**Hormonal regulation of biomineralisation**

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 **[Au: Please note, I’ve moved the ethics declaration and the author contributions to below the main text for formatting reasons.]**

**Abstract [Au: I edited the abstract in order to add a definition for biomineralisation and to add PTH and FGF23 abbreviations, as these are key terms. Increasing the number of key terms in the abstract will increase the visibility of your article in web databases. In addition, minor edits were made to break up the long sentence and for style. Please check for accuracy, OK?]**

Biomineralisation is the process by which organisms produce mineralised tissues. This crucial process enables 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, whereas 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 biomineralisation. We discuss the roles of calcium and inorganic phosphate, dietary intake of minerals and the delicate balance between activators and inhibitors of mineralisation. We also highlight the importance of tight regulation of serum concentrations of calcium and phosphate, and the major regulators of biomineralisation: parathyroid hormone (PTH), the vitamin D system, vitamin K, fibroblast growth factor-23 (FGF23), and phosphatase enzymes. Finally, we summarise how developmental stresses in the fetus and neonate, and in the mother during pregnancy and lactation, invoke alternate hormonal regulatory pathways to control mineral delivery, skeletal metabolism and biomineralisation.

 **[Au: Thank you for writing this fantastic article for Nature Reviews Endocrinology! I have really enjoyed reading and editing your Review, which is fascinating and well written. All our articles are thoroughly and often heavily edited, taking into account clarity, language, scientific correctness, consistency and house style, to ensure they meet our high publication standards. The majority of changes have been made to bring the article in line with house style and to tighten up the narrative flow. These changes are just suggestions and you should feel free to discuss them with me. At places marked [Au:OK?], I’d be grateful if you could check especially carefully that the meaning of what you wrote has not been altered by the editing. I have also asked a few questions where I felt a bit more information or clarity was needed.**

**[H1] Introduction [Au: Hx heading markers have been added to keep track of the heading level. Please leave these as they are for now.]**

Biomineralisation, the process by which organisms produce mineralised tissues,**[Au:OK to define biomineralisation in the first sentence of the introduction?]** is critical in animals**[Au:OK?]** for enabling the rigidity and flexibility of the skeleton, which is required for ambulation, to anchor organs, tendons and muscles,**[Au:OK? I’ve moved this text up from below.]** and to protect vital organs. Furthermore, biomineralisation is essential to produce hard teeth, which are required to tear and grind food **[Au: Edit OK? I split the long sentence into two parts for flow. Edits also made to avoid repetition with the abstract text.]** . In contrast to fishes **[Au: can we change ‘fishes’ to ‘marine organisms’, as this covers whales and other marine mammals that swim in abundant minerals? Or do marine mammals also use the skeleton as a source of mineral in times of scarcity? Please clarify.]** that are swimming in abundant minerals, humans and other terrestrial organisms **[Au: see previous comment.]** also use the skeleton as a source of mineral in times of short supply, whereas 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 biomineralisation. We address the interplay among multiple component systems, including the roles of calcium and inorganic phosphate(alternatively referred to as ‘phosphate’ hereafter), and dietary intake and renal excretion of minerals, which has implications for bone growth, maintenance and nutritional health. **[Au: I’ve split this very long sentence up for flow, OK?]** We cover the delicate balance between activators and inhibitors of mineralization, such as phosphate, ionized calcium, and pyrophosphate. Furthermore, we summarise 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 biomineralisation: parathyroid hormone (PTH), vitamin D **[Au: calcitriol requires further explanation and is defined later, so I’ve just used vitamin D here.]** , vitamin K, fibroblast growth factor 23 (FGF23) **[Au: FGF23 now defined at first use, OK?]** and phosphatase enzymes. Importantly, we discuss the ways in which these key systems represent vulnerabilities for pathologic disruption. Such disruptions 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 alternate regulatory pathways to ensure adequate mineral delivery and adjust skeletal metabolism and biomineralisation. Finally **[Au: Streamlining edit OK?]** , we highlight areas of uncertainty for which we expect future investigations to make important discoveries **[Au:OK?]** , both in pathophysiological understanding and advancement of human health.

**[H1] Calcium and skeletal mineralisation [Au:OK? Please note, H1 heading can use a maximum of 39 characters including the white spaces. Heading edited for length. Please feel free to amend my suggestion, but keep to the character count.]**

Calcium is an essential element that has numerous biological functions in the body, including skeletal mineralisation **[Au: Streamlining edit OK?]** .1 It is the major component of bone **[Au: I’ve deleted this text to avoid repetition, as the following paragraph discusses the composition of bone in detail.]** and provides strength and structure to the skeleton. Bone **[Au: Long sentence split for clarity, OK?]** is a metabolic reservoir that maintains the intracellular and extra-cellular calcium pool **[Au: The systemic calcium pool?]** . Specifically, both bone forming and resorbing cells **[Au: do you mean 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 **[Au: I’ve deleted this sentence to avoid repetition and moved some text up to the introduction; you’ve already mentioned in the introduction that bone is crucial for ambulation and protection of vital organs, OK?]** . By weight, ~70% of bone is mineral and 30% is organic. The composition of the mineral phase is about 95% 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, and by a ground substance **[Au: please clarify what is meant here by ‘ground substance’.]** formed by glycoproteins and proteoglycans **[Au: Does reference 1 support the whole paragraph? If so, please can we cite it again at the end of the paragraph?]**.

***[H2] Skeletal mineralisation.*** **[Au: I suggest breaking up this long section with some subheadings. This will guide non-specialist readers through the narrative and give your article a stronger structure. H2 headings occur in-line and can use up to 80 characters including white spaces.]** Skeletal mineralisation is a well-regulated biological process, which occurs in two steps. The first rapid step, called primary mineralisation, takes place on the mineral front **[Au: please clarify the mineral front. Does the term mean the same thing as the mineral phase, which is used in the paragraph above?]** and involves the deposition of amorphous calcium–phosphate salts. A second slow step, called secondary mineralisation, contributes to ~50% of the final mineral content of bone matrix and is characterized by a progressive mineral maturation towards hydroxyapatite.2 Although the precise mechanism of bone mineralisation 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 **[Au: Edits for flow OK?]** .3,4 Inorganic phosphate is transported into matrix vesicles via sodium dependent and 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*) **[Au:OK? Please note, we default to protein nomenclature from uniprot.org. The Pit-1 and Pit-2 abbreviations are also used for pituitary-specific positive transcription factor 1 and 2, so to avoid confusion, I’ve written out the names in full at first use and added the genes.]** .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 mineralisation by breaking down pyrophosphate (which inhibits hydroxyapatite formation), adenosine triphosphate, and the protein-bound form of phosphate, to produce inorganic phosphate.3,4 **[Au:OK? I assumed TNSALP was the same as TNAP and combined the two sentences into one to reduce repetition.]**. Although studies carried out **[Au: Please note, journal style is to avoid using recent as it loses context rapidly and means different things to different people. Please feel free to add more specific time context here, OK?]** in mice has implicated another phosphatase, PHOSPHO1,6,7 its role in humans is not yet fully clear.

In growing children, new bone is formed at two sites. Bone modelling occurs due to apposition of bone tissue, for example at the outer ridge of long bones to allow growth in thickness. By contrast, bone remodeling occurs in cavities that form inside the mineralized matrix, where tissue is replaced **[Au: I’ve split this long sentence into three parts for flow and clarity. Please check for accuracy, OK?]** . A 2020 study **[Au:OK? ‘recently’ edited out as per our journal style. Please note, it is also not our style to name colleagues in back half reviews, unless the names are of historical significance.]** demonstrated that mineral crystal shape and composition are not the same between these two sites, which is a consequence of differences in mineralisation precursors. This finding could**[Au:OK? Long sentence split for flow.]** reflect a longer mineral transport distance to sites of new bone formation, as compared to remodelling, where mineral can be locally recycled.8

Bone is classified into two types: cortical bone, which forms a dense layer on **[Au:OK?]** 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

***[H2] Regulation of blood concentrations of calcium.*** **[Au: New subheading OK?]** The regulation of blood calcium levels is controlled by PTH. In the parathyroid, **[Au:OK?]** calcium in the extracellular fluid binds and activates the calcium sensing receptor (CaSR) on parathyroid cells, leading to an increase in intracellular calcium concentration **[Au:OK?]** and a reduction in PTH **[Au: PTH secretion? Or production? Please clarify.]** . By contrast, hypocalcemia leads to a decrease in intracellular calcium levels and increased PTH secretion **[Au:OK? For clarity.]** . PTH acts by increasing renal calcium reabsorption **[Au: Is this a more rapid effect? E.g. does it occur over a few minutes or hours rather than hours to days? For completeness, it might be nice to specify this.]** and over a period of hours to days increases osteoclastic bone resorption and FGF23 **[Au: FGF23 now defined at first use, OK?]** release from mature osteoblasts and osteocytes (**Figure 1**). PTH also stimulates the renal conversion of 25-hydroxyvitamin D (25[OH]D) to 1,25(OH)2D (known as calcitriol), probably over several hours, which in turn results in increased intestinal calcium and phosphate absorption.1

***[H2] Calcium intake.*** **[Au: New subheading OK?]** Genetic factors have a critical role in growth and development of peak bone mass **[Au:OK? Is this what you mean here?]** ; however, an adequate intake of nutrients essential for bone is crucial **[Au: Edits OK?]** for the full expression of a given genetic potential and for bone maintenance during adulthood. Among the various nutrients, calcium and vitamin D are essential for normal bone growth and development in children and adolescents, and their supplementation can slow bone mineral loss in postmenopausal women **[Au: Edits OK? Is this what you mean here? Please note, as efficacy refers to the performance of an intervention under ideal and controlled circumstances, I edited it out from this general statement. Please also clarify: are you referring to supplements or normal dietary intake here?]**. 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 and bone maintenance, which occur at varying levels throughout life **[Au: Edits for clarity OK?]** , being highest during childhood and adolescence.

Preterm babies are particularly vulnerable to bone mineralisation defects **[Au:OK?]** as their intestines are not capable of absorbing adequate amounts of mineral, which would normally be provided by the placenta **[Au: Edits for flow, OK?]** . Thanks to advances in neonatal care, many of these infants now survive. The foetus has a higher rate of skeletal growth than newborn infants **[Au:OK?]** , especially during the last trimester. On average, 30 grams of calcium 13-22 and 20 grams of phosphate13,15,20 is deposited in the foetus **[Au: in the skeleton?]** by term, with 80% of that accretion **[Au:OK?]** occurring during the third trimester. This accumulation corresponds to an overall **[Au:OK? Do these ranges correspond to the overall rate?]** calcium accretion rate of 100–150 mg/kg/day and a phosphate accretion rate of 50–65 mg/kg/day **[Au: Edits for flow OK?]** .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 calcium per day between the 35th and 40th weeks of gestation.13 Similarly, phosphate accretion increases from 40 mg per day at week 24 to 200 mg per day over the final five weeks of gestation **[Au:OK?]** .13 Bone volume increases very notably **[Au:OK? Please note, journal style is to avoid using the term significant unless in the context of statistical significance.]** with advanced gestational age due to bone remodelling and increased bone formation; the rate of trabecular thickening is 240 times more rapid in the foetus than in children, with the activity of osteoblasts increasing **[Au:OK?]** exponentially (involving 80% of mineral accretion) during 24 to 37 weeks of gestation.25-29

Calcium can be obtained from the diet **[Au: Is it possible to include some example non-vegan and vegan high calcium foods for context?]** and also through supplementation, and supplementation 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 **[Au: please note, journal style is to avoid ‘may’.]** be more beneficial in those **[Au: please clarify ‘those’: ‘children’ or ‘people’?]** that have lower baseline levels, **[Au: lower baseline levels of intake? Or lower baseline serum levels of calcium?]** although this hypothesis **[Au:OK? Please note, journal style does not permit a hanging ‘this…’.]** 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 **[Au: Please clarify, do you mean in adults, or in older adults?]** , the benefits of calcium supplementation without adjuvant vitamin D supplementation on fracture risk are not well demonstrated. **[Au: Long sentence split for flow, OK?]** 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 intakes are shown in **Table 1**.

Further studies are required to establish whether calcium supplementation might be more beneficial in those that have lower baseline levels **[Au: intake levels, serum levels or skeletal levels, please clarify.]**. In addition, clear evidence is needed regarding whether any gains in bone mass remain once calcium supplementation ceases, particularly in children. **[Au: Edits for style, OK?]**

**[H1] Phosphate and pyrophosphate [Au: Heading edited for length, feel free to amend but keep to the character count.]**

The restriction of mineralisation to the skeleton and teeth and the absence of mineralisation 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 biomineralisation research, found that native collagen, supersaturated calcium **[Au: please clarify supersaturated calcium. Do you mean a supersaturated calcium solution or something else here?]** , and inorganic phosphate were all that was needed to induce mineralisation (that is, hydroxyapatite formation) in vitro. This finding was true **[Au:OK? Long sentence split for flow.]** even when collagen from soft tissues was used.30,31 The addition of plasma **[Au: human or animal plasma?]** inhibited this process, but the inhibition was lost upon treatment with alkaline phosphatase. The specific inhibitor was later identified as pyrophosphate (PPi) **[Au: Please reference this statement. Do refs 30 and 31 still apply here?]** . 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 mineralisation. Mineralisation requires multiple factors, including an adequate supply of mineral ions (that is, phosphate and calcium), the regulated removal of inhibitors of mineralisation and the presence of fibrillar collagen (**Figure 2**).

***[H2] Inorganic phosphate: homeostasis and hormonal regulation.*** Phosphate is kept within a tight concentration range in the serum. Prolonged **[Au: Is it possible to be more specific here than ‘prolonged’? E.g., do you mean deviations of minutes, hours or days?]** 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 **[Au:OK?]** of phosphate and calcium–phosphate product **[Au: would ‘complexes’ work better here than ‘product’? Or ‘products’?]** are elevated, extraskeletal calcifications 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 (CKD),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 (encoded by*SLC34A2*) **[Au: Edited to conform with uniprot nomenclature, OK? Expressed on enterocytes?]** . Once absorbed into the bloodstream, **[Au:OK to add linking words here to improve the flow?]** phosphate is freely filtrated 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 sodium-dependent phosphate transport protein 2A (NPT2A, encoded by *SLC34A1*) and sodium-dependent phosphate transport protein 2C (NPT2C, encoded by *SLC34A3*) **[Au: Protein names edited to conform with uniprot, OK?]** are responsible for regulating phosphate reabsorption and excretion through the kidney, according to the needs of the body **[Au: Please reference this statement. Does ref 36 still apply here?]** . Regulation of phosphate intestinal absorption and the release of phosphate from bone also contribute to phosphate homeostasis, although to a much lesser degree than kidney filtration and reabsorption **[Au:OK? The use of ‘lesser’ requires a comparator for clarity.]** .

 **[Au: New paragraph OK? We prefer to keep paragraphs fairly short, to keep the text accessible.]** The primary hormonal regulators of serum phosphate levels are the phosphaturic hormones **[Au:OK? This term is used below. I’ve just moved it up.]** PTH and bone-derived FGF23.37 In the kidney, **[Au:OK?]** 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*) **[Au:OK?]** , a scaffold protein that stabilizes NPT2A on the BBM **[Au: Please reference this statement. Does reference 37 still apply here?]** . Humans with mutations in *SLC9A3R1* **[Au:OK?]** 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. **[Au: Long sentence split for clarity, OK?]** By contrast, prolonged **[Au: please can you provide more specific time context here?]** hyperphosphatemia leads to a decrease, or hypophosphatemia leads to an increase, in the transcription and translation of *SLC34A1* and *SLC34A3* **[Au:OK to add the gene names here, as they are defined in the previous paragraph?]** **[Au: Please reference this statement. Does ref 37 still apply here?]**.

Of note, **[Au: I’ve moved this term up to the start of the previous paragraph for flow.]** 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) **[Au: I’ve added this in to avoid the need to go into extra details here, OK?]** and, to a lesser degree, phosphate. By contrast, FGF23 inhibits production of calcitriol. The production **[Au:OK? Or secretion?]** of both PTH and FGF23 are stimulated by hyperphosphatemia; PTH is also stimulated by hypocalcemia, and FGF23 by iron deficiency, erythropoietin, and inflammatory cytokines.39-41

Other phosphate transporters include PiT1 and PiT2 **[Au: The genes for these proteins are now defined at first use in the text above, OK?]** , which are ubiquitously expressed; however, their regulation is only partially understood. PiT2 is expressed **[Au: highly expressed? The previous sentence says that both receptors are ubiquitously expressed, so the wording here requires clarification. Does this work: ‘PiT2 expression on the apical membrane of cells in the proximal tubule is regulated by dietary phosphate and PTH’.]** in the apical membrane of cells in the proximal tubule, where its expression is regulated by dietary phosphate and PTH.42 A role of PiT2 in skeletal health was demonstrated by low bone mineralisation and impaired bone quality in mice with *Slc20a1* knockout **[Au:OK?]** .43

The multi-pass cell surface membrane protein xenotropic and polytropic retrovirus receptor 1 (encoded by *XPR1*) **[Au: Edits OK?]** is the only known phosphate exporter in mammals. XPR1 **[Au:OK?]** is expressed in all tissues examined and is evolutionary conserved. 44-46 Of note, loss-of-function mutations in humans **[Au:OK?]** are associated with primary familial brain calcifications.47 Furthermore, Xpr1 **[Au:OK?]** knockout mice are embryonic lethal **[Au: Please reference this statement. Does ref 47 still apply here]** . **[Au: Paragraphs merged.]** Inactivation of XPR1 **[Au: should this be gene or protein nomenclature?]** in the kidneys of mice leads to a generalized proximal tubulopathy, impaired renal phosphate reabsorption and hypophosphatemic rickets.44,45 However, not much is known about the regulation of XPR1. In the last two years studies have found that **[Au:OK? Recently edited out as per our journal style.]** members of the inositol pyrophosphate signaling family regulate XPR1-dependent cellular phosphate efflux.48,49

***[H2] 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 FGF23, **[Au: increase in FGF23 secretion?]** and phosphate restriction lead to a decrease?50 In yeast and bacteria, numerous proteins that form a phosphate-sensing apparatus have been identified and studied in detail.51 However, phosphate-sensing machinery has not yet been established in animal cells. Three promising molecules that sense phosphate have **[Au: Feel free to add specific time context here.]** been identified. FGF receptor 1 is activated by high extracellular phosphate **[Au: on any specific cell type or tissue?]** and is involved in the regulation of FGF23 production **[Au: by bone?]** ;52 FGF23 in turn leads to a decrease in serum concentrations of phosphate. The lysophosphatidic acid **[Au: Abbreviation removed as its only used 3 times.]** receptor 1 **[Au: this abbreviation also only used once, so I edited it out.]** 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 1.53

A 2019 study **[Au:OK?]** showed that not only calcium, but also phosphate mediates its effect on the parathyroid glands through binding to the CaSR.54 In vitro, phosphate inhibits the CaSR via non-competitive antagonism. **[Au: I split the two findings into two sentences for flow, OK?]** Furthermore, in ex vivo PTH secretion measurements, phosphate elicits rapid and reversible increases in PTH secretion from freshly-isolated human parathyroid cells. This effect is also seen in isolated parathyroid glands from wild-type mice, but not from mice with a *Casr* knockout54 **[Au:OK to cite the relevant reference again here?]** . The relevance of these novel results for the physiological regulation of serum concentrations of phosphate will need to be established.

***[H2] 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 critical 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 **[Au:OK?]** lead to a reduction in renal phosphate reabsorption; this reduction **[Au: I split this long sentence up with a semi-colon for flow.]** enhances calcitriol production, thereby increasing intestinal calcium absorption, which can result in nephrocalcinosis and kidney stones.55,56 Moreover, in inherited and acquired forms of hypoparathyroidism, basal ganglia calcifications are common, which are probably caused by **[Au:OK?]** hyperphosphatemia.57 Finally, tumour-induced osteomalacia is an acquired condition of FGF23-producing tumours that leads to renal phosphate wasting, hypophosphatemia, and often severe osteomalacia with bone fractures.58

***[H2] Pyrophosphate.*** Pyrophosphate (PPi) is one of the most potent inhibitors of mineralisation.59 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 (**Figure 2**). ATP, and other nucleotide triphosphates, are hydrolyzed by extracellular pyrophosphatases (for example, ENPP1) to ADP and PPi. The key role of the mineralization inhibitor PPi **[Au:OK?]** is evident by inactivating mutations of *ENPP1* ***[Au:OK?]*** that lead to a decrease in extracellular PPi, **[Au:OK?]** resulting in extraskeletal calcifications **[Au: in an animal model, or is this an observation from human genetic disease?]** .60 In addition to inhibiting mineralisation locally, circulating PPi has been shown to have an important role. For example, *Enpp1*-null mice **[Au:OK?]** have very low plasma concentrations of PPi and develop aortic calcifications61 **[Au:OK to cite the relevant reference again here?]** . When aortas from *Enpp1*-null mice **[Au:OK?]** 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 mineralisation.61

Loss-of-function of the ectoenzyme TNAP, the enzyme that hydrolyzes 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.62

***[H2] Future Research.***Although enormous progress has been made over the last few decades in the identification of key players in mineralisation, work to identify mammalian phosphate and pyrophosphate 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 PF06869206.63 Inhibitors of phosphate transporters have potential for clinical use, such as lowering phosphate **[Au: blood phosphate levels?]** in chronic kidney disease.

**[H1] Role of Vitamin D in biomineralisation**

Vitamin D is an important regulator of calcium and phosphate homeostasis. Synthesized in the skin after exposure to ultraviolet light **[Au:OK? Is this what you mean here?]** , 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-1 alpha hydroxylase **[Au:OK? This enzyme is mentioned later without mentioning its function, so I think we should introduce it here.]** at position 1 in the kidney, which generates the active metabolite calcitriol.64 The latter step is stimulated by PTH, insulin-like growth factor 1 **[Au: term expanded as the abbreviation is only used once.]** and low calcium or phosphate intakes, or low extracellular concentrations of calcium or phosphate **[Au: Edits to paragraph OK? For style and clarity, please check for accuracy.]** .

By promoting optimal extra-cellular calcium and phosphate concentrations, the vitamin D system ensures the mineralisation of newly deposited bone and of hypertrophic cartilage matrix.65 The role of this system in preventing rickets in childhood and osteomalacia in adults is well established.66 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 **[Au: Edits OK?]** .

 **[Au: New paragraph, OK? The previous paragraph was on the long side and required a paragraph break.]** The vitamin D active metabolite calcitriol stimulates intestinal trans-epithelial transport of dietary calcium and phosphate, through both genomic and non-genomic mechanisms.64 For example, trans-apical membrane transport of calcium through the calcium channel TRPV6 **[Au:OK?]** is stimulated by calcitriol, whereas extrusion at the basolateral membrane is carried out by calcium ATPase 1b **[Au: is this the correct protein: plasma membrane calcium-transporting ATPase 1 (**[**https://www.uniprot.org/uniprot/P20020**](https://www.uniprot.org/uniprot/P20020)**)?]** (**Figure 3**). Calcitriol might **[Au:OK? or ‘can’ or ‘could’?]** also regulate paracellular calcium transport through the intercellular space **[Au:OK?]** by acting on various tight junction proteins.64 However, the effects of calcitriol on the intestinal epithelium might be more complex than the classic three-stage regulated process **[Au: Do the yellow highlighted sentences refer to the classic three-stage regulated process? If so, we can edit the text to ‘this classic three stage-regulated process’. If not, please clarify what the classic three-stage regulated process is.]** .67 The large intestine is equipped with a potent vitamin D-dependent calcium transport system, which is usually **[Au: please clarify when this system is poorly used and/or under what conditions that calcium is complexed with anions: e.g. under homeostatic conditions?]** poorly used, since the calcium substrate is complexed with anions like oxalate, which prevent calcium from accessing the transport system.68 The gut microbiota can be modified with prebiotics **[Au: Please clarify: in humans or an animal model?]** , which are metabolised by the microbiota resulting in decreased large intestine content pH, increased calcium bioavailability **[Au: do you mean that the calcium becomes complexed with anions and is therefore available to the vitamin D-dependent calcium transport system described in the previous sentence?]** and increased calcium absorption in the large intestine **[Au: Edits OK? For clarity.]** .69 Of note, calcitriol also stimulates intestinal phosphate absorption.70 A severe impairment of mineralisation is observed in selective intestinal *VDR* **[Au: please define VDR. Vitamin D3 receptor?]** deletion **[Au: in a mouse model? If so, we should change the gene notation to *Vdr*]** , and this defect can be rescued **[Au: Edit OK?]** by selective expression of *VDR* in the distal intestine.71 This finding underlines the major role of vitamin D-dependent intestinal calcium and phosphate transport systems in the mineralisation process.

A very old observation highlights the important role of extracellular phosphate concentration in the mineralisation process: by mobilizing phosphate from soft tissue stores, starvation improved rickets in experimental animals.72 An adequate phosphate level is necessary for normal growth plate development, owing to the phosphate-dependent apoptosis of hypertrophic chondrocytes.73 Infusion of calcium and phosphate in vitamin D-deficient rats results in normal mineralisation.74 Furthermore, in a systemic VDR knock-out model **[Au: mouse model?]** , rickets and osteomalacia can be prevented by a diet rich in calcium and phosphate.64,75 Similarly, a calcium and phosphate rescue diet improves bone and cartilage in *Cyp27b1* knock out mice that lack the 25-hydroxyvitamin D-1 alpha hydroxylase **[Au: Uniprot name used, OK?]** .76 However, the bone disorders of these animals are not totally normalized by providing minerals, suggesting some possible direct effect of calcitriol as well.77

Calcitriol is a potent stimulator of bone resorption78 that acts **[Au:OK?]** by increasing expression and production of the osteoclast differentiation and activation factor RANKL **[Au:OK to add functional information for RANKL?]** by osteoblasts.79 In addition, calcitriol regulates osteoblast differentiation and function by interacting with WNT signalling.80 Thus, by mobilizing calcium and phosphate from the intestine and from bone, calcitriol increases extracellular calcium and phosphate concentrations to supersaturated levels, allowing the mineralisation of hypertrophic cartilage and bone.64,65

In *in vitro* studies, calcitriol promotes mineralisation by enhancing **[Au: inducing? Or stimulating?]** osteoblast differentiation and accelerating the production of mature matrix vesicles, which are involved in the initiation of the calcification process.81,82 Calcitriol stimulates the production of a variety of different non-collagenous matrix proteins **[Au: by osteoblasts? Please clarify]** , which have a role in either the promotion or inhibition of mineralisation. For instance, activin A, osteopontin or osteocalcin are matrix proteins that inhibit **[Au:OK?]** mineralisation.81 In a 2020 **[Au:OK?]** report of an infant with hypophosphatasia and vitamin D deficiency-induced rickets, serum concentrations of calcium and phosphate were always normal.83 In this infant, rickets **[Au:OK?]** 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 mineralisation. However, under certain circumstances, vitamin D could also exert a direct effect on the mineralisation process.

**[H1] Role of vitamin K in biomineralisation**

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 **[Au: Streamlining edits OK?]** . Both are cofactors for a post-translational carboxyl moiety that is added on to protein bound glutamate residues, through a mechanism called gamma-glutamylcarboxylation.84 The gamma-carboxylation **[Au: We should keep terminology consistent. Should we use gamma-carboxylation or gamma-glutamylcarboxylation here and elsewhere?]** of the glutamate residues (that is, carboxyglutamic acid **[Au:OK to add full name as well as three letter code?]** 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.84

Osteocalcin is the most abundant non-collagenous component in the mineralized matrix of bone. The presence of three Gla-residues enables post-translational gamma-carboxylation at positions 17, 21 and 24. Calcification is inhibited by the fully carboxylated form of osteocalcin.85 By contrast, the undercarboxylated form of osteocalcin seems to regulate energy metabolism and reproductive function **[Au: Please reference this statement. Does ref 85 still apply here?]** . Studies of osteocalcin-deficient mice models, which display high bone mineral content, show that osteocalcin influences hydroxyapatite crystal growth and structure.84

 **[Au: New paragraph OK?]** The extrahepatic vitamin K-dependent protein matrix gla protein (encoded by *MGP*) **[Au:OK?]** , which is synthesized by vascular smooth muscle cells, is a very potent inhibitor of soft tissue and arterial calcification.86 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.87,88 Importantly, depletion of this protein **[Au: in mouse models]** is associated with massive arterial calcification.89 Furthermore, the prevention of matrix gla protein carboxylation by vitamin K antagonists is also associated with arterial calcification **[Au: I split the two findings into two sentences, OK? Does the second finding on VKAs relate to an animal model or humans? Please reference the second statement, does ref 89 still apply?]** (**Figure 4**). Another carboxylated protein, the unique cartilage matrix-associated protein **[Au:OK to use uniprot name here?]** , is found in bone and in calcified cartilage.84 Thus, vitamin K deficiency might **[Au:OK?]** be associated with an increased **[Au:OK? ‘higher’ requires a comparator.]** risk of soft tissue calcification.84

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.90 In 2015, **[Au:OK?]** vascular calcification was revealed as a detrimental effect of vitamin K antagonists.91 Compared with vitamin K-independent anticoagulants, warfarin is associated with higher progression of arteriosclerotic **[Au: I feel like a word is missing here. Arteriosclerotic plaques? Or should this say arteriosclerosis?]** and calcification in the coronary artery.92 However, prevention of vascular calcification by vitamin K supplementation is inconsistently reported.88,93

 **[Au: New paragraph for the summary statements, OK?]** Thus, vitamin D deficiency is associated with impaired cartilage and bone mineralisation; 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.

**[H1] PTH: physiology and disorders [Au: We can use abbreviations in headings if it brings them in-line with the character count.]**

Due to the essential and evolutionarily conserved role for calcium in many of the most fundamental processes in biology **[Au: Edits for style, OK?]** , 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 **[Au: Edits OK?]** . In the tetrapod transition **[G] [Au: I have highlighted suggestions for glossary terms throughout your manuscript. Please provide succinct, one-sentence definitions for these specialist terms.]** , bone took on increasing importance for support, resisting gravity, locomotion and also crucially as a storage depot for calcium. New physiological systems **[Au:OK?]** 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 parathyroid hormone and the extracellular calcium receptor **[Au: Is this the same as CaSR?]**.94

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 the some of the parathyroid primordia, but the distinct cell fates of parathyroid are marked very specifically by expression of GCM2 **[Au: edited for brevity here as the paragraph above mentions that GCM2 is a transcription factor.]** .94,95 An essential role for GCM2 is evidenced by the congenital hypoparathyroid phenotype found in humans and mice that lack GCM2 expression.96-99

***[H2] PTH biology and the extracellular calcium-sensing receptor.*** PTH **[Au: Abbreviation now defined at first use.]** , an 84-amino acid peptide, is the major and crucial product of the parathyroid glands. The human *PTH* gene, located on the distal short arm of chromosome 11, is expressed almost exclusively in parathyroid tissue. **[Au: Is this what you mean here?]** A precursor peptide preproPTH is produced **[Au: Long sentence split into two parts for flow, OK?]** , which is processed in the Golgi to the mature bioactive PTH 1–84 peptide, and subsequently collected in storage granules **[Au: please clarify the cell type that contains these storage granules.]**.

 **[Au: New paragraph OK?]** 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, and vice versa when PTH release is suppressed. The main targets for PTH action (**Figure 1**) are the kidneys, bone and the gut. **[Au:OK? Edits to break up the long sentence for flow.]** In the kidneys, PTH increases fractional reabsorption of calcium and induces 25-hydroxyvitamin D-1 alpha hydroxylase expression, **[Au:OK? Or activation?]** which leads to increased calcitriol production. **[Au:OK?]** 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 **[Au: Edited for flow, OK?]**. 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 (**Figure 1**).

The ability of the parathyroid glands to release PTH and precisely maintain homeostasis depends heavily on the CaSR, a G protein-coupled receptor whose function and downstream signalling determines the calcium–PTH setpoint **[G]** , measured by plotting the relationship of serum calcium versus PTH levels. The resulting prototypical sigmoidal curve is shown in **Figure 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 hypercalcemia type 1 (FHH1).100

 **[Au: New paragraph OK?]** In patients with FHH1, the insufficiency of functional CaSR molecules causes **[Au: relative requires a comparator, so I’ve edited it out. I’ve replaced it with ‘mildly insensitive’. You could also have ‘fairly insensitive’ or ‘somewhat insensitive’.]** the parathyroid glands to become mildly insensitive to increased extracellular calcium levels; that is, the setpoint curve is shifted to the right and increased calcium is required to suppress PTH **[Au: Edits OK? For style and clarity]** (**Figure 5**). Affected individuals therefore tend to exhibit mild to moderate hypercalcemia together with modestly elevated or inappropriately normal PTH levels. In addition, as *CASR* is expressed in the kidney (among other non-parathyroid tissue types including brain, gastrointestinal tract, and bone), FHH1 is characterized by an increased fractional reabsorption of calcium and **[Au: relative edited out as it requires a comparator. Would ‘mild’ work here?]** hypocalciuria.100 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 **[Au:OK? Long sentence split into two for flow.]** is because patients are heterozygous for *CASR* mutations and will therefore have one remaining normal *CASR* allele. 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 **[Au: Please reference this statement. Does ref 100 still apply here?]**. 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 (**Figure 5**) with hypocalcemia and hypercalciuria, in a syndrome called autosomal dominant hypocalcemia **[Au: this abbreviation is only used once in the main text, so I’ve edited it out.]** .100

***[H2] 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**).

 **[Au: New paragraph, OK?]** Primary hyperparathyroidism, a biochemical diagnosis classically defined as hypercalcemia with excessive PTH levels, is caused by a variety of pathological processes affecting one or more parathyroid glands. **[Au: Long sentence split for flow, OK?]** Of these, sporadic benign adenomatous growth in a single parathyroid gland is by far the most common, accounting for more than 80% of patients **[Au: Please reference this statement.]**. Sporadic parathyroid adenomas can result from driver mutations in a number of oncogenes and tumour suppressor genes, most notably *MEN1* (encoding menin) and *CCND1* (otherwise known as *PRAD1*, which encodes cyclin D1 **[Au: Edited for style, OK?]** ) (**Table 2**).101 *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 syndrome **[G]** **[Au: Deleted text has been moved into the glossary term, to streamline the narrative here.]** .102

 **[Au: New paragraph, OK?]** *CCND1* **[Au:OK? As this sentence refers to the gene rather than the protein.]** 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.101 A subset of sporadic parathyroid adenomas bear a clonal inversion of chromosome 11, in which the active 5’ regulatory region of the *PTH* gene 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.103,104 The temporal sequence of these findings **[Au:OK? Long sentence split for flow.]** 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 genes are well defined in large part (**Table 2**). The distinct syndromes can manifest hyperparathyroidism with distinct **[Au:OK? Or specific?]** clinical characteristics, which bears importantly on management. **[Au: Long sentence split for flow, OK?]** 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 **[Au: Please reference this statement.]**. By contrast, a diagnosis of familial multiple endocrine neoplasia type 1 syndrome **[Au:OK?]** , given its strong predisposition to multigland parathyroid tumours and ectopic parathyroid cell growth in the thymus, often changes the approach to parathyroid surgery **[Au: Please reference this statement. For clarity here, can we edit the text to ‘warrants serious consideration of parathyroid surgery’ or something similar? Is surgery usually carried out for patients with MEN1 syndrome?]**. Diagnosis of the hyperparathyroidism-jaw tumour syndrome **[Au: I’ve edited out this abbreviation as it is only used once in the main text.]** , caused by germline mutation in the *CDC73* (also known as *HRPT2*) **[Au:OK?]** gene, is especially important **[Au: please clarify why this diagnosis is important. Are patients normally advised to undergo surgery to prevent future malignancy, or just closely followed up for signs of malignancy?]** in that patients are at increased risk for parathyroid malignancy, which is otherwise exceedingly rare in the general population **[Au: Please reference this statement.]**.

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 biomineralisation issues in bone **[Au: Edits for flow OK?]**. Such consequences can be understood from the normal physiological actions of PTH described earlier. For example, chronically high PTH levels will cause hypercalcemia in a multi-pronged fashion by pathologically increasing renal calcium reabsorption, inducing **[Au: inducing the expression?]** high levels of 25-hydroxyvitamin D-1 alpha hydroxylase **[Au:OK?]** and thus the production of calcitriol **[Au:OK?]** , with the latter acting to heighten dietary calcium absorption. **[Au: Long sentence split for flow, OK?]** In addition, excessive PTH also causes increased bone turnover, might increase **[Au:OK? Is this what you mean by ‘chances for increased’?]** movement of calcium from bone to the circulation and, over time, increases the likelihood of osteoporosis **[Au: Please reference these statements.]**.

 **[Au: New paragraph, OK?]** Chronic hyperparathyroidism is characterized by increased serum concentrations of calcium, decreased or normal serum concentrations of phosphorus, and inappropriately increased PTH.105 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. **[Au: Long sentence split for clarity, OK?]** 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.106 In patients in whom the filtered calcium load exceeds the reabsorption ability of the kidney, urine calcium levels increase, sometimes quite dramatically **[Au: Edits for style, OK?]**. These changes in mineral metabolism cause bone loss, predominantly through thinning of cortical bone; by contrast, trabecular bone throughout the skeleton is relatively preserved. **[Au: Edits OK? Is this what you mean here? Relative required a comparator.]** These changes result in an increased risk of fracture **[Au: Edits OK?]**.107 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 **[Au:OK? Long sentence split for flow.]** be seen occasionally at the lumbar spine or hip.

 **[Au: New paragraph, OK?]** Severe bone disease can occur due to chronic hyperparathyroidism, which is known as osteitis fibrosa cystica. **[Au: Long sentence split for flow, OK?]** 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.108 Osteitis fibrosa cystica **[Au:OK?]** is much less common now in patients with primary hyperparathyroidism than in the past **[Au: due to PTH replacement?]**. If urine concentrations of calcium increase sufficiently, calcium-containing kidney stones can form quickly and cause kidney damage **[Au: Edits for style, OK?]**. Patients with classical primary hyperparathyroidism typically develop osteoporosis and kidney stones as a result of these changes in physiology **[Au: Please reference this statement.]**. Surgical removal of the parathyroid glands and PTH replacement can cure chronic **[Au:OK? Is this what you mean here?]** hyperparathyroidism, and typically reverses these changes over several years.

***[H2] Hypoparathyroidism and inadequate PTH action.*** Deficiency in PTH results in hypoparathyroidism, which is characterized by hypocalcemia and hyperphosphatemia, with absent or inappropriately decreased PTH levels, in the absence of impaired renal function. The concentrations **[Au: serum 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.109 Causes of hypoparathyroidism include surgical removal of the parathyroid glands **[Au:OK?]** , autoimmune destruction and congenital agenesis, which frequently is associated with a genetic disorder. The genetic forms of hypoparathyroidism can **[Au:OK?]** occur as part of syndromic disorders or as a non-syndromic solitary endocrinopathy, called isolated or idiopathic hypoparathyroidism (**Table 2**).

 **[Au: New paragraph for PHP, OK?]** Pseudohypoparathyroidism **[Au: journal style is to generally avoid abbreviating single words.]** is a genetic **[Au:OK?]** disorder of PTH resistance and three main variants — pseudohypoparathyroidism type Ia (PHPIa), pseudohypoparathyroidism type 1b (PHPIb), and pseudopseudohypoparathyroidism (PPHP) **[Au: I’ll leave this one abbreviated for style consistency with the other pseudohypoparathyroidism variants.]** — are recognized on the basis of biochemical and somatic features.57 PHPIa is characterized by occurrence of hypocalcaemia, hyperphosphataemia, and elevated serum concentrations of PTH, with the features of Albright’s hereditary osteodystrophy **[G]** (AHO) **[Au: Deleted text has been moved into the glossary term, to streamline the narrative here.]**. In PHPIb, PTH resistance occurs without the somatic features of AHO, whereas in PPHP, the somatic features of AHO occur in the absence of PTH resistance. PHPIa and PPHP are due to parentally imprinted inactivating mutations of *GNAS1,* which encodesGsα **[Au: Please reference this statement. Does ref 57 still apply here?]** (**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 **[Au: Please reference this statement.]**.

Chronic hypoparathyroidism causes fairly **[Au:OK? relatively requires a comparator.]** low bone turnover due to the complete or relative absence of PTH in the circulation.110,111 As a result, bone formation and resorption decrease, and bone density tends to increase due to increased mineral deposition. **[Au: Long sentence split for flow, OK?]** Patients treated with conventional therapy **[Au: by conventional therapy, do you mean PTH replacement therapy? Or do you mean that the calcium and vitamin D supplementation are the conventional therapy? Please clarify.]**, with calcium and vitamin D supplementation, show thicker cortices and more plate-like trabecular microarchitecture **[Au: Sentence structure flipped for flow, OK?]**. 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 **[Au: in patients with chronic hypoparathyroidism?]** , and restores a more normal bone microarchitecture.112 Long-term therapy with recombinant human PTH (1–84) over 4 years **[Au: please clarify the patient group.]** has been shown to increase bone density further at the lumbar spine and femoral neck, and to cause small decreases toward the normal range **[Au:OK?]** at the total hip and ultradistal radius.113

**[H1] Fetal and post-natal biomineralisation [Au: Heading edited for length. Please feel free to amend my suggestion but keep to the character count, OK?]**

***[H2] The Fetus.***The fetal skeleton undergoes very rapid longitudinal growth and mineral accretion, with 80% of its mineral content accreted during the third trimester.114,115 Calcium and phosphate are maintained ~0.5 mmol/L higher in cord blood than adult normal values **[Au:OK?]** to facilitate mineralisation; a fetal blood calcium concentration **[Au: Edits OK?]** that is reduced to adult values results in low skeletal mineral content.114,115 The fetus obtains mineral by active placental transport of calcium and phosphate, and can usually achieve adequate levels **[Au:OK?]** even when maternal mineral concentrations are low.114,115

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 **[Au:OK?]** 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 (**Figure 6**). The fetal circulation is characterized by low PTH and calcitriol concentrations, and normal to modestly low concentrations of intact **[Au: Edits OK? please clarify what is meant by intact FGF23.]** FGF23.114,115 The parathyroid expresses CaSR, which suppresses PTH secretion in response to the high serum concentrations of calcium **[Au: Edits OK?]**.114,116 Absence of the parathyroid glands or of PTH in the fetus **[Au: Edits OK?]** causes mild hypocalcemia, hyperphosphatemia and under-mineralized skeletons with normal limb lengths.117-120 Calcitriol levels are kept low by high levels of calcium and phosphate, low PTH concentrations, and increased 24-hydroxylated catabolism **[Au: do you mean increased 24 hydroxylated vitamin D catabolism? I feel like a word might be missing here.]**.114,115 Without vitamin D,121-127 calcitriol,128-130 or the vitamin D receptor,131,132 **[Au: Are these mice studies or human studies?]** the fetus maintains normal serum concentrations of calcium, phosphate and PTH, and develops a skeleton with normal lengths and mineral content. In addition, without FGF23133 or its signalling partner Klotho **[Au:OK? If not, please clarify the relevance of klotho here.]** ,134 **[Au: please clarify, mouse or human studies?]** or with up to 100-fold excess FGF23,133,135 fetal **[Au:OK?]** mineral metabolism and skeletal development are normal.

Parathyroid hormone-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 (**Figure 6**). The existence of PTHrP was predicted when human cord blood revealed high PTH-like bioactivity but low to undetectable immunoreactive PTH.114 PTHrP has both paracrine and endocrine functions in the fetus. Deletion of PTHrP **[Au: in mice]** leads to a lethal skeletal dysplasia characterized by accelerated endochondral bone formation,136 as well as hypocalcemia, hyperphosphatemia, reduced placental calcium transport and secondary hyperparathyroidism.118,137 By acting on PTH1R, PTHrP regulates fetal bone metabolism,138 and by acting on a mid-region receptor **[Au: Is it possible to be more specific about this mid-region receptor?]** , PTHrP stimulates placental calcium transport **[Au: Edits OK? For flow.]**.117,137,139,140 The placenta is likely the dominant source of circulating PTHrP, although there is some evidence that the fetal parathyroid glands and liver might **[Au:OK?]** contribute.117,119,137 **[Au: I’m just curious as it wasn’t mentioned previously: does PTHrP have any known functions on biomineralisation in adults (apart from lactation)?]**

***[H2] The Neonate.*** After birth,cutting the umbilical cord stops the placental infusion of hormones and minerals, whereas breathing causes a rise in pH and fall in ionized calcium **[Au: in the blood?]**. Serum and ionized calcium **[Au: Ionized calcium in the serum? Or are serum calcium and ionized calcium two separate things here? If so, where is the ionized calcium found if not in blood?]** typically drop 20–30% over 12–24 hours141-143 and then rise to neonatal values after several days.114 Serum concentrations of phosphate increase over 24–48 hours and then slowly decline to neonatal norms.114 The intestines are now the source of minerals **[Au: The intestines are now the site of mineral intake?]** , whereas the kidneys begin to reabsorb calcium and bone turnover contributes mineral to the circulation (**Figure 6**).114

The falling concentration of ionized calcium **[Au:OK?]** 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 **[Au: in mother’s milk?]** , but this process **[Au:OK? To clarify ‘this..’]** later becomes active and calcitriol-dependent.114 In mouse models, **[Au:OK?]** loss of FGF23 or Klotho, and excess FGF23, begin to show effects after birth.134,144

In the fetus, low ionized calcium **[Au: concentrations in blood? Please clarify.]** does not affect survival to term,117,118,145,146 but it might **[Au: Edit OK?]** lead to a critically low level **[Au: lower requires a comparator so I edited it to ‘low’.]** being reached after birth **[Au: please clarify: what occurs if a critically low level of ionized calcium is reached after birth?]**.

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 hypocalcemia, hypophosphatemia and rickets.114,115

**[H1] Pregnancy and Lactation [Au: Heading edited for length. Please feel free to amend my suggestion but keep to the character count of 39 including white spaces.]**

Pregnancy and lactation place considerable demands on the mother to provide adequate minerals to herself and the fetus **[Au: Edits OK?]**. During the last six 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 **[Au: Edit for clarity OK?]**.19,21 After birth, the neonate requires about 200 mg of calcium daily from milk during the first six months. With the normal 25% fractional absorption of calcium,147 a pregnant women would need 1,200 mg extra per day during the third trimester, whereas a lactating women would require 800 mg more per day.125 Instead, specific reproductive adaptations meet the demands for mineral delivery.

***[H2] Pregnancy.*** **[Au: New subheading OK?]** During pregnancy, the efficiency of intestinal calcium absorption doubles to provide more than the fetus requires, resulting in maternal hypercalciuria.125,148 Total and free calcitriol levels **[Au: please clarify the difference between total and free calcitriol]** more than double during pregnancy and contribute to upregulating the intestinal absorption of calcium;125,148 however, animal models indicate that this upregulation occurs without vitamin D, calcitriol, or its receptor **[Au: please clarify the receptor.]**.149-152 Moreover, PTH is normally the dominator regulator of calcitriol synthesis, but intact PTH levels are typically low or undetectable in pregnant women.125,148 This finding underscores that novel factors must be stimulating calcitriol production as well as intestinal calcium absorption.

 **[Au: New paragraph, OK?]** Histomorphometric assessments suggest that bone turnover in the maternal skeleton increases in the first trimester,153. **[Au: Long sentence split for flow, OK?]** By contrast, serial assessments of bone mass by DXA 125,154 and structure by pQCT155 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 **[Au: Edit OK?]** to provide for the combined needs of the mother and fetus; **[Au: semi-colon added to break up the long sentence for flow, OK?]** some of these women present with marked skeletal demineralization and fractures in late pregnancy or the puerperium.156

***[H2] Lactation.*** **[Au: New subheading OK?]** 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.157-159 Systemic estradiol 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.125,148 The dominant effect of PTHrP is exemplified by women with hypoparathyroidism, **[Au:OK?]** who normalize mineral homeostasis while breastfeeding, independently from oral calcium supplements.160 Of note, aBMD **[Au: this abbreviation is defined at first use.]** of the spine typically declines 5–10% during six months of lactation and by half that amount at the hip and radius. This aBMD loss is **[Au: Long sentence split for flow, OK?]** accompanied by loss of radial and tibial microarchitecture as seen by HR-pQCT, but aBMD and apparent strength are restored within a year after weaning through unknown mechanisms.125,148,161-163

***[H2] An evolutionary perspective. [Au: New subheading OK?]*** Why do biomineralisation adaptations **[Au:OK?]** differ between pregnancy and lactation? From an evolutionary perspective, reproductive cycles **[Au: in tetropods? Please clarify.]** 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 **[Au:OK? If not, please clarify the ‘/’.]** calcitriol ensures that women can absorb calcium regardless of their latitude and sunlight exposure. If mating occurs in spring, **[Au:OK?]** 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 **[Au:OK?]** provide important insights into novel ways of treating malabsorptive disorders and conditions that cause skeletal fragility.

**[H1] Conclusions [Au: Please note, the ‘Conclusions’ heading is rigid.]**

Biomineralisation is critically important to ensure that the skeleton and teeth are fully mineralized to enable ambulation and feeding. Inhibition of biomineralisation is also important to avoid disabling and even fatal mineralisation of soft tissues and organs, such as the electrical conducting system in the heart, joints, blood vessels and the brain. This Review has discussed the main regulators of biomineralisation and also how disorders of mineralisation can be caused by deficiency or excess of these same factors. Over 99% of the calcium content of the human body **[Au:OK?]** is contained within the skeleton, which enables bone to be used as a storehouse of mineral during times of increased need, such as 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 **[Au: do you mean systemic mineral metabolism?]** and skeletal metabolism. In addition, pregnant and lactating women might **[Au:OK?]** 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 biomineralisation has revealed receptors and intracellular molecules that are potential targets for future drugs for the treatment of mineral and skeletal disorders.

 **[Au: Please note, the acknowledgements have been moved below the reference list for formatting reasons.]**

**References [Please ensure that references are cited sequentially in the following order: main text, tables, figure legends and then boxes. The numbered references should be listed at the end of the article in the format: 1. Author, A. B. & Author, B. C. Title of the article. Nat. Cell Biol. 6, 123–131 (2001). (with journal abbreviation italic, and volume bold). If there are six or more authors to a reference, only the first author should be listed followed by ‘et al.’. For more details on reference format please consult the Guidelines to Authors.]**

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 **[Au: Please note, our formatting does not permit two column tables, so this table has been reformatted to include three columns. Please check for accuracy, OK?]**

**Table 1: IOM 2010 Institute of Medicine of the UA National Academy of Sciences recommendations for daily dietary calcium intake**a

|  |  |  |
| --- | --- | --- |
| **Category** | **Age** | **Recommended intake (mg/day)** |
| Infancy to adolescence | 0–12 months | 200 mg first 6 months; 260 mg thereafter |
| 1–3 years | 700 mg |
| 4–8 years | 1000 mg |
| 9–13 years | 1300 mg |
| 14–18 years | 1300 mg |
| Women | 19–50 years | 1000 mg |
| Post menopause | 1200 mg |
| Men | 19–70 years | 1000 mg |
| >71 years | 1200 mg |

aData are originally presented in 164,165 **[Au:OK?]**

**Table 2. Major germline and somatic genetic foundations in familial and sporadic parathyroid disorders [Au: I’ve edited this table to conform with our formatting rules. Please check for accuracy, OK?]**

|  |  |  |  |
| --- | --- | --- | --- |
| **METABOLIC ABNORMALITY** | **DISEASE** | **INHERITANCE** | **GENE (GENE PRODUCT)****a** |
| **PRIMARY HYPERPARATHYROIDISM** | Multiple endocrine neoplasia type 1 | Autosomal dominant | *MEN1* (menin), *CDKN1B* (p27)e, other CDKI genes |
| Hereditary hyperparathyroidism and jaw tumors (HPT-JT) | Autosomal dominant | *CDC73* (parafibromin) |
| Familial isolated hyperparathyroidism | Autosomal dominant | *MEN1, CDC73, CASR*, otherb |
| Sporadic parathyroid adenoma | Sporadic | ***[Au: alternative gene name removed]*** *CCND1*c*, MEN1*c |
| Parathyroid carcinoma | Autosomal dominant or sporadic | *CDC73* |
| Familial hypocalciuric hypercalcemia (FHH) |  **[Au: Please note, we cannot leave cells empty. Please add something into this cell and the other empty cells, even if it is ‘unknown’.]**  |  |
| FHH1 | Autosomal dominant | *CASR* ***[Au:OK?]*** |
| FHH2 | Autosomal dominant | Gα11 **[Au: please add gene]**  |
| FHH3 | Autosomal dominant | *AP2S1* |
| Neonatal severe hyperparathyroidism (NSHPT) | Autosomal recessive or autosomal dominant | *CASR* ***[Au:OK?]*** |
| Jansen’s disease | Autosomal dominant | PTH receptor, PTHrP receptor **[Au:OK? Can you add the genes?]**  |
| **HYPOPARATHYROIDISM** | Isolated hypoparathyroidism | Autosomal dominant | *PTH*d*, GCM2* |
|  **[Au: please add something into this empty cell.]**  | Autosomal recessive | *PTH*d*, GCM2* |
| Autosomal dominant hypocalcemia type 1 (ADH1) | Autosomal dominant | *CASR* |
| Autosomal dominant hypocalcemia type 2 (ADH2) | Autosomal dominant | Gα11 **[Au: please add the gene]**  |
| Hypoparathyroidism associated with polyglandular autoimmune syndrome (APECED) | Autosomal recessive | *AIRE1* |
| Hypoparathyroidism associated with complex congenital syndromes |  **[Au: please add something into these empty cells]**  |  |
| DiGeorge syndrome type 1 | Autosomal dominant | *TBX1* |
| HDR syndrome | Autosomal dominant | *GATA3* |
| Blomstrand’s lethal chondrodysplasia | Autosomal recessive | PTH receptor, PTHrP receptor **[Au: can you add the genes?]**  |
| Pseudohypoparathyroidism (type 1a) | Autosomal dominant parentally imprinted | *GNAS* exons 1-3 |
| Pseudohypoparathyroidism (type 1b) | Autosomal dominant parentally imprinted | *GNAS* upstream deletion |
| HDR, hypoparathyroidism, deafness, and renal dysplasia.aMajor established pathogenic mutations and **[Au:OK?]** variants are germline unless otherwise indicated |
| bSpecific germline variants of *GCM2* have been associated with FIHP and **[Au:OK?]** primary hyperparathyroidism, with undetermined penetrance, and their role in clinical management is not yet establishedcsomatic mutationsdMutations of *PTH* gene are identified only in some families.eMEN1-like phenotype caused by germline mutation of *CDKN1B* has been termed MEN4 |

**Figure legends [Au: I’ve carried out a light edit on the figure legends but I will take another look at them once the figures are redrawn, in case any further changes need to be made.]**

**Figure 1. Regulation of calcium and phosphate economy and bone mass.** Within bone cells, parathyroid hormone (PTH) stimulates osteoblasts to form bone, indirectly stimulates osteoclasts to resorb bone and bring calcium and phosphate into the circulation, and acts on osteocytes to stimulate fibroblast growth factor 23 (FGF23) release. Within kidney tubules, PTH increases reabsorption of calcium and renal excretion of phosphate, while FGF23 also promotes renal phosphate excretion. Within intestines, calcitriol increases intestinal calcium and phosphate absorption. The integration of these regulators includes that PTH increases FGF23 and calcitriol, while FGF23 has opposing effects to inhibit PTH and lower calcitriol. Green arrows indicate stimulatory actions while red arrows are inhibitory. Reproduced with permission from:....166 **[Au: Please can I ask you to fill out a third party rights table and send it to use ASAP so that we can apply for this permission? We must obtain permission even though ref 166 is a Springer Nature publication.]**

**Figure 2. Activators and inhibitors of mineralization.** The figure shows activators (inorganic phosphate (Pi),, ionized calcium (Ca2+) and collagen) and inhibitors (pyrophosphate (PPi)) of mineralisation. 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 **[Au: Please clarify, is this process relevant for all cells or just bone cells? Can we label the cell in the figure?]** by the transmembrane pyrophosphate transporter ANK. TNAP (tissue non-specific alkaline phosphatase) hydrolyzes PPi.

**Figure 3. Calcitriol-VDR stimulated and VDR-independent calcium transport in intestinal epithelium.** TRPV6: apical membrane calcium channel; PMCA1b: basolateral plasma membrane protein. From 65. **[Au: please add a figure legend of 150–200 words that fully describes all elements of the figure, as you have for figures 1 and 2. In addition, please include this figure in the third party rights table.]**

**Figure 4: Role of Matrix-Gla-Protein in mineralisation.** Adapted from Siltari and Vapaatalo 90. **[Au: please add a figure legend of 150–200 words that fully describes all elements of the figure, as you have for figures 1 and 2. In addition, please include this figure in the third party rights table]**

**Figure 5: Relationship of serum concentrations of calcium and PTH.** Solid black 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 midway 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 green dashed curve depicts altered setpoint curve in FHH **[Au: please expand the abbreviation.]**. Germline disruption of the parathyroid glands’ calcium-sensing or downstream signaling apparatus, most notably via inactivating mutation of the CaSR **[Au: please define this term. All abbreviations must be redefined in each display item.]** 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, and setpoint, to the right. The red dashed curve depicts altered setpoint curve in autosomal dominant hypocalcemia (ADH). Germline activating mutation of CaSR or Gsα results 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.

**Figure 6. Regulation of mineral homeostasis before and after birth.** On the left, placental transport of calcium (regulated by parathyroid hormone-related protein (PTHrP)) and phosphate (regulated by unknown factors) provide the required mineral and contribute to the fetus maintaining higher circulating mineral concentrations than in the adult. Circulating PTHrP is produced by the placenta and possibly the fetal parathyroid glands, whereas PTH is suppressed by the hypercalcemia and hyperphosphatemia. FGF23 is not shown because it does not play a role in fetal mineral homeostasis. Green arrows depict the dominant flow of mineral across the placenta and into the developing skeleton. Red arrows depict back-flow of mineral from the fetus to the mother, and the minor autophagic circuit that includes excretion of mineral into the amniotic fluid (which largely comprises urine), and reabsorption into the circulation after swallowing that fluid. The fetal kidneys do not appreciably reabsorb calcium, such that the concentration of calcium in amniotic fluid correlates to the fetal serum calcium and implied renal filtered load. On the right, 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. It may suppress mineral absorption in the neonate since milk devoid of PTHrP led to increased skeletal mineral accretion in mice. **[Au: please could you cite the relevant main text refs here again to support this figure legend?]**

**Glossary** **[Au: I have highlighted suggestions for glossary terms throughout your manuscript with a [G]. Please provide succinct, one-sentence definitions for these specialist terms.]**

Albright’s hereditary osteodystrophy: a genetic syndrome characterized by short stature, obesity, subcutaneous calcification, mental retardation, round faces, dental hypoplasia and brachydactyly. **[Au:OK?]**

Tetrapod transition

Calcium–PTH setpoint

Familial multiple endocrine neoplasia type 1 syndrome: A condition characterized by predisposition to primary hyperparathyroidism in association with neuroendocrine tumours of the pancreas, pituitary adenomas and adrenal tumours. **[Au:OK?]**

This Review focuses on physiological and pathological aspects of the hormonal regulation of biomineralisation, which is crucial for skeletal health, during adulthood, fetal and neonatal development and pregnancy. The role of mineral intake, serum concentrations of mineral and hormonal regulators of biomineralisation are highlighted. **[Au: ToC blurb OK? Please note, we cannot make this any longer.]**