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PEDIATRICS AND SKELETAL DEVELOPMENT (CRAIG LANGMAN AND MARIA LUISA BIANCHI, SECTION EDITORS)

Bone Development in the Fetus and Neonate: Role of the Calciotropic Hormones

Christopher S. Kovacs

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Abstract During embryonic and fetal development much of the skeleton initiates as a cartilaginous scaffold, which is progressively resorbed and replaced by bone. Endochondral bone formation continues until the growth plates fuse during puberty. At all life stages adequate delivery of mineral is required for the skeleton to achieve and maintain appropriate mineral content and strength. During fetal development the placenta actively transports calcium, phosphorus, and magnesium. Postnatally passive and then active absorption from the intestines becomes the main supply of minerals to the skeleton. Animal and human data indicate that fetal bone development requires parathyroid hormone (PTH) and PTH-related protein but not vitamin D/calcitriol, calcitonin, or (possibly) sex steroids. During the postnatal period, when intestinal calcium absorption becomes an active process, skeletal development begins to depend upon vitamin D/calcitriol but this requirement can be bypassed by increasing the calcium content of the diet or by administering intermittent calcium infusions.

Keywords Fetus · Neonate · Calcium · Phosphorus · Endochondral bone development · Skeletal development · Mesenchymal bone development · Parathyroid hormone · Parathyroid hormone-related protein · PTH/PTHrP receptor · Estradiol · Estrogen receptor · Calcitonin · Calcitonin receptor · Vitamin D · 25-hydroxyvitamin D · Calcitriol · Knockout mice · Sheep · Humans · Randomized clinical trials · Observational studies · Associational studies · RANKL · Osteoprotegerin · Indian hedge hog · Placental calcium transfer · Bone formation · Bone resorption

C. S. Kovacs (✉)
Medicine (Endocrinology and Metabolism), Obstetrics & Gynecology, and BioMedical Sciences, Faculty of Medicine, Memorial University of Newfoundland, Health Sciences Centre, 300 Prince Philip Drive,
St. John's, Newfoundland A1B 3V6, Canada
e-mail: ckovacs@mun.ca

Introduction

Skeletal patterning begins in the embryo but it is in the fetal and neonatal periods that bone formation and mineralization accelerate. The placenta actively transports calcium, magnesium, and phosphorus to appropriately mineralize the skeleton before birth. With loss of the placental pump at birth, the neonate becomes dependent upon intestinal absorption of minerals. Initially, the intestines absorb calcium passively but with increased postnatal age this becomes active and vitamin D-dependent. The calciotropic hormones play different roles during the fetal and neonatal periods in regulating skeletal development and mineralization.

Overview of Fetal and Neonatal Mineral Metabolism

Within the developing human, serum calcium, ionized calcium, magnesium, and phosphorus are raised above maternal values [1••]. Studies in fetal mice suggest that these high mineral concentrations are needed for normal skeletal accretion of minerals [2, 3•, 4] but not for fetal survival to term [2, 5, 6].

The fetal circulation in humans and other mammals is characterized by low levels of parathyroid hormone, 1,25-dihydroxyvitamin D (calcitriol), and fibroblast growth factor-23, and high levels of parathyroid hormone–related protein

(PTHrP) and calcitonin [1••], [7]. 25-hydroxyvitamin D [25 (OH)D] readily crosses the placenta and results in cord blood 25(OH)D levels that are within 75% to 100% of the maternal value at term [8, 9, 10•]. The low calcitriol levels are due to suppression of the renal 1α-hydroxylase (CYP27b1) by high serum calcium and phosphorus, and low parathyroid hormone. But if fetal hyperparathyroidism is present then fetal calcitriol levels markedly increase [11].

Animal studies have shown that absence of parathyroids, parathyroid hormone, or PTHrP each cause fetal hypocalcemia and hyperphosphatemia [1••, 2, 3•, 5, 6], whereas absence of calcitonin [4], vitamin D [12–14], calcitriol [15], or the vitamin D receptor [16] do not disturb fetal blood calcium or phosphorus.

Minerals enter the fetus predominantly via the placenta, whereas kidneys and intestines enable calcium to be voided into the amniotic fluid and then swallowed and reabsorbed. PTHrP regulates placental calcium and possibly magnesium transfer, although parathyroid hormone (PTH) may also play a role [3•, 5, 17–19]. During the interval of active calcium transfer the placenta markedly upregulates expression of genes involved in calcium transport, and the fetal skeleton accretes mineral rapidly [16, 20–22]. Human skeletons accrete 80% of required calcium in the third trimester [23], whereas rats attain 95% of skeletal calcium during the last 5 days of gestation [24].

At birth the total and ionized calcium levels fall due to loss of the placental calcium infusion and calciotropic hormones, and a breathing-induced rise in blood pH [25]. Phosphorus increases over the same interval and then declines. PTH rises to near adult normal values by 24 to 48 h after birth and this precedes an increase in calcitriol [1••].

The neonate is dependent upon the intestines for supply of minerals. Calcium absorption in newborns is largely passive and nonsaturable [26, 27]. The high lactose content of milk increases paracellular diffusion of calcium in the distal small bowel and net bioavailability of dietary calcium in humans [28, 29] and rodents [30]. As the neonate matures, passive absorption of calcium declines, enterocytes upregulate expression of the vitamin D receptor and associated calcium-transporting genes and proteins, and calcium absorption becomes active and vitamin D-dependent [26, 31, 32]. This developmentally programmed maturation of the neonatal intestines explains why preterm babies do not respond to calcitriol but are dependent upon passive absorption of mineral until they are more mature.

Overview of Endochondral Bone Development

Early patterning of the embryonic skeleton is dependent upon a multitude of signaling pathways, which include Hox genes, Wnts, Hedgehogs, bone morphogenetic proteins, fibroblast growth factors, Notch/Delta, and other factors [33•]. Mesenchymal cells are laid down in spatiotemporal patterns where bones of the axial and appendicular skeletons will form. These osteochondral progenitors become osteoblasts at the site of intramembranous bone formation (vault of the skull and a few other bones), whereas in the bulk of the developing skeleton they become chondroblasts that initiate endochondral bone formation.

By 8 weeks of gestation in humans a complete cartilaginous scaffold with digits and joints is present. The scaffold of each long bone lengthens at both ends, with the oldest cells nearer the center undergoing differentiation, hypertrophy, and then apoptosis. Chondroclasts and osteoclasts resorb the apoptosed chondrocytes and surrounding matrix, vascular invasion occurs, and osteoblasts lay down the primary spongiosa (osteoid) that will become mineralized. These primary ossification centers form in the vertebrae and long bones between the 8th to 12th weeks, but it is not until the third trimester that the bulk of skeletal mineralization occurs. Resorption and remodeling of the primary spongiosa to create secondary spongiosa occurs in utero and resorption will abnormally increase when the fetus is stressed by maternal hypocalcemia. At the 34th week of gestation, secondary ossification centers form in the femurs—creating true growth plates—but otherwise most epiphyses are cartilaginous until after birth [34]. The growth plates become fused during puberty, after which linear growth is no longer possible.

Role of PTHrP

PTHrP is produced by the perichondrium and proliferating chondrocytes, whereas the PTH/PTHrP receptor (PTH1R) is expressed further down the growth plate within prehypertrophic chondrocytes [35, 36, 37•]. The critical role that PTHrP plays in regulating endochondral bone formation was first made evident by *Pthrp*-null fetuses, which have a chondrodysplasia characterized by dwarfed long bones and deformed growth plates [38]. Further study has determined that PTHrP acts locally to delay terminal differentiation and hypertrophy of chondrocytes. Without PTHrP, hypertrophy begins early and bone formation starts before the cartilaginous template has reached its intended length; consequently, the long bones are shortened [37•, 39, 40]. Conversely, overexpression of PTHrP or expression of an activating mutation of the PTH1R within fetal chondrocytes results in delayed chondrocyte hypertrophy, and skeletons that are largely cartilaginous at birth [41, 42]. PTHrP and Indian hedgehog interact in a negative feedback loop that determines the length of the columns of proliferative chondrocytes, and PTHrP also interacts with

the Wnt signaling pathway to determine the lifespan and fate of chondrocytes [37•, 40].

Preosteoblasts and osteoblasts express PTHrP and the PTH1R, and thus one might expect the *Pthrp*-null skeleton to show decreased expression in osteoblast-specific genes and reduced bone formation and mineralization. However, *Pthrp*-null growth plates have normal expression of collagen α1(I) and collagenase-3, and upregulated expression of osteocalcin and osteopontin [38, 43]. Acceleration of skeletal maturation in the *Pthrp*-null results in bones becoming mineralized in utero that normally do not mineralize until after birth, and the normally cartilaginous parts of the ribs and sternum also become bone [38]. *Pthrp* nulls have threefold higher levels of PTH than normal [2] and this hyperparathyroidism likely rescues any deficit in osteoblast function that loss of PTHrP might otherwise cause. Further studies in mice lacking PTH or the PTH1R support this notion (see below).

Targeted deletion of PTHrP within preosteoblasts and osteoblasts leads to reduced bone formation and low bone mass at 6 weeks of age, confirming that osteoblast-derived PTHrP regulates bone formation postnatally [44]. However, the fetal skeletons of these mice have not been examined and thus it remains unknown whether osteoblast-derived PTHrP is important for fetal bone formation.

Pthrp-null fetuses lack circulating PTHrP, are hypocalcemic (ionized calcium reduced to the maternal level), and have a lower rate of placental calcium transfer [2, 5]. Despite these changes and shorter long bones, the *Pthrp*-null skeleton has a normal ash weight and content of calcium, magnesium, and phosphorus [2, 5]. Why is skeletal mineral content not reduced? The *Pthrp*-null skeleton accretes more mineral than normal due to its accelerated and abnormal calcification of skeletal structures, and thus its mineral content cannot be meaningfully compared with the normal skeleton.

Overall, the evidence is that PTHrP participates in fetal skeletal development by upregulating placental calcium transfer, maintaining a high serum calcium, determining the fate of chondrocytes within the scaffold, and possibly regulating preosteoblasts and osteoblasts.

Role of PTH

There is mixed evidence for parathyroid hormone's role in regulating endochondral bone formation and mineralization. Aparathyroid *Hoxa3*-null fetuses in a Black Swiss background have low skeletal mineral content but otherwise show no abnormality of endochondral bone development or expression of osteoblast-specific genes including collagen α1(I), collagenase-3, osteocalcin, and osteopontin [2, 6]. They are markedly hypocalcemic with an ionized calcium reduced well below the maternal level, whereas the serum phosphorus is increased above the fetal norm [6]. Placental calcium transport and circulating PTHrP levels are normal [2, 6]. It is evident that, despite its normally low circulating levels in the fetus, PTH plays an important role in regulating serum minerals and skeletal mineral accretion.

Double mutants lacking *Hoxa3* and *Pthrp* have an undermineralized form of the *Pthrp*-null chondrodysplasia and are globally smaller than either single mutant [2]. These findings confirm that the chondrodysplasia seen in *Pthrp* nulls is largely due to absence of PTHrP and not the compensatory secondary hyperparathyroidism, whereas undermineralization of the skeleton results from loss of parathyroids and/or PTH [2].

In contrast to the aparathyroid *Hoxa3* phenotype, *Pth*-null fetuses in a C57BL/6 background have slightly shortened tibial metaphyseal lengths, shorter metacarpals and metatarsals, smaller vertebrae, reduced trabecular bone volumes, and fewer osteoclasts and osteoblasts [45]. Modest changes are also seen in expression of genes involved in chondrocyte maturation and apoptosis, mineralization, and vascular invasion of the growth plate [45]. Overall, the structural changes are largely in bone parameters whereas the cartilaginous indices are little different from wild-type (wt) siblings. *Pth/Pthrp* double mutants are smaller and display an undermineralized form of *Pthrp*-null chondrodysplasia (similar to the *Hoxa3/Pthrp* double mutants) [45]. The data from this study indicate that PTH regulates bone formation in utero but not chondrocyte development.

A third study backcrossed *Pth* nulls and *Gcm2* nulls (which have little or no circulating PTH) from the original inbred C57BL/6 strains into outbred Black Swiss to compare to *Hoxa3*-null fetuses [3•]. In both *Pth* and *Gcm2* nulls the ionized calcium is reduced to the maternal level, equal to the *Pthrp* null but not to the extreme seen in the *Hoxa3* null [2, 3•, 5]. Skeletons of both mutants are undermineralized but to a lesser extent than in *Hoxa3* null [2, 3•]. There is no shortening of the long bones or alteration in trabecular bone volumes or skeletal morphology [3•]. However, studies of gene expression within the *Pth*-null and *Gcm2*-null growth plates were not done. It is possible that alterations in serum calcium (which is approximately 0.25 mmol/L higher in Black Swiss [5]), or genetic differences in background strain, may explain why some alterations in bone parameters are seen in *Pth*-null fetuses within one but not both background strains.

Placental calcium transfer is normal in *Pth*-null fetuses (the same as in *Hoxa3* nulls) but upregulated significantly in *Gcm2* nulls [3•]. Circulating PTHrP is normal in both mutants [3•]. Further study found that the *PTH* gene is expressed in wt placenta and upregulated in *Gcm2*-null placentas, whereas *Pth*-null placentas have reduced expression of genes involved in the transport of calcium and other cations [3•]. PTH may therefore regulate placental calcium and cation transport independent of PTHrP.

Overall, the evidence indicates that PTH maintains the fetal blood calcium at a level sufficient to facilitate mineral accretion by the developing skeleton. Within developing endochondral bones PTH does not control chondrocytes, whereas its effects on osteoblast development vary with the genetic background.

Role of PTHrP and PTH in Combination

Loss of the PTH1R blocks action of amino-terminal PTH and PTHrP, leading to a phenotype that combines aspects of *Pthrp*-null and *Pth*-null fetuses. These *Pthr1*-null fetuses are globally smaller than wt and display the *Pthrp*-null phenotype of accelerated endochondral ossification and dysplasia combined with the *Hoxa3*- or *Pth*-null phenotype of significant undermineralization of the skeleton [39]. The human equivalent of absence of the PTH1R, Blomstrand chondrodysplasia, shows similar features [46, 47].

There are notable differences between *Pthrp*- and *Pthr1*-null skeletons. *Pthrp*-null fetuses have normal expression of collagenase-3, upregulation of osteocalcin and osteopontin, and increased mineralization [43]. In contrast, *Pthr1* fetuses have reduced expression of collagenase-3, osteocalcin, and osteopontin, and reduced mineralization [43]. This suggests that PTH upregulates expression of these osteoblast-specific genes in the *Pthrp* null, whereas blocking PTH action downregulates these genes in the *Pthr1* nulls.

Pthr1-null fetuses have the lowest ionized calcium level, equivalent to *Hoxa3/Pthrp* double mutants [2, 5]. PTH and PTHrP evidently have additive roles in regulating the fetal blood calcium and skeletal mineralization because loss of both ligands or their common receptor leads to the greatest decline in blood calcium and skeletal mineral content [2]. However, it remains unclear why the ionized calcium is lower in aparathyroid *Hoxa3*-null fetuses than it is in *Pth* or *Gcm2*-null fetuses within the same genetic background. Placental calcium transfer is upregulated 50% in *Pthr1*-null fetuses, presumably reflecting the 11-fold increase in systemic PTHrP and its actions on a mid-molecular receptor [2, 5]. This increase is relative to placental blood flow as inferred from the diffusion of ^{51}Cr-EDTA across the placenta; the absolute amount of ^{45}Ca transferred to the *Pthr1*-null fetuses is reduced compared with their wt littermates, in keeping with their much smaller size and lower skeletal mineral content [48•].

Role of Estradiol

Sex steroids (estradiol and testosterone) circulate at low levels during fetal development [49] and the role that each might play in fetal calcium homeostasis and skeletal development is unclear. Mice lacking estrogen receptor α or β, or the aromatase, have normal skeletal lengths at birth and do not develop altered skeletal metabolism until later [50–52]. However, no studies have examined whether absence of sex steroids alters fetal calcium homeostasis or endochondral bone development and mineralization.

Role of Calcitonin

Calcitonin appears unimportant for fetal and neonatal skeletal development because in its absence the mutant fetuses have normal placental calcium transfer, ionized calcium, calciotropic hormone levels, endochondral bone development, gene expression, and mineral content [4]. However, serum magnesium and skeletal mineral content are both lower than normal, suggesting that absence of calcitonin disturbs magnesium homeostasis through unknown mechanisms [4].

Role of Vitamin D/Calcitriol

The role of vitamin D/calcitriol in regulating fetal and neonatal skeletal development has come under more intense interest in recent years and will be discussed in greater detail.

Animal Data

Severe vitamin D deficiency, ablation of vitamin D receptor, and absence of 1α-hydroxylase each result in normal skeletal lengths, morphology, ash weight, mineral content (by atomic absorption spectroscopy), and radiologic bone mineral content at term; moreover, the blood calcium, phosphorus, and PTH are normal. Animals examined include offspring of severely vitamin D-deficient rats [12–14], 1α-hydroxylase-null pigs [15], and both *Vdr* heterozygous and *Vdr*-null mice [16]. 1α-hydroxylase-null mice appear normal at birth but extensive fetal studies have not been reported [53, 54]. The normal, heterozygous, and *Vdr*-null fetuses born of *Vdr* heterozygous mothers are indistinguishable from each other, whereas heterozygous and null fetuses born of *Vdr*-null mothers are smaller and weigh less, but have normal mineral content after adjusting for smaller size [16].

The placenta evidently provides calcium to the fetus without requiring calcitriol, as suggested by studies in vitamin D-deficient rats [55] and *Vdr*-null fetuses [16]. Calbindin-D 9 K and Ca^{2+}-ATPase both show normal placental expression in severely vitamin D-deficient and *Vdr*-null placentas [16, 55–57]. Placental calcium transfer and placental expression of PTHrP and the calcium channel TRPV6 are upregulated in placentas of *Vdr*-null mice [16].

It is in the weeks after birth—particularly after weaning—that hypocalcemia, hypophosphatemia, secondary hyperparathyroidism, and rickets begin to develop [12, 13, 15, 53, 54, 58, 59]. This parallels the developmental change of intestinal calcium absorption from a nonsaturable, passive process in the newborn to an active, saturable, calcitriol-dependent process [60, 61]. Additional animal data have shown that calcitriol's role can be completely bypassed by using high-dose oral calcium or calcium infusions, resulting in a normal skeleton despite absence of vitamin D, vitamin D receptor, or 1α-hydroxylase [62–64].

Collectively these findings indicate that fetal calcium homeostasis, skeletal development, and mineralization do not require vitamin D, calcitriol, or its receptor. The animal studies predict that human babies born of vitamin D-replete and vitamin D-deficient mothers should be indistinguishable in blood calcium, phosphorus, skeletal morphology, and mineral content at birth. Calcitriol controls active intestinal calcium absorption in the older neonate but its role can be completely bypassed by increasing the calcium content of the diet.

Human Data: Observational Studies and Case Reports

A systematic study of newborns who died of obstetrical accidents found skeletal ash weight and mineral content (by atomic absorption spectroscopy) to be the same between neonates born of normal mothers versus those with extreme vitamin D deficiency and osteomalacia; moreover, there were no radiologic signs of rickets and centers of ossification were normal [65]. A few case reports indicate that skeletal changes suggestive of rickets (usually craniotabes) can be detected at birth [66–68], but other investigators have concluded that craniotabes is a nonspecific finding that should not be used to indicate the presence of rickets [69]. In multiple reports that describe craniotabes or rickets being present "at birth" the diagnosis was actually made within the first or second week [68, 70–74]. In one such case radiographic findings were absent at day 2 after birth but had developed by day 16 [74]. In many cases in which skeletal abnormalities were found soon after birth, the mothers had significant malnutrition, malabsorption (eg, celiac disease, pancreatic insufficiency), or very low intakes of both calcium and vitamin D [67, 68, 73]. Consequently, the skeletal changes may not have been due to vitamin D deficiency alone in cases of early neonatal rickets.

The reported global experience is that vitamin D-deficient rickets usually does not develop (or become recognized) until weeks to months after birth with a peak incidence between 6 and 18 months, even in regions where severe vitamin D deficiency during pregnancy is endemic and clinicians are especially vigilant for it [75–78]. The maturation of intestinal calcium absorption to a vitamin D-dependent process likely explains why vitamin D-deficient rickets does not usually develop until later.

Children with 1α-hydroxylase deficiency (vitamin D-dependent rickets type I; VDDR-I) or those lacking the vitamin D receptor (vitamin D-dependent rickets type II or hereditary vitamin D-resistant rickets; VDDR-II) are normal at birth [79–82]. In both conditions hypocalcemia, hypophosphatemia, and rickets eventually develop. VDDR-I presents late in the first year, whereas VDDR-II presents in the second year or later [79–82]. The deficiency in intestinal calcium absorption in VDDR-II patients can be bypassed by repeated calcium infusions or high oral dose calcium, thereby correcting the biochemical abnormalities and preventing or healing rickets [81, 83, 84]. Thus, the genetic disorders in humans confirm the animal data that hypocalcemia and rickets are not present at birth and can be prevented with calcium alone.

Congdon et al. [69] found that forearm bone mineral content, measured within 5 days of birth, did not differ by use of vitamin D supplementation during pregnancy. More recently, Weiler et al. [85•] measured bone mineral content of the lumbar spine, femur, and whole body within 15 days after birth in 50 healthy term infants. There was no difference in bone mineral content at any site of vitamin D-sufficient versus -insufficient whites, whereas the mineral content of the lumbar spine (but not whole body or femur) was lower in Asian and First Nations babies compared with whites. The authors concluded that genetic differences affected bone mineral content whereas vitamin D insufficiency did not [85•].

Human Data: Intervention Studies

Several randomized clinical trials of vitamin D supplementation during pregnancy found no effect on cord blood calcium or phosphorus, anthropometric measurements, or radiologic evidence of rickets [86–91]. This includes a study of 126 babies in which controls were severely vitamin D deficient (10 nmol/L 25(OH)D on cord blood), whereas babies from vitamin D-treated mothers had 25 (OH)D levels of 138 nmol/L [86].

Hollis et al. [92••] recently completed two large randomized trials of vitamin D supplementation beginning at 12 to 16 weeks of pregnancy. In the first study, 494 women received 400, 2,000, or 4,000 IU of vitamin D_3 daily, whereas in the second study, 257 women received 2,000 or 4,000 IU of vitamin D_3 per day. Results of the first study have been recently published and show that use of 400 to 4,000 IU of vitamin D had no effect on gestational age at delivery, birth weight, mode of delivery, or need for level II or III neonatal care [92••]. Moreover, at a presentation to the Centers for Disease Control and

Prevention in Atlanta, Wagner [93••] revealed that neither trial showed an effect on cord blood calcium, preterm birth, preterm labor, preeclampsia, or infection. The first study's primary objective included measuring bone mineral density of the newborns but this was apparently not carried out. The limited published results clearly indicate the amount of maternal vitamin D intake required to achieve various target levels of 25(OH)D in mother and cord blood, but do not show any clinical benefit of higher intakes for mother or baby or provide any evidence as to which target level of 25 (OH)D is required for optimal skeletal health of the fetus or neonate.

Beyond preventing rickets, at best a transient effect on bone mass has been seen through the use of vitamin D supplements during infancy. In one randomized study healthy term infants achieved 25(OH)D levels of 95 nmol/L on vitamin D versus 50 nmol/L on placebo. Bone mineral content of the radius and ulna was 23% higher than in the placebo group by 12 weeks of age but this difference vanished by 6 and 12 months [94, 95]. Another randomized study found breastfed infants achieved 25(OH)D levels of 92.4 nmol/L on vitamin D versus 47 nmol/L on placebo, but this did not affect bone mineral content at 3 or 6 months of age [96].

Human Data: Associational Studies

Several studies have examined associations between single measurements of serum 25(OH)D during pregnancy in the mother and various skeletal outcomes in the fetus, neonate, or child. In none of these was an association found with birth weight, skeletal lengths, and bone mineral density [10•, 97, 98•, 99•]. Morley et al. [97] measured 25(OH)D at 28 to 32 weeks in 374 mothers and reported in a subanalysis that a 25(OH)D level below 28 nmol/L was associated with a slightly shorter knee-heel length. However, the difference was not statistically significant after correcting for gestational age [97]. Mahon et al. [98•] measured maternal 25(OH)D levels at 36 weeks in 424 women and found that 25(OH)D levels below 50 nmol/L were associated with greater distal metaphyseal cross-sectional area and a novel parameter called the "femoral splaying index." The greater cross-sectional area was inferred by the authors to represent early rachitic change. In contrast, Viljakainen et al. [10•] found that mothers above a median 25(OH)D level of 42.6 nmol/L had babies with a slightly greater tibial cross-sectional area. These authors interpreted the increased cross-sectional area to indicate stronger bone. The two studies exemplify how subjective the interpretation has been, with greater cross-sectional area of the metaphysis considered an adverse effect in one study and a benefit in another.

A well-publicized study by Javaid et al. [99•] found no associations between maternal serum 25(OH)D and birth weight, length, placental weight, abdominal circumference, head circumference, or cord blood calcium. At the 9-month follow-up there was still no association of maternal 25(OH)D with skeletal and anthropometric parameters in the infants. However, a maternal serum 25(OH)D level below 27.5 nmol/L during pregnancy was associated with a modestly lower bone mineral content in offspring at 9 years of age compared with offspring of mothers whose 25(OH)D levels were 50 nmol/L or higher. These findings have been used to promote the theory that vitamin D exposure during fetal development programs childhood peak bone mass [100].

There are significant caveats about these associational studies. Unstated are which radiologic parameters were prespecified and how many were examined before a single statistically significant finding was reported in each study. Why the tibia in one study and the femur in another, and not both sites? The possibility of chance findings cannot be excluded. Notably associational studies are hypothesis-generating and do not prove causality. Moreover, these associational studies are confounded by factors that contribute to low 25(OH)D levels, including maternal obesity, lower socioeconomic status, poorer nutrition, lack of exercise, prenatal care or vitamin supplementation, etc. Therefore, is lower 25(OH)D simply a marker of a less healthy pregnant woman? In the Javaid et al. [99•] study, much time elapsed between birth (when no effect was seen) and 9 years of age (when lower bone mineral content was found). Did low 25(OH)D in utero program lower bone mineral content at 9 years of age? Or does the lower maternal 25(OH)D status in late pregnancy indicate that lower socioeconomic status, poorer nutrition, and other factors in the mother remain unchanged and will be shared with the child?

Overall, the current evidence (reviewed in more detail in [101••]) indicates that the human fetus may suffer no skeletal problems as a consequence of vitamin D deficiency and insufficiency, but after birth hypocalcemia and progressive rickets will develop in those with severe vitamin D deficiency. It remains unclear whether 25(OH)D levels in the fetus or pregnant mother have a direct influence on neonatal or childhood bone mass. Large randomized controlled trials of vitamin D supplementation during pregnancy are needed to control the confounding and answer these questions. The American Academy of Pediatrics and the Institute of Medicine both concluded that a target 25(OH)D blood level of 50 nmol/L is appropriate for pediatric ages and to date there is no conclusive evidence that higher target 25(OH)D blood levels confer any additional benefit [102••].

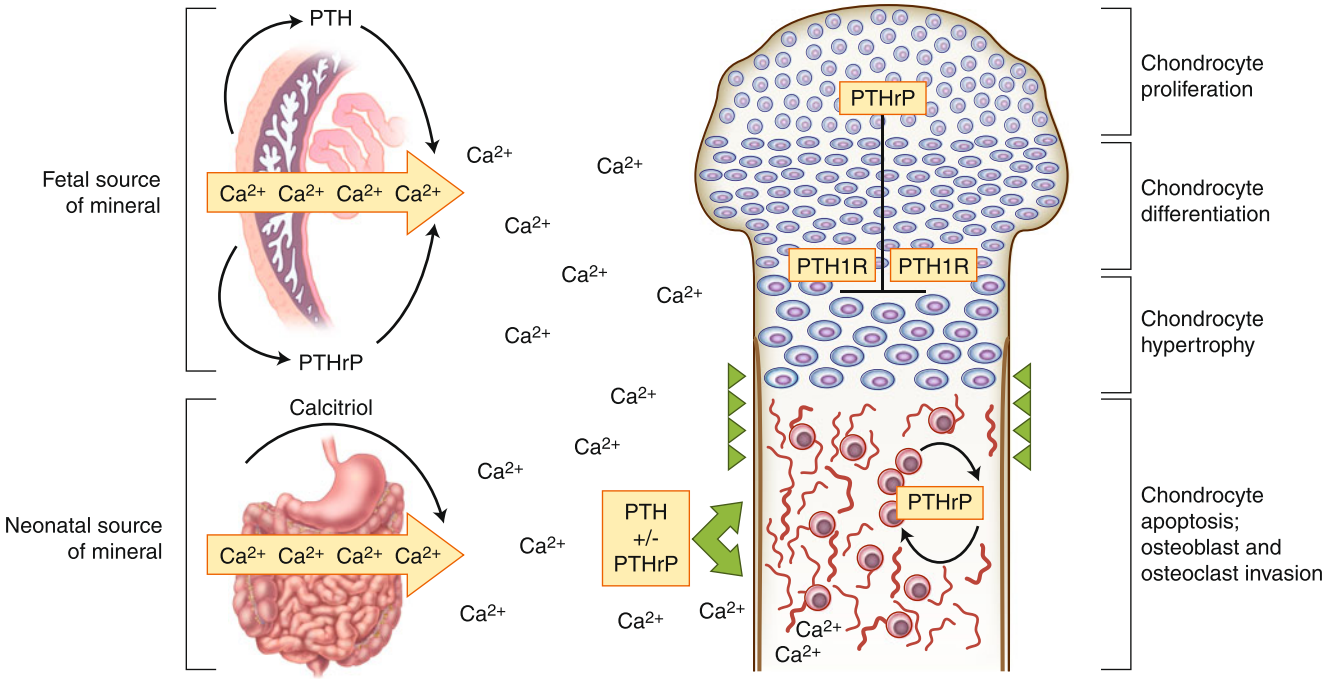

Fig. 1 Relative roles of PTH, PTHrP, and calcitriol during fetal and neonatal life. The placenta is the main source of mineral during fetal life. PTH and PTHrP are expressed within the placenta but may also act on it from systemic sources to stimulate calcium transfer. The intestines are a trivial source of mineral in the fetus but are the main source for the neonate. Intestinal calcium absorption is initially passive but later becomes active, saturable, and calcitriol-dependent. Within the endochondral skeleton, PTHrP is produced by proliferating chondrocytes and perichondrial cells (*arrowheads*) and delays terminal differentiation of pre-hypertrophic chondrocytes. PTHrP is also produced within pre-osteoblasts and osteoblasts and stimulates bone formation (*semicircular arrows*). During fetal life PTHrP and PTH both maintain high blood calcium and phosphorus levels to facilitate mineralization. Calcitriol is not required to regulate blood calcium, endochondral bone formation, or skeletal mineralization in the fetus. PTH—parathyroid hormone; PTHrP—parathyroid hormone–related protein; PTH1R— parathyroid hormone receptor type 1 AKA PTH/PTHrP receptor

Conclusions

At all life stages adequate delivery of mineral is required for the skeleton to achieve and maintain appropriate mineral content and strength (Fig. 1). The placenta supplies mineral to the fetus while passive and then active absorption from the intestines supplies the neonate. Fetal and postnatal bone development require PTH and PTH-related protein. When intestinal calcium absorption becomes an active process, skeletal development begins to depend upon vitamin D/calcitriol but this requirement can be bypassed by increasing the calcium content of the diet or by administering intermittent calcium infusions. Key messages of interest to clinicians are summarized in Table 1. Much remains unknown about the roles of the calciotropic hormones during fetal development, particularly potential skeletal and extraskeletal roles of vitamin D, and thus suggested priorities for future research are outlined in Table 2.

Table 1 Key messages of interest to clinicians

- High serum mineral levels in the fetus facilitate mineralization of the skeleton
- PTH and PTHrP are both required for normal development and mineralization of the endochondral skeleton
- The fetus does not require vitamin D, calcitriol, or the vitamin D receptor because in their absence, serum minerals and skeletal development are normal at term
- The neonate requires vitamin D/calcitriol for optimal intestinal calcium absorption to mineralize the skeleton and avoid hypocalcemia
- The neonatal requirement for vitamin D/calcitriol can be bypassed through use of a high calcium diet or intermittent calcium infusions

PTH parathyroid hormone; *PTHrP* parathyroid hormone–related protein

Table 2 Research agenda

- Randomized trials to determine if vitamin D supplementation during pregnancy improves calcium/bone-related outcomes in the fetus or neonate (skeletal development, mineralization, hypocalcemia)
- Randomized trials to determine if vitamin D supplementation during pregnancy improves any of the proposed extraskeletal outcomes of vitamin D in the baby (reduced type 1 diabetes, preterm birth, etc.)
- Randomized trials to determine if neonatal supplementation with calcium or vitamin D achieves any long-term benefit in bone mass and strength
- Randomized trials to determine whether a 25(OH)D blood level of 75 nmol/L or higher confers any clinical benefit to the fetus, neonate, or infant versus the current pediatric target of 50 nmol/L

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- • Of importance
- •• Of major importance

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