biology, to the extent that we now have a much more detailed picture of the delicate balance between the activators and inhibitors of mineralization. Mineralization requires multiple factors, including an adequate supply of mineral ions (that is, phosphate and calcium), the regulated removal of inhibitors of mineralization and the presence of fibrillar collagen (FIG. 2).

Table 1 | **IOM 2010 recommendations for daily dietary calcium intake**

Category	Age (years)	Recommended intake (mg/day)
Infancy to adolescence	0–1	200 first 6 months; 260 thereafter
	1–3	700
	4–8	1,000
	9–13	1,300
	14–18	1,300
Women	19–50	1,000
	Post menopause	1,200
Men	19–70	1,000
	≥71	1,200

IOM, Institute of Medicine of the US National Academy of Sciences. Data originally presented in REFS[170,171].

Phosphate: homeostasis and hormonal regulation. Phosphate is kept within a tight concentration range in the serum. Long-term deviations from the normal range can have considerable negative consequences. For example, low levels of serum phosphate lead to osteomalacia (that is, undermineralized bone) in adults and rickets in children. By contrast, when blood concentrations of phosphate and calcium–phosphate product are elevated, extraskeletal calcifications can occur, which lead to organ malfunction and permanent damage to the soft tissues. Increased serum concentrations of phosphate are associated with increased mortality in patients with chronic kidney disease[32,33] and in the general population[34,35].

The identification of phosphate transporters was key to our understanding of the regulation of serum concentrations of phosphate[36]. Dietary phosphate is absorbed in the intestine, mainly through the sodium-dependent phosphate transport protein 2B (NPT2B, encoded by *SLC34A2*) expressed on enterocytes. Once absorbed into the bloodstream, phosphate is freely filtered by the kidney and reabsorbed along the nephrons. In healthy humans, the primary physiological regulation of the levels of serum phosphate occurs at the proximal tubule. The proximal renal tubule-expressed NPT2A (encoded by *SLC34A1*) and NPT2C (encoded by *SLC34A3*) are responsible for regulating phosphate reabsorption and excretion through the kidney, according to the needs of the body[36]. Regulation of intestinal phosphate absorption and the release of phosphate from bone also contribute to phosphate homeostasis, although to a much lesser degree than regulation of renal phosphate reabsorption.

The primary hormonal regulator of serum phosphate levels is the phosphaturic hormone bone-derived FGF23 (REF.[37]). PTH, which is primarily a calciotropic hormone, also regulates phosphate homeostasis. In the kidney, PTH and FGF23 lead to the relatively rapid removal of NPT2A and NPT2C from the brush border membrane (BBM) of the proximal tubule. Endocytosis and lysosomal degradation of these transporters results in renal phosphate wasting and a decrease in blood phosphate levels. The removal of NPT2A from the surface of the BBM by PTH is achieved through phosphorylation of the PDZ domain-containing protein NHERF1 (encoded by *SLC9A3R1*), a scaffold protein that stabilizes NPT2A on the BBM[37]. Humans with mutations in the gene encoding NHERF1 are reported to have renal phosphate wasting, confirming its role in phosphate homeostasis[38]. The acute regulation of NPT2A and NPT2C is based on the translocation of these proteins from the cell surface to endosomes. By contrast, long-term hyperphosphataemia leads to a decrease, or hypophosphataemia leads to an increase, in the transcription and translation of the genes encoding NPT2A and NPT2C[39].

Of note, PTH and FGF23 have opposite effects on vitamin D metabolism. PTH increases the production of calcitriol, which increases the intestinal absorption of calcium (discussed in detail later) and, to a lesser degree, phosphate. By contrast, FGF23 inhibits production of calcitriol. The release of both PTH and FGF23 are stimulated by hyperphosphataemia; FGF23 is also stimulated by iron deficiency, erythropoietin and inflammatory cytokines, and PTH mainly by hypocalcaemia[40–42].

Other phosphate transporters include PiT1 and PiT2, which are ubiquitously expressed; however, their regulation is only partially understood. PiT2 is expressed in the apical membrane of cells in the proximal tubule, where its expression is regulated by dietary phosphate and PTH[43]. A role of PiT2 in skeletal health was demonstrated by low bone mineralization and impaired bone quality in mice with *Slc20a2* knockout[44].

The multi-pass cell surface membrane protein xenotropic and polytropic retrovirus receptor 1 (encoded by *XPR1*) is the only known phosphate exporter in mammals. XPR1 is expressed in all tissues examined and is evolutionarily conserved[45–47]. Of note, loss-of-function mutations in humans are associated with primary familial brain calcifications[48]. Furthermore, *Xpr1* knockout in mice is embryonic lethal[46,49]. Inactivation of *Xpr1* in the kidneys of mice leads to a generalized proximal tubulopathy, impaired renal phosphate reabsorption and hypophosphataemic rickets[45,46]. However, not much is known about the regulation of XPR1. In the past 2 years studies have found that members of the inositol pyrophosphate signalling family regulate XPR1-dependent cellular phosphate efflux[50,51].

Phosphate sensing. The tight regulation of serum levels of phosphate requires, in addition to hormonal regulators and phosphate transporters, a sensing mechanism to complete the feedback loop. For example, how does high phosphate intake in the diet lead to an increase in circulating FGF23 concentrations, and phosphate restriction lead to a decrease[52]? In yeast and bacteria, numerous proteins that form a phosphate-sensing apparatus have been identified and studied in detail[53]. However, phosphate-sensing machinery has not yet been established in animal cells. Three promising molecules that sense phosphate have been identified. Unliganded FGF receptor 1 is activated by high extracellular phosphate in UMR106 rat osteosarcoma cells and is involved in the regulation of FGF23 production in mice[54]; FGF23 in turn leads to a decrease in serum concentrations of phosphate. The lysophosphatidic acid receptor 1 in bone is activated by lysophosphatidic acid, which is converted

from glycerol-3-phosphate produced in the kidneys and other organs. Increased serum phosphate concentrations lead to increased local production of lysophosphatidic acid, which stimulates the production of FGF23 in bone through activation of lysophosphatidic acid receptor 1 (REF.[55]).

A 2019 study proposed that not only calcium but also phosphate mediate their effect on the parathyroid glands through binding to the CaSR[56]. In vitro, phosphate inhibits the CaSR via non-competitive antagonism. Furthermore, in ex vivo PTH secretion measurements using freshly isolated human parathyroid cells, phosphate elicits rapid and reversible increases in PTH secretion. This effect is also seen in isolated parathyroid glands from wild-type mice, but not from mice with a *Casr* knockout[56]. The relevance of these novel results for the physiological regulation of serum concentrations of phosphate will need to be established.

Diseases of high and low serum levels of phosphate. Acquired and inherited human diseases of phosphate homeostasis, as well as genetic manipulations in mice, have confirmed the crucial roles that phosphate transporters and their hormonal regulators have in maintaining normal serum phosphate concentrations[37]. For example, mutations in the genes encoding NPT2A and NPT2C lead to a reduction in renal phosphate reabsorption; this reduction enhances calcitriol production, thereby increasing intestinal calcium absorption, which can result in nephrocalcinosis and kidney stones[57,58]. Moreover, in inherited and acquired forms of hypoparathyroidism, basal ganglia calcifications are common, which are probably caused by hyperphosphataemia[59]. Finally, tumour-induced osteomalacia is an acquired condition of FGF23-producing tumours that leads to renal phosphate wasting, hypophosphataemia and often severe osteomalacia with bone fractures[60].

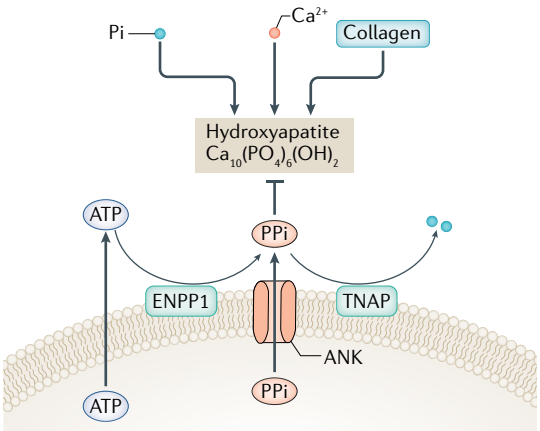

Fig. 2 | **Activators and inhibitors of mineralization.** The figure shows activators (inorganic phosphate (PO_4^{3-}), Ca^{2+} and collagen) and inhibitors (pyrophosphate (PPi)) of mineralization. PPi is derived from nucleotide triphosphates (such as ATP) by pyrophosphatases (for example, ENPP1). Small amounts of PPi are also transported from the intracellular to the extracellular space by the transmembrane pyrophosphate transporter ANK. Tissue non-specific alkaline phosphatase (TNAP) hydrolyses PPi.

Pyrophosphate. PPi is one of the most potent inhibitors of mineralization[61]. Only a tiny fraction of the PPi produced during protein synthesis is transported, through the transmembrane pyrophosphate transporter ANK, to the extracellular space. Most extracellular PPi, however, is derived from ATP that is secreted from cells through vesicular exocytosis (FIG. 2). ATP and other nucleotide triphosphates are hydrolysed by extracellular pyrophosphatases (for example, ENPP1) to ADP and PPi. The key role of the mineralization inhibitor PPi is evident by inactivating mutations of *ENPP1* that lead to a decrease in extracellular PPi, resulting in extraskeletal calcifications[62]. In addition to inhibiting mineralization locally, circulating PPi has been shown to have an important role. For example, *Enpp1*-null mice have very low plasma concentrations of PPi and develop aortic calcifications[63]. When aortas from *Enpp1*-null mice are transplanted into normal mice, the calcifications of the aorta are completely arrested, which shows that systemic levels of PPi are sufficient to prevent further vascular calcifications even when local production of PPi is curtailed. By contrast, normal aortas transplanted into *Enpp1*-null mice calcify despite the presence of normal localized ENPP1 activity, demonstrating an essential role for circulating PPi levels in preventing mineralization[63].

Loss-of-function of the ectoenzyme TNAP, the enzyme that hydrolyses and decreases PPi concentrations, leads to an accumulation of PPi and causes the inherited disorder hypophosphatasia. Severely affected infants can present with life-threatening rickets[64].

Future research. Although enormous progress has been made over the past few decades in the identification of key players in mineralization, work to identify mammalian phosphate and PPi sensors is still in its infancy. An important agenda for future research is the discovery and characterization of a receptor, or sensor, for PPi. Inhibitors of phosphate transporters have been useful tools for in vitro studies, and some have been used for animal studies, for example, the selective NPT2A inhibitor PF06869206 (REF.[65]). Inhibitors of phosphate transporters have potential for clinical use, such as decreasing blood phosphate levels in chronic kidney disease.

Role of vitamin D in biomineralization

Vitamin D is an important regulator of calcium and phosphate homeostasis. Synthesized in the skin after exposure to ultraviolet light, vitamin D (cholecalciferol) then undergoes a first hydroxylation at position 25 in the liver, which generates 25-hydroxyvitamin D (calcifediol). Calcifediol then undergoes a second hydroxylation by 25-hydroxyvitamin D-1α-hydroxylase at position 1 in the kidney, which generates the active metabolite calcitriol[66]. The latter step is stimulated by PTH, insulin-like growth factor 1 and low calcium or phosphate intake, or low extracellular concentrations of calcium or phosphate.

By promoting optimal extracellular calcium and phosphate concentrations, the vitamin D system ensures the mineralization of newly deposited bone and of hypertrophic cartilage matrix[67]. The role of this system in preventing rickets in childhood and osteomalacia

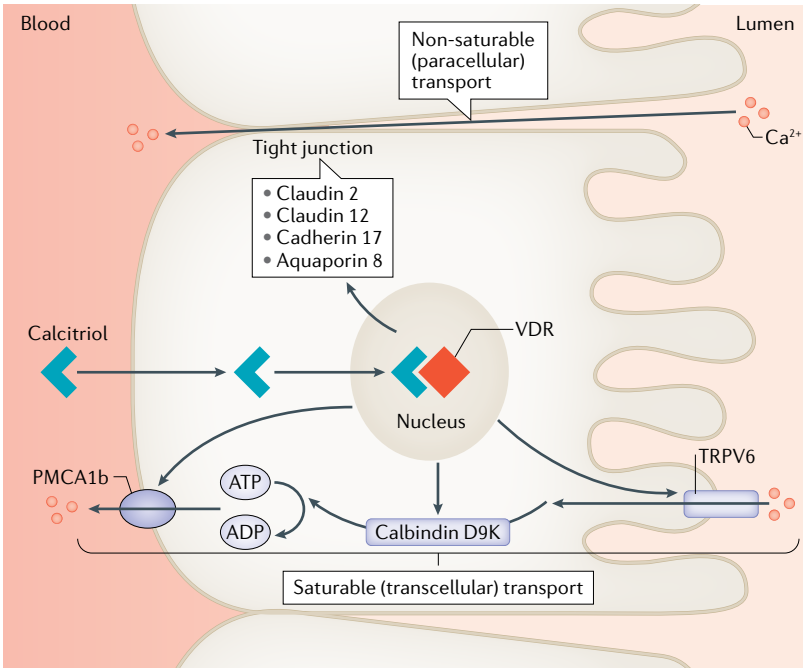

Fig. 3 | **Calcitriol–VDR-stimulated calcium transport in intestinal epithelium.** Calcitriol ($1,25(OD)_2D$) stimulates saturable transcellular and non-saturable paracellular calcium transport. Calcitriol binds to nuclear vitamin D receptor (VDR) and increases gene transcription of apical membrane calcium channel TRPV6, calbindin D9k and basolateral plasma membrane protein PMCA1b. In this three-step process, calcitriol stimulates calcium uptake from the intestinal lumen, the transfer from apical membrane to basolateral membrane and calcium extrusion into the blood through an ATP-mediated process. By increasing gene expression of the tight junction proteins claudin 2 and claudin 12, cadherin 17 and aquaporin 8, the VDR–calcitriol interaction contributes to increase paracellular passive calcium passage. Adapted from REF.[67], Springer Nature Limited.

in adults is well established[68]. The question arises as to whether the effects of vitamin D in the prevention and treatment of rickets and osteomalacia are both direct and/or indirect; that is, through a direct cellular effect and/or through changes in extracellular calcium and phosphate concentrations.

The vitamin D active metabolite calcitriol stimulates intestinal trans-epithelial transport of dietary calcium and phosphate, through both genomic and non-genomic mechanisms[66]. For example, trans-apical membrane transport of calcium through the calcium channel TRPV6 is stimulated by calcitriol, whereas extrusion at the basolateral membrane is carried out by calcium ATPase 1b (FIG. 3). Calcitriol could also regulate paracellular calcium transport through the intercellular space by acting on various tight junction proteins[66]. However, the effects of calcitriol on the intestinal epithelium might be more complex than this classic three-stage regulated process[69]. The large intestine is equipped with a potent vitamin D-dependent calcium transport system, which is poorly used under usual conditions, as the calcium substrate is complexed with anions like oxalate, which prevent calcium from accessing the transport system[70]. In animal models and humans, the gut microbiota can be modified with prebiotics, which are metabolized by the microbiota, resulting in decreased large intestine content pH, increased calcium bioavailability and increased calcium absorption in the large intestine[71]. Of note, calcitriol also stimulates intestinal phosphate absorption[72]. A severe impairment of mineralization is observed in selective intestinal *Vdr* (encoding vitamin D3 receptor) deletion, and this defect can be rescued by selective expression of *Vdr* in the distal intestine[73]. This finding underlines the major role of vitamin D-dependent intestinal calcium and phosphate transport systems in the mineralization process.

A very old observation highlights the important role of extracellular phosphate concentration in the mineralization process: by mobilizing phosphate from soft tissue stores, starvation improved rickets in experimental animals[74]. An adequate phosphate level is necessary for normal growth plate development, owing to the phosphate-dependent apoptosis of hypertrophic chondrocytes[75]. Infusion of calcium and phosphate in vitamin D-deficient rats results in normal mineralization[76]. Furthermore, in a systemic *Vdr*-knockout model, rickets and osteomalacia can be prevented by a diet rich in calcium and phosphate[66,77]. Similarly, a calcium and phosphate rescue diet improves bone and cartilage in *Cyp27b1*-knockout mice that lack the 25-hydroxyvitamin D-1α-hydroxylase[78]. However, the bone disorders of these animals are not totally normalized by providing minerals, suggesting some possible direct effect of calcitriol as well[79].

Calcitriol is a potent stimulator of bone resorption[80] that acts by increasing expression and production of the osteoclast differentiation and activation factor RANKL by osteoblasts[81]. In addition, calcitriol regulates osteoblast differentiation and function by interacting with WNT signalling[82]. Thus, by mobilizing calcium and phosphate from the intestine and from bone, calcitriol increases extracellular calcium and phosphate concentrations to supersaturated levels, allowing the mineralization of hypertrophic cartilage and bone[66,67].

In in vitro studies, calcitriol promotes mineralization by stimulating osteoblast differentiation and accelerating the production of mature matrix vesicles, which are involved in the initiation of the calcification process[83,84]. Calcitriol stimulates the production by osteoblasts of a variety of different noncollagenous matrix proteins, which have a role in either the promotion or inhibition of mineralization. For instance, activin A, osteopontin and osteocalcin are matrix proteins that inhibit mineralization[83]. In a 2020 report of an infant with hypophosphatasia and vitamin D deficiency-induced rickets, serum concentrations of calcium and phosphate were always normal[85]. In this infant, rickets was cured by vitamin D treatment alone. Taken together, the present evidence indicates that optimal extracellular calcium and phosphate concentrations are necessary for hypertrophic cartilage and bone mineralization. However, under certain circumstances, vitamin D could also exert a direct effect on the mineralization process.

Role of vitamin K in biomineralization

There are two main species of vitamin K: phylloquinones (vitamin K1), which are found in green leafy vegetables; and menaquinones (vitamin K2), which are found in fermented foods. Both are cofactors for a post-translational carboxyl moiety that is added on to

Tetrapod transition
The water-to-land transition, involving evolutionary adaptations such as four legs and joints that enabled walking on land.

protein-bound glutamate residues, through a mechanism called γ-carboxylation[86]. The γ-carboxylation of the glutamate residues (that is, carboxyglutamic acid or Gla residues) is necessary for prothrombin activity, by providing a calcium-binding site involved in the coagulation cascade. However, at least 17 other proteins contain vitamin K-dependent Gla residues, among them osteocalcin[86].

Osteocalcin is the most abundant noncollagenous component in the mineralized matrix of bone. The presence of three Gla residues enables post-translational γ-carboxylation at positions 17, 21 and 24. Calcification is inhibited by the fully carboxylated form of osteocalcin[87]. By contrast, the undercarboxylated form of osteocalcin seems to regulate energy metabolism and reproductive function[87]. Studies of osteocalcin-deficient mice models, which display high bone mineral content, show that osteocalcin influences hydroxyapatite crystal growth and structure[86].

The extrahepatic vitamin K-dependent matrix Gla protein, which is synthesized by vascular smooth muscle cells, is a very potent inhibitor of soft tissue and arterial calcification[88]. Overexpression of matrix Gla protein in the growth plate inhibits cartilage calcification and endochondral ossification. The spontaneous precipitation of calcium and phosphate, which are present in supersaturated concentrations in extracellular fluids, is prevented by inhibitors like matrix Gla protein[89,90]. Importantly, depletion of this protein in mouse models is associated with massive arterial calcification[91]. Furthermore, the prevention of matrix Gla protein carboxylation by vitamin K antagonists is also associated with arterial calcification[91] (FIG. 4). Another carboxylated protein, the unique cartilage matrix-associated protein, is found in bone and in calcified cartilage[86].

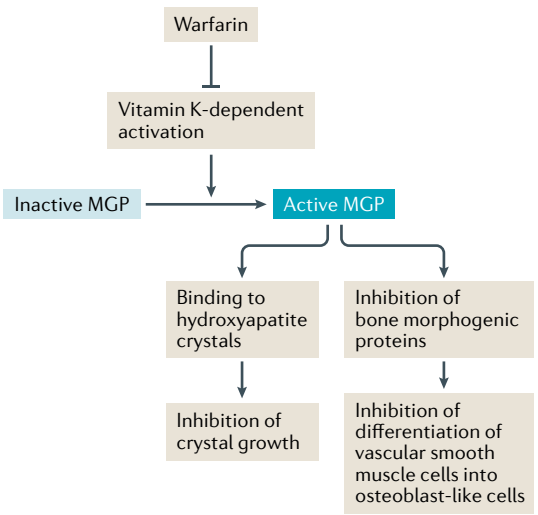

Fig. 4 | **Role of matrix Gla protein in mineralization.** Inactive extrahepatic matrix Gla protein (MGP) is activated through a vitamin K-dependent process. The latter is blocked by warfarin and dicoumarol agents. Active MGP prevents hydroxyapatite crystal growth by binding to hydroxyapatite crystals. It also inhibits vascular smooth muscle cell differentiation into osteoblast-like cells by interacting with bone morphogenetic proteins. Adapted with permission from REF.[92], Wiley.

Thus, vitamin K deficiency might be associated with an increased risk of soft tissue calcification[86].

Coumarins are vitamin K antagonists that inhibit vitamin K recycling and deplete tissue vitamin K stores, which leads to undercarboxylation of vitamin K-dependent proteins[92]. In 2015, vascular calcification was revealed as a detrimental effect of vitamin K antagonists[93]. Compared with vitamin K-independent anticoagulants, warfarin is associated with higher progression of arteriosclerosis and calcification in the coronary artery[94]. However, prevention of vascular calcification by vitamin K supplementation is inconsistently reported[90,95].

Thus, vitamin D deficiency is associated with impaired cartilage and bone mineralization, whereas, vitamin K depletion predisposes to soft tissue and arterial calcifications. In the future, understanding the mechanisms by which vitamin D and vitamin K influence the process of soft tissue calcification could provide important insights into novel ways of preventing atherosclerosis and arterial wall calcification.

PTH: physiology and disorders

Owing to the essential and evolutionarily conserved role of calcium in many of the most fundamental processes in biology, including neurotransmitter release, stimulus–secretion coupling and muscle contraction, the transition of life from aquatic to terrestrial environments required and selected for major adaptations. Such adaptations were needed to maintain extracellular calcium levels within a very tight range compatible with normal physiological demands, when it was no longer possible to simply take up minerals from the surrounding ocean. In the tetrapod transition, bone took on increasing importance for support, resisting gravity, locomotion and also crucially as a storage depot for calcium. New physiological systems were needed to interact with this storage depot and regulate the exchange of calcium with the extracellular fluid to maintain homeostasis, leading to the evolutionary emergence of the parathyroid glands in tetrapods. Interestingly, this emergence seems to have its tissue origins in the gills of fish, which express GCM2 (a parathyroid-specific transcription factor in mice and humans), a form of PTH and extracellular CaSR[96].

The parathyroid glands develop embryologically from endoderm, specifically the third and fourth pharyngeal pouches in humans. Cells destined to form the thymus are found in close proximity to some of the parathyroid primordia, but the distinct cell fates of parathyroid are marked very specifically by expression of GCM2 (REFS[96,97]). An essential role for GCM2 is evidenced by the congenital hypoparathyroid phenotype found in humans and mice that lack GCM2 expression[98–101].

PTH biology and the extracellular calcium-sensing receptor. PTH, an 84-amino acid peptide, is the major and crucial product of the parathyroid glands. The human *PTH* gene is located on the distal short arm of chromosome 11 and is expressed almost exclusively in parathyroid tissue. A precursor peptide preproPTH is produced in parathyroid chief cells that is processed in the Golgi to the mature bioactive PTH(1–84) peptide,

Calcium–PTH setpoint
Refers to the relationship between extracellular calcium concentration and parathyroid hormone (PTH) release. Setpoint is the calcium concentration at which PTH release (or circulating concentration) is mid-way between its maximum and minimum values.

and subsequently collected in storage granules that await secretion.

PTH has a short half-life in the circulation and acts as a classic hormone, with exquisitely modulated release that is designed to regulate the level of extracellular calcium in a rapid, minute-to-minute, time frame. Following an increase in release, PTH acts in a coordinated fashion on target tissues to increase extracellular calcium concentration, and vice versa when PTH release is suppressed. The main targets for PTH action (FIG. 1) are the kidneys, bone and the gut. In the kidneys, PTH increases fractional reabsorption of calcium and induces 25-hydroxyvitamin D-1α-hydroxylase expression, which leads to increased calcitriol production. In bone, PTH increases bone turnover and the accessibility of calcium to the circulation. In the gut, PTH-induced calcitriol increases absorption of dietary calcium. Notably, the circulating concentrations of calcium and phosphate are quite close to levels that would cause their damaging precipitation in soft tissues. Thus, it should not be surprising that systems that evolved to regulate extracellular calcium, including 'calciotropic hormones' like PTH, also have effects on phosphate handling. Indeed, PTH has a notably phosphaturic effect on the kidney, and calcitriol increases levels of FGF23, a potent phosphaturic hormone (FIG. 1).

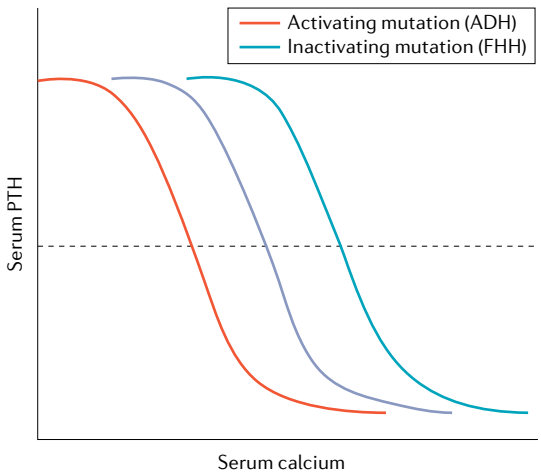

Fig. 5 | **Relationship of serum concentrations of calcium and PTH.** The solid violet curve depicts normal physiology. The steep part of this classic sigmoidal 'setpoint curve' describes the exquisite sensitivity of the parathyroid hormone (PTH) response to small perturbations in and near the normal range for serum concentrations of calcium. The dashed black line is mid-way between maximal and minimal PTH levels, and the setpoint is defined as the calcium concentration corresponding to the point where this line intersects the sigmoidal curve. The teal curve depicts the altered setpoint curve in familial hypocalciuric hypercalcaemia (FHH). Germline disruption of the calcium-sensing or downstream signalling apparatus of the parathyroid glands, most notably via inactivating mutation of the gene encoding calcium-sensing receptor (CaSR) as shown, results in the parathyroid glands being relatively insensitive to suppression of PTH release by serum calcium. This pathophysiology is seen as a shift of the normal setpoint curve (violet), and setpoint, to the right. The red curve depicts an altered setpoint curve in autosomal dominant hypocalcaemia (ADH). Germline activating mutations of *CASR* or *GNA11* (encoding Gα11) result in heightened suppressibility of PTH release by serum concentrations of calcium. This pathophysiology is seen as a shift of the normal setpoint curve, and setpoint, to the left.

The ability of the parathyroid glands to release PTH and precisely maintain homeostasis depends heavily on CaSR, a G protein-coupled receptor. The function and downstream signalling of CaSR determines the calcium–PTH setpoint, measured by plotting the levels of serum calcium against PTH levels. The resulting prototypical sigmoidal curve is shown in FIG. 5, and the steepness of the slope around the central setpoint shows how small perturbations in serum levels of calcium elicit substantial responses in PTH release. The pivotal role for the CaSR is underscored by clinical features and outcomes in individuals with activating or inactivating germline mutations in *CASR*. For example, heterozygous germline inactivation of *CASR* causes an autosomal dominant syndrome called familial hypocalciuric hypercalcaemia type 1 (FHH1)[102].

In patients with FHH1, the insufficiency of functional CaSR molecules causes the parathyroid glands to become somewhat insensitive to extracellular calcium levels; that is, the setpoint curve is shifted to the right and increased calcium is required to suppress PTH (FIG. 5). Affected individuals therefore tend to exhibit mild to moderate hypercalcaemia together with modestly elevated or inappropriately normal PTH levels. In addition, as *CASR* is expressed in the kidney (among other non-parathyroid tissues including brain, gastrointestinal tract and bone), FHH1 is characterized by an increased fractional reabsorption of calcium and relative hypocalciuria[102]. Patients with FHH1 do not have bone abnormalities, even though CaSR is expressed by bone cells, for example, osteoblasts, osteocytes, osteoclasts and chondrocytes. This lack of bone phenotype is because patients are heterozygous for *CASR* mutations and will therefore have one remaining normal *CASR* allele, sufficient to retain adequate function in the bone cell context. However, patients with neonatal severe primary hyperparathyroidism, who have compound heterozygous or homozygous loss-of function *CASR* mutations, and *Casr*-null mice have undermineralized bone and fractures[102]. Conversely, patients with heterozygous mutations that constitutively activate the CaSR, and hence its downstream signalling pathways in the parathyroid glands, exhibit a left-shifted setpoint (FIG. 5) with hypocalcaemia and hypercalciuria, in a syndrome called autosomal dominant hypocalcaemia[102].

Hyperparathyroidism and excessive PTH action. The phenotypes of patients with parathyroid tumours that release excessive amounts of PTH highlight the crucial role of this hormone in calcium homeostasis and in skeletal biology. Furthermore, much has been learned about the genetics of familial syndromes in which patients are strongly predisposed to develop hyperparathyroidism, as well as the somatic mutations that underlie the growth of common sporadic parathyroid neoplasms (TABLE 2).

Primary hyperparathyroidism, a biochemical diagnosis classically defined as hypercalcaemia with excessive

Table 2 | **Major germline and somatic genetic foundations in familial and sporadic parathyroid disorders**

Metabolic abnormality	Disease	Inheritance	Gene (gene product)[a]
Primary hyperparathyroidism	MEN1	Autosomal dominant	*MEN1* (menin), *CDKN1B* (p27)[b], other CDKI genes
	Hereditary hyperparathyroidism and jaw tumours (HPT–JT)	Autosomal dominant	*CDC73* (parafibromin)
	Familial isolated hyperparathyroidism	Autosomal dominant	*MEN1*, *CDC73*, *CASR*, other[c]
	Sporadic parathyroid adenoma	Sporadic	*CCND1*[d], *MEN1*[d]
	Parathyroid carcinoma	Autosomal dominant or sporadic	*CDC73*[e]
	Jansen disease	Autosomal dominant	*PTH1R*
	Familial hypocalciuric hypercalcaemia (FHH1)	Autosomal dominant	*CASR*
	Familial hypocalciuric hypercalcaemia (FHH2)	Autosomal dominant	*GNA11*
	Familial hypocalciuric hypercalcaemia (FHH3)	Autosomal dominant	*AP2S1*
	Neonatal severe hyperparathyroidism (NSHPT)	Autosomal recessive or autosomal dominant	*CASR*
Hypoparathyroidism	Isolated hypoparathyroidism	Autosomal dominant	*PTH*[f], *GCM2*
	Isolated hypoparathyroidism	Autosomal recessive	*PTH*[f], *GCM2*
	Autosomal dominant hypocalcaemia type 1 (ADH1)	Autosomal dominant	*CASR*
	Autosomal dominant hypocalcaemia type 2 (ADH2)	Autosomal dominant	*GNA11*
	Hypoparathyroidism associated with polyglandular autoimmune syndrome (APECED)	Autosomal recessive	*AIRE1*
	Pseudohypoparathyroidism type 1a	Autosomal dominant parentally imprinted	*GNAS* exons 1–3
	Pseudohypoparathyroidism type 1b	Autosomal dominant parentally imprinted	*GNAS* upstream deletion
	DiGeorge syndrome type 1[g]	Autosomal dominant	*TBX1*
	HDR syndrome[g]	Autosomal dominant	*GATA3*
	Blomstrand lethal chondrodysplasia[g]	Autosomal recessive	*PTH1R*

HDR, hypoparathyroidism, deafness and renal dysplasia; MEN1, multiple endocrine neoplasia type 1. [a]Major established pathogenic mutations and variants are germline unless otherwise indicated. [b]MEN1-like phenotype caused by germline mutation of *CDKN1B* has been termed MEN4. [c]Specific germline variants of *GCM2* have been associated with familial isolated hyperparathyroidism, with undetermined penetrance, and the potential role of their detection in clinical management is not yet established. [d]Somatic mutations. [e]Germline and somatic mutations. [f]Mutations of *PTH* are identified only in some families. [g]Hypoparathyroidism associated with complex congenital syndromes.

PTH levels, is caused by a variety of pathological processes affecting one or more parathyroid glands. Of these, sporadic benign adenomatous growth in a single parathyroid gland is by far the most common, accounting for more than 80% of patients[103]. Sporadic parathyroid adenomas can result from driver mutations in a number of oncogenes and tumour suppressor genes, most notably *MEN1* (encoding menin) and *CCND1* (also known as *PRAD1*, which encodes cyclin D1)[104] (TABLE 2). *MEN1* is a classic tumour suppressor gene that is subject to acquired biallelic inactivating defects in 20–30% of parathyroid adenomas. It was initially cloned as the responsible gene in which germline mutations cause familial multiple endocrine neoplasia type 1 (MEN1)[105].

CCND1 is a direct-acting oncogene and established driver in many tumours in addition to parathyroid adenomas, including breast cancer, mantle cell lymphoma, myeloma and squamous cell cancer of the head and neck[104]. A subset of sporadic parathyroid adenomas bear a clonal inversion of chromosome 11, in which the active 5′ regulatory region of *PTH* is rearranged upstream of the coding exons of *CCND1*, leading to cyclin D1 overexpression. Notably, transgenic, parathyroid-targeted overexpression of cyclin D1 in mice results in parathyroid hypercellularity and biochemical hyperparathyroidism[106,107]. The temporal sequence of these findings demonstrates that a primary proliferative drive in the parathyroid glands can secondarily result in PTH–calcium setpoint deregulation, and nicely models the phenotype of human sporadic primary hyperparathyroidism.

Familial syndromes account for up to 10% of patients with primary hyperparathyroidism, and the responsible

Multiple endocrine neoplasia type 1 (MEN1). A disorder characterized by predisposition to primary hyperparathyroidism in association with neuroendocrine tumours of the pancreas, pituitary adenomas and adrenal tumours.

genes are, in large part, well defined (TABLE 2). The distinct syndromes can manifest as hyperparathyroidism with specific clinical characteristics, which bears importantly on management. DNA diagnostic testing is widely available and recommended in specific contexts. For example, the biochemical hyperparathyroidism of FHH is generally benign, so an important outcome of proper diagnosis is to spare the patient unnecessary and futile parathyroid surgery[102]. By contrast, a diagnosis of familial MEN1, given its strong predisposition to multigland parathyroid tumours and ectopic parathyroid cell growth in the thymus, often changes the surgical approach to parathyroidectomy[108]. Hyperparathyroidism–jaw tumour syndrome is caused by germline mutation in *CDC73* (also known as *HRPT2*). Diagnosis of this condition is especially important in that patients (and relatives who carry the mutation) are at increased risk of parathyroid malignancy, which is otherwise exceedingly rare in the general population, and of multigland or recurrent parathyroid tumours[109].

In many instances, these diverse causes of sporadic and familial hyperparathyroidism converge phenotypically, in terms of the consequences of PTH excess on the major target organs, including biomineralization issues in bone. Such consequences can be understood from the normal physiological actions of PTH described earlier. For example, chronically high PTH levels will cause hypercalcaemia in a multi-pronged fashion by pathologically increasing renal calcium reabsorption and by inducing the expression of high levels of 25-hydroxyvitamin D-1α-hydroxylase and thus the production of calcitriol, with the latter acting to heighten dietary calcium absorption. In addition, excessive PTH also causes increased bone turnover and can increase movement of calcium from bone to the circulation, which, over time, will increase the likelihood of osteoporosis[103].

Chronic hyperparathyroidism is characterized by increased serum concentrations of calcium, decreased or normal serum concentrations of phosphorus and inappropriately increased PTH[103]. The increase in serum calcium levels results from the actions of PTH on the skeleton, where it stimulates the release of calcium and phosphorus into the circulation. Furthermore, PTH acts on the proximal renal tubule, stimulating reabsorption of calcium from the glomerular filtrate and inhibiting phosphorus reabsorption, so that phosphorus is lost in the urine[110]. In patients in whom the filtered calcium load exceeds the reabsorption ability of the kidney, urine calcium levels increase, sometimes quite dramatically. These changes in mineral metabolism cause bone loss, predominantly through thinning of cortical bone; by contrast, trabecular bone throughout the skeleton is relatively preserved. These changes result in an increased risk of fracture[111]. Bone loss is particularly pronounced at the distal third of the forearm, as this is a site with predominantly cortical bone; however, bone loss can be seen occasionally at the lumbar spine or hip.

A form of severe bone disease called osteitis fibrosa cystica can occur as a result of pronounced chronic hyperparathyroidism. This condition is characterized by subperiosteal resorption of the distal phalanges, tapering of the distal clavicles, 'salt-and-pepper' appearance of the skull, bone cysts and brown tumours in the long bones[112]. Osteitis fibrosa cystica is much less common now in patients with primary hyperparathyroidism than in the past, because of factors like earlier detection and dietary improvements. If urine concentrations of calcium increase sufficiently, calcium-containing kidney stones can form and cause kidney damage. Patients with classical primary hyperparathyroidism typically develop osteoporosis and kidney stones as a result of these changes in physiology[113]. Curative surgical removal of the parathyroid tumour or tumours reduces the high levels of PTH and typically reverses these changes over several years.

Hypoparathyroidism and inadequate PTH action. Deficiency in PTH results in hypoparathyroidism, which is characterized by hypocalcaemia and hyperphosphataemia, with absent or inappropriately decreased PTH levels, in the absence of impaired renal function. The concentrations of calcitriol and bone turnover markers, including alkaline phosphatase activity, are usually in the low-normal to low range, and the fractional excretion of calcium is increased[114]. Causes of hypoparathyroidism include surgical removal of the parathyroid glands, autoimmune destruction and congenital agenesis, which frequently is associated with a genetic disorder. The genetic forms of hypoparathyroidism can occur as part of syndromic disorders or as a non-syndromic solitary endocrinopathy, called isolated or idiopathic hypoparathyroidism (TABLE 2).

Pseudohypoparathyroidism is a genetic disorder of PTH resistance and three main variants — pseudohypoparathyroidism type 1a (PHP1a), pseudohypoparathyroidism type 1b (PHP1b) and pseudopseudohypoparathyroidism (PPHP) — are recognized on the basis of biochemical and somatic features[59]. PHP1a is characterized by occurrence of hypocalcaemia, hyperphosphataemia and elevated serum concentrations of PTH, with the features of Albright's hereditary osteodystrophy (AHO). In PHP1b, PTH resistance occurs without the somatic features of AHO, whereas in PPHP, the somatic features of AHO occur in the absence of PTH resistance. PHP1a and PPHP are due to parentally imprinted inactivating mutations of *GNAS1*, which encodes Gsα[59] (TABLE 2). The most extreme example of PTH resistance is observed when both alleles encoding the type 1 PTH receptor are mutated, as occurs in Blomstrand lethal chondrodysplasia[115].

Chronic hypoparathyroidism causes diminished bone turnover owing to the complete or relative absence of PTH in the circulation[116,117]. As a result, bone formation and resorption decrease, and bone density tends to increase owing to increased mineral deposition. Patients treated conventionally (with calcium and vitamin D supplementation) show thicker cortices and more plate-like trabecular microarchitecture. Despite the increase in bone density due to these changes in mineral metabolism, it is not yet clear whether fracture risk decreases. PTH-based therapy with recombinant human PTH(1–84) reverses these changes over time in patients with chronic hypoparathyroidism and restores a more normal bone

Albright's hereditary osteodystrophy
(AHO). A genetic syndrome characterized by short stature, obesity, subcutaneous calcification, mental retardation, round face, dental hypoplasia and brachydactyly.

microarchitecture[118]. Long-term therapy with recombinant human PTH(1–84) over 4 years has been shown to increase bone density further at the lumbar spine and femoral neck, and to cause small decreases towards the normal range at the total hip and ultradistal radius[119].

Fetal and post-natal biomineralization

The fetus. The fetal skeleton undergoes very rapid longitudinal growth and mineral accretion, with 80% of its mineral content accreted during the third trimester[120,121]. Calcium and phosphate are maintained at levels ~0.5 mmol/l higher in cord blood than adult normal values to facilitate mineralization; a fetal blood calcium concentration that is reduced to adult values results in low skeletal mineral content[120,121]. The fetus obtains minerals by active placental transport of calcium and phosphate, and can usually achieve adequate levels even when maternal mineral concentrations are low[120,121].

Preceding sections indicated the roles of PTH, calcitriol and FGF23 in adult mineral metabolism. Remarkably, extensive animal studies and supportive human data have shown that these hormones have modest or absent roles during fetal development. These findings reflect that the intestines are a trivial route for mineral delivery in the fetus, absorbing only mineral that is voided into the amniotic fluid and swallowed (FIG. 6). The fetal circulation is characterized by low PTH and calcitriol concentrations, and normal to modestly low concentrations of intact FGF23 (REFS[120,121]). The parathyroid expresses CaSR, which suppresses PTH secretion in response to the high serum concentrations of calcium[120,122]. Absence of the parathyroid glands or of

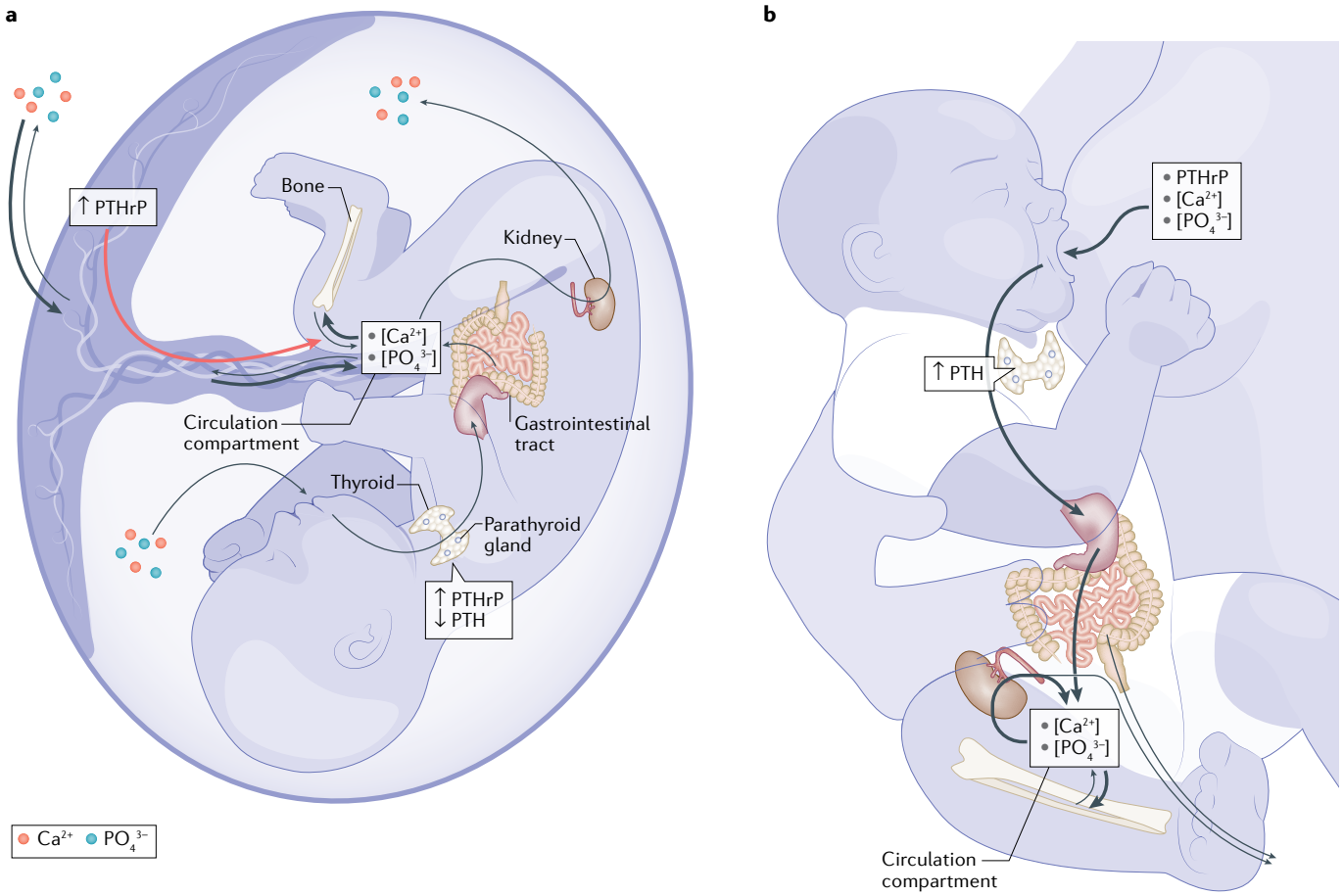

Fig. 6 | **Regulation of mineral homeostasis before and after birth.** **a** | Placental transport of calcium (Ca^{2+}, regulated by parathyroid hormone-related protein (PTHrP)) and phosphate (PO_4^{3-}, regulated by unknown factors) provide the required minerals and contribute to the fetus maintaining higher circulating mineral concentrations than in the adult. Circulating PTHrP is produced by the placenta (red arrow) and possibly the fetal parathyroid glands, whereas parathyroid hormone (PTH) is suppressed by hypercalcaemia and hyperphosphataemia. Thick black arrows depict the dominant flow of mineral across the placenta and into the developing skeleton. Thin arrows depict other pathways, including backflux of mineral from the fetus to the mother, resorption of mineral from the fetal skeleton into the fetal circulation and the minor autophagic circuit that includes excretion of mineral into the amniotic fluid, and reabsorption into the circulation after swallowing that fluid. The fetal kidneys do not appreciably reabsorb calcium. **b** | Immediately after birth, the loss of the placental pump and its hormones, and the onset of breathing, provoke a fall in ionized calcium and a further rise in serum levels of phosphate. These changes programme a developmental awakening of the parathyroid glands to release PTH and in turn to stimulate the production of calcitriol, thereby making the intestines the main route of mineral delivery. The high lactose content of breast milk also facilitates passive calcium absorption before the calcitriol-dependent active absorption pathways are fully developed. The neonatal kidneys undergo developmental changes that lead to progressively increased renal calcium reabsorption and phosphate excretion. Milk contains a high concentration of PTHrP, but its role in the neonate is unclear. Adapted with permission from REF.[173], Elsevier.

PTH in the fetus causes mild hypocalcaemia, hyperphosphataemia and undermineralized skeletons with normal limb lengths[123–126]. Calcitriol levels are kept low by high levels of calcium and phosphate, low PTH concentrations and increased 24-hydroxylated catabolism[120,121]. Studies in animal models and humans show that without vitamin D[127–133], calcitriol[134–136] or the vitamin D receptor[137,138], the fetus maintains normal serum concentrations of calcium, phosphate and PTH, and develops a skeleton with normal lengths and mineral content. In addition, in mice without FGF23 (REF.[139]) or its co-receptor Klotho[140], or with up to 100-fold excess FGF23 (REFS[139,141]), fetal mineral metabolism and skeletal development are normal.

PTH-related protein (PTHrP) is produced by a gene located on chromosome 12, related to but different from the gene encoding PTH on chromosome 11. PTHrP circulates at high levels in fetal blood and has a key role in mineral homeostasis (FIG. 6). The existence of PTHrP was predicted when human cord blood revealed high PTH-like bioactivity but low to undetectable immunoreactive PTH[120]. PTHrP has both paracrine and endocrine functions in the fetus. Deletion of *Pthlh* (encoding PTHrP) in mice leads to a lethal skeletal dysplasia characterized by accelerated endochondral bone formation[142], as well as hypocalcaemia, hyperphosphataemia, reduced placental calcium transport and secondary hyperparathyroidism[124,143]. By acting on PTH1R, PTHrP regulates fetal bone metabolism[144], and by acting on a mid-region receptor (as yet uncloned), PTHrP stimulates placental calcium transport[123,143,145,146]. The placenta is likely the dominant source of circulating PTHrP, although there is some evidence that the fetal parathyroid glands and liver might contribute[123,125,143].

The neonate. After birth, cutting the umbilical cord stops the placental infusion of hormones and minerals, and breathing causes a rise in pH and fall in blood concentrations of ionized calcium. Levels of serum calcium and whole blood ionized calcium typically drop 20–30% over 12–24 hours[147–149] and then rise to neonatal values after several days[120]. Serum concentrations of phosphate increase over 24–48 hours and then slowly decline to neonatal norms[120]. The intestines are now the source of minerals, and the kidneys begin to reabsorb calcium and bone turnover contributes mineral to the circulation (FIG. 6)[120].

The falling concentration of ionized calcium provokes the parathyroid glands to release PTH and upregulate calcitriol synthesis. In addition, FGF23 begins to control renal phosphate excretion and the synthesis and catabolism of calcitriol. Calcium is at first absorbed passively, facilitated by lactose in milk, but this process later becomes active and calcitriol dependent[120]. In mouse models, loss of FGF23 or Klotho, and excess FGF23, begin to show effects after birth[140,150].

In the fetus, low blood concentrations of ionized calcium does not affect survival to term[123,124,151,152], but it could lead to a critically low level being reached after birth, resulting in seizures, tetany or death.

As time passes, neonatal mineral metabolism becomes indistinguishable from adult mineral metabolism. Deficiencies of calciotropic hormones begin to show their expected effects, such as disorders of vitamin D physiology that cause hypocalcaemia, hypophosphataemia and rickets[120,121].

Pregnancy and lactation

Pregnancy and lactation place considerable demands on the mother to provide adequate minerals to herself and the fetus. During the last 6 weeks of pregnancy, 300–350 mg of calcium and 200 mg of phosphate cross the placenta each day, with 5–10% of what is present in the mother's circulation crossing the placenta each hour[19,21]. After birth, the neonate requires about 200 mg of calcium daily from milk during the first 6 months. With the normal 25% fractional absorption of calcium[153], a pregnant woman would need 1,200 mg extra per day during the third trimester, whereas a lactating woman would require 800 mg more per day[131]. Instead, specific reproductive adaptations meet the demands for mineral delivery.

Pregnancy. During pregnancy, the efficiency of intestinal calcium absorption doubles to provide more than the fetus requires, resulting in maternal hypercalciuria[131,154]. Total and free calcitriol levels more than double during pregnancy and contribute to upregulating the intestinal absorption of calcium[131,154]; however, animal models indicate that this upregulation occurs without vitamin D, calcitriol or the vitamin D receptor[155–158]. Moreover, PTH is normally the dominator regulator of calcitriol synthesis, but intact PTH levels are typically low or undetectable in pregnant women[131,154]. This finding underscores that novel factors must be stimulating calcitriol production as well as intestinal calcium absorption.

Histomorphometric assessments suggest that bone turnover in the maternal skeleton increases in the first trimester[159]. By contrast, serial assessments of bone mass by dual-energy X-ray absorptiometry[131,160] and structure by peripheral quantitative CT[161] indicate that the net effect on bone mineral content and structure is small and usually negligible by term. However, women who maintain habitually low intake of calcium throughout pregnancy (for example, <300 mg per day) must resorb minerals from the skeleton to provide for the combined needs of the mother and fetus; some of these women present with marked skeletal demineralization and fractures in late pregnancy or the puerperium[162].

Lactation. During lactation, intestinal calcium absorption is normal and the skeleton provides much of the calcium needed for milk. Randomized interventional studies have demonstrated that this marked skeleton resorption occurs independently of maternal calcium intake[163–165]. Systemic oestradiol levels are low and the breasts produce abundant PTHrP that escapes into the circulation. These two factors upregulate osteoclast-mediated skeletal resorption and osteocytic osteolysis to resorb mineral from bone[131,154]. The dominant effect of PTHrP is exemplified by women with hypoparathyroidism, who normalize mineral homeostasis while breastfeeding, independently of oral calcium supplements[166]. Of note, aBMD of the spine

typically declines 5–10% during 6 months of lactation and by half that amount at the hip and radius. This aBMD loss is accompanied by loss of radial and tibial microarchitecture as seen by high-resolution peripheral quantitative CT, but aBMD and apparent strength are restored within a year after weaning through unknown mechanisms[131,154,167–169].

An evolutionary perspective. Why do biomineralization adaptations differ between pregnancy and lactation? From an evolutionary perspective, reproductive cycles in mammals were originally tied to the seasons. Mating in spring means pregnancy when food sources are abundant, and the mother's physiology adapts to absorb it all. Moreover, having intestinal calcium absorption upregulate independently of vitamin D and calcitriol ensures that women can absorb calcium regardless of their latitude and sunlight exposure. If mating occurs in spring, birth would follow in autumn and winter when food sources are not plentiful, and so making use of the mineral content of the mother's skeleton ensures that the neonate obtains what it needs. Understanding the mechanisms that upregulate intestinal mineral absorption during pregnancy, and the rapid full recovery of the maternal skeleton after weaning, could provide important insights into novel ways of treating malabsorptive disorders and conditions that cause skeletal fragility.

Conclusions

Biomineralization is critically important to ensure that the skeleton and teeth are fully mineralized to enable ambulation and feeding. Inhibition of biomineralization is also important to avoid disabling and even fatal mineralization of soft tissues and organs, such as the electrical conduction system in the heart, joints, blood vessels and the brain. This Review discusses the main regulators of biomineralization and also how disorders of mineralization can be caused by deficiency or excess of these same factors. More than 99% of the calcium content of the human body is contained within the skeleton, which enables bone to be used as a storehouse of the mineral during times of increased need, such as during lactation. The fetus and neonate demonstrate how developmentally programmed changes in the route of mineral delivery (that is, placenta versus intestines) in turn affect the regulation of system mineral and skeletal metabolism. In addition, pregnant and lactating women might demonstrate how mineral and skeletal metabolism have adapted to the seasonally driven availability of nutrients and reproductive cycles. Furthermore, understanding the mechanisms of the hormonal regulation of biomineralization has revealed receptors and intracellular molecules that are potential targets for future drugs for the treatment of mineral and skeletal disorders.

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