REVIEW

Endochondral bone growth, bone calcium accretion, and bone mineral density: how are they related?

Kannikar Wongdee · Nateetip Krishnamra ·
Narattaphol Charoenphandhu

Abstract Endochondral bone growth in young growing mammals or adult mammals with persistent growth plates progresses from proliferation, maturation and hypertrophy of growth plate chondrocytes to mineralization of cartilaginous matrix to form an osseous tissue. This complex process is tightly regulated by a number of factors with different impacts, such as genetics, endocrine/paracrine factors [e.g., PTHrP, $1,25(OH)_2D_3$, IGF-1, FGFs, and prolactin], and nutritional status (e.g., dietary calcium and vitamin D). Despite a strong link between growth plate function and elongation of the long bone, little is known whether endochondral bone growth indeed determines bone calcium accretion, bone mineral density (BMD), and/or peak bone mass. Since the process ends with cartilaginous matrix calcification, an increase in endochondral bone growth typically leads to more calcium accretion in the primary spongiosa and thus higher BMD. However, in lactating rats with enhanced trabecular bone resorption, bone elongation is inversely correlated with BMD. Although BMD can be increased by factors that enhance endochondral bone growth, the endochondral bone growth itself is unlikely to be an important determinant of peak bone mass since it is strongly determined by genetics.

K. Wongdee · N. Krishnamra · N. Charoenphandhu
Center of Calcium and Bone Research (COCAB),
Faculty of Science, Mahidol University, Bangkok, Thailand

K. Wongdee
Office of Academic Management,
Faculty of Allied Health Sciences, Burapha University,
Chonburi, Thailand

N. Krishnamra · N. Charoenphandhu (✉)
Department of Physiology, Faculty of Science,
Mahidol University, Rama VI Road, Bangkok 10400, Thailand
e-mail: naratt@narattsys.com

Therefore, endochondral bone growth and bone elongation are associated with calcium accretion only in a particular subregion of the long bone, but do not necessarily predict BMD and peak bone mass.

Introduction

As a primary structural framework of the body, bone formation takes place by two distinct mechanisms, i.e., intramembranous and endochondral ossifications. Most flat bones such as the skull are formed by intramembranous bone formation, in which bone tissue is laid down directly in primitive connective tissue or mesenchymes without being preceded by the formation of cartilage template [1–3]. On the other hand, long bones such as the tibia and femur are formed by endochondral bone formation, in which bone tissue replaces the preexisting cartilage template. Thus, endochondral bone growth requires precise timing of the sequential steps of proliferation and differentiation of growth plate chondrocytes [1–3]. Despite a considerable amount of information regarding endochondral bone growth, evidence of its influence over the subsequent bone calcium accretion, bone mineral density (BMD; mineral amount per a unit area of bone) or peak bone mass is sparse. In this review, we elaborate that the process of endochondral bone growth does affect bone calcium accumulation in the primary spongiosa near the growth plate as well as an increase in total bone length. Thus, under normal conditions, the resultant BMD should exhibit a positive correlation with bone elongation. However, under certain

Fig. 1 Longitudinal section of the growth plate (epiphyseal plate) of the proximal tibial metaphysis. The growth plate separates the epiphysis from the metaphysis, and is important for endochondral bone formation. The growth plate is divided into reserve zone (*RZ*), proliferative zone (*PZ*) and the hypertrophic zone (*HZ*), which is next by the primary spongiosa (*PS*)—the initial trabecular network formed after vascular invasion and matrix calcification (hematoxylin–eosin staining; *scale bar* 30 μm)
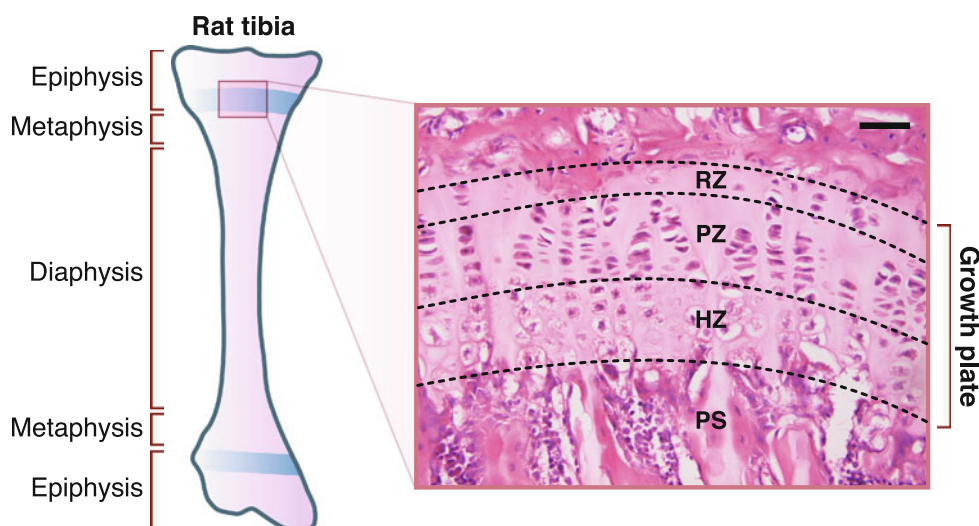
physiological conditions, such as in lactation, a correlation between bone elongation and BMD may be inversed.

Growth plate chondrocytes and endochondral bone growth

The growth plates located in the proximal and distal epiphyses near the ends of the long bone contain chondrocytes at different stages of development. Proliferation and differentiation of growth plate chondrocytes lead to growth and elongation of the bone [1, 2, 4]. Histologically, the growth plate is divided into 3 zones from metaphysis to diaphysis, i.e., reserve or resting zone, proliferative zone, and hypertrophic zone (Fig. 1) [1–3]. The reserve zone is rich in extracellular matrix proteins, e.g., proteoglycans and type-IIb collagen with embedded small, uniform, low mitotically active chondroblasts [2]. The reserved chondroblasts, in turn, go through the proliferative zone, where chondrocytes have highly proliferative capacity and are packed into parallel vertical columns. The synthesis of type-II and -XI collagens is increased in this zone [1, 2]. Towards the end of the proliferation phase, chondrocytes which progressively differentiate into mature cells, by increasing cell size (hypertrophy) and accumulating glycogen in their cytoplasm, start to secrete type-X collagen in abundance. This zone is called a hypertrophic zone [2]. Ultrastructurally, the mature chondrocytes contain abundant rough endoplasmic reticulum and developing Golgi apparatus with numerous filopodia on the plasma membrane [2, 4]. Mature chondrocytes then undergo degeneration by apoptosis, and the matrix is later invaded by capillaries, osteoblasts, and hematopoietic cells from the marrow [1, 2]. Cartilaginous matrix also acts as a scaffold for hydroxyapatite formation, and matrix calcification ensues. The rate of matrix production by hypertrophic chondrocytes and

calcification are important determinants of bone elongation [1, 2, 4]. The endochondral bone growth is thus the primary mechanism that determines the skeletal framework, bone morphology, and bone mineral accretion, while other factors, such as nutritional status, (patho)physiological conditions (e.g., lactation), physical activity, or exercise can impact the degree of calcium accumulation and bone microstructure. Exercise training is also capable of inducing chondrocyte proliferation and osteoblast-mediated bone formation in humans and rodents [5, 6]. Having said that, is it reasonable to propose that endochondral bone growth is an important determinant of bone calcium accretion, and thus BMD? Supporting evidence comes from Gafni and colleagues [7] who investigated bone recovery in dexamethasone-treated 5-week-old rabbits, and found that, although dexamethasone induced growth retardation and severe osteoporosis (low BMD), after stopping such a treatment, endochondral bone growth (represented by bone length), and cortical and trabecular bone mass were concurrently recovered within 16 weeks, with endochondral bone growth and BMD finally showing no difference between control and dexamethasone-treated groups. Therefore, endochondral bone growth and bone calcium accretion as represented by bone elongation and BMD, respectively, should generally show positive correlation. However, under certain physiological conditions, such as lactation, these two parameters may be inversely correlated (see below).

Local regulators of growth plate development and endochondral bone growth

Development of growth plate chondrocytes, i.e., proliferation, differentiation, maturation, and apoptosis, is tightly regulated by several factors, e.g., genetics, hormones, local
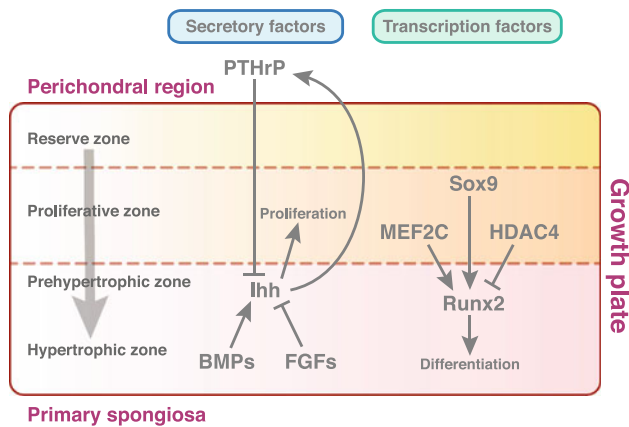
Fig. 2 An overview of the regulation of growth plate chondrocyte development by secretory and transcriptional factors. Parathyroid hormone-related peptide (PTHrP) synthesized from the perichondral cells in the epiphysis and growth plate chondrocytes acts directly on PTHrP receptor in the proliferative chondrocytes to keep chondrocytes in the proliferative stage. The prehypertrophic chondrocytes express Indian hedgehog homolog (Ihh) which acts as a negative feedback regulator that suppresses its own synthesis by stimulating PTHrP production, thereby preventing further differentiation of proliferative chondrocytes. Bone morphogenetic proteins (BMPs) promote Ihh production, whereas fibroblast growth factors (FGFs) decrease Ihh production. A proper chondrocyte development also requires regulation from transcription factors, such as Sox9, Runx2, and MEF2C, which induce chondrocyte proliferation and maturation. On the other hand, chondrocyte maturation is suppressed by the enzyme HDAC4

cytokines, and nutritional status, as well as individual lifestyle [1, 8, 9]. In addition to circulating hormones known to have crucial roles in the regulation of endochondral bone growth [e.g., growth hormone, insulin-like growth factor (IGF)-1, thyroid hormone, estrogen and androgens], transcription factors (e.g., Sox9 and Runx2), and secretory local factors, e.g., Indian hedgehog protein (Ihh), parathyroid hormone-related peptide (PTHrP), fibroblast growth factors (FGFs), and bone morphogenetic proteins (BMPs) produced by chondrocytes are also important for proper development and regulation of morphological heterogeneity of growth plate chondrocytes [1, 8–10]. An overview of important local factors and their interactions is depicted in Fig. 2.

In the proliferative zone, an obligatory protein for chondrocyte development is PTHrP. PTHrP is expressed and secreted by periarticular perichondrium and proliferative growth plate chondrocytes, which can bind to PTH/PTHrP receptor on both late proliferative and hypertrophic chondrocytes [1, 8, 11]. Binding of PTHrP and its receptor maintains chondrocytes in their proliferative state and prevents progression of proliferative chondrocytes to hypertrophic chondrocytes [1, 12]. However, the proliferative chondrocytes distant from the site of PTHrP production are able to escape from the influence of PTHrP,

thereby transforming into prehypertrophic and hypertrophic cells [1, 8, 9]. Meanwhile, the prehypertrophic chondrocytes begin to express Ihh as a negative feedback regulator to prevent proliferative chondrocyte progression into the hypertrophic stage [1, 8, 9, 11]. Ihh belongs to the family of hedgehog proteins, which play roles in embryonic patterning and development [1, 9, 10]. Once released, Ihh maintains PTHrP expression in proliferative chondrocytes, which in turn, slows down or inhibits Ihh production in a negative feedback manner [1, 2, 8]. When the prehypertrophic chondrocytes undergo hypertrophy, they produce type-X collagen, alkaline phosphatase, and transglutaminase (TG), which can be used as markers of hypertrophic chondrocytes [2, 13]. It is noteworthy that this is the first zone that produces alkaline phosphatase, an essential enzyme for the matrix calcification process [2]. TG2 produced by prehypertrophic chondrocytes acts partly to promote chondrocyte hypertrophy [2, 14].

In addition to PTHrP/Ihh, other important local secretory proteins that regulate growth plate development are FGFs and BMPs. BMPs are members of the transforming growth factor (TGF)-β family that have diverse roles in bone development (for review, see [9]). BMP-7 is expressed in proliferative chondrocytes, whereas BMP-6 is expressed in prehypertrophic and hypertrophic chondrocytes [1, 9, 15]. BMP signaling appears to promote Ihh production in prehypertrophic and hypertrophic chondrocytes, thereby increasing the number of proliferative chondrocytes and the thickness of the proliferative zone [9, 16]. On the other hand, FGF signaling through four isoforms of FGF receptors (FGFR) has an opposite effect to BMPs. For example, activation of FGFR3, which is expressed in proliferative and hypertrophic chondrocytes, inhibits chondrocyte proliferation and accelerates chondrocyte hypertrophy [1, 17]. The reciprocal effects of BMPs and FGFs on terminal differentiation of hypertrophic chondrocytes are essential for proper matrix calcification [9].

Besides the secretory proteins, an important transcription factor required for maturation of chondrocytes is the sex-determining region Y-box 9 (Sox9), one of the earliest markers of chondrocyte condensation, which is expressed in chondroprogenitors and proliferating chondrocytes [1, 9, 18]. Sox9 is essential for chondrocyte proliferation and delays the onset of chondrocyte hypertrophy [1, 19]. Inactivation of Sox9 in limb buds prior to mesenchymal condensation led to a complete absence of cartilage and bone formation. The embryo also manifested severe generalized chondrodysplasia and dwarfism [19]. Downstream to Sox9 is *runt*-related transcription factor (Runx)-2, an essential transcription factor that drives differentiation and hypertrophy of proliferative chondrocytes [2, 20, 21]. Runx2 is also an early transcription factor for commitment

of osteoblast lineage; therefore, an absence of Runx2 expression results in no osteoblast formation [9, 22]. In vivo evidence of delayed chondrocyte hypertrophy in Runx2 null mice confirmed its function by showing a delayed chondrocyte hypertrophy [15, 21]. Another evidence in Runx2/Runx3 double knockout mice showed a complete absence of chondrocyte maturation [21]. An in vitro study in prechondrogenic ATDC5 cells also suggested that Runx2 induced chondrogenic differentiation and hypertrophy [20]. Moreover, the function of Runx2 could be modulated by several intracellular proteins, such as myocyte enhancer factor-2C (MEF2C, a transcription factor) [23] and histone deacetylase-4 (HDAC4, an enzyme that removes acetyl groups from a histone), which enhances Runx2 expression and inhibits Runx2 activity, respectively [24]. Runx2 also promotes vascular invasion and initiates commitment of mesenchymal stem cell differentiation into osteoblasts [22, 25].

Anomalous up- or downregulation of Ihh and PTHrP expressions not only impair growth plate development but also affect whole body osteogenesis [26–29]. Target deletion of PTHrP or its receptor in mice was lethal and the fetus showed premature chondrocyte differentiation, leading to abnormally excessive bone formation at birth [27]. In contrast, overexpressed PTHrP mice showed chondrodysplasia, characterized by short-limbed dwarfism and delayed chondrocyte differentiation, leading to retardation of endochondral bone growth [28, 29]. Similarly, Ihh null mice showed dwarfism of all appendicular and axial skeletal elements, decreased chondrocyte proliferation, maturation of chondrocyte at inappropriate location, and failure of osteoblast development in cortical and trabecular parts of the long bones [26]. Although BMD was not directly determined in the aforementioned studies, deletion or overexpression of Ihh and PTHrP could have deleterious effect on bone structure and bone formation.

Endochondral bone growth and bone calcium accretion in vitamin deficiency

It is widely accepted that genetic background is a major factor controlling endochondral bone growth [8, 30]. However, nutritional status can also have effects on endochondral bone growth and calcium accretion [8, 31, 32]. A number of nutrients, e.g., vitamins, calcium, phosphate, and lactose, cooperatively provide sufficient amount of precursors required for bone growth [32–35].

Regarding the roles of vitamins in normal bone growth and development, vitamins A and D regulate endochondral bone growth presumably through their actions on growth plate chondrocytes [32, 36, 37]. The biologically active metabolite of vitamin D [i.e., 1,25-dihydroxyvitamin D_3;

$1,25(OH)_2D_3$] also indirectly induces matrix mineralization, calcium accretion, and bone growth by enhancing intestinal calcium absorption [38]. However, the direct roles of $1,25(OH)_2D_3$ on growth plate chondrocyte proliferation and differentiation are not completely understood. Vitamin D deficiency can induce abnormal bone growth known as rickets in the young and osteomalacia in adults. In rickets, failure of two separate processes are evident: (1) the growth plate chondrocytes fail to complete a sequential process of proliferation, differentiation and degeneration; and (2) the matured chondrocytes persist in the hypertrophic state without undergoing degeneration; therefore, there is no capillary invasion and matrix mineralization [34, 39–41]. An investigation in vitamin D receptor (VDR) knockout mice revealed the rachitic changes throughout the body. The growth plate of VDR knockout mice showed extensive disorganization of the chondrocyte column with an increase in the growth plate thickness [42]. Without proper bone formation, the accumulated osteoid (nonmineralized matrix) and cartilage are distorted by the pressure from weight bearing, thus producing pathological features such as bowed legs [34, 39, 41]. The severity depends on duration and degree of vitamin D deficiency.

In vivo studies of 8-day-old vitamin D-deficient chick showed an absence of PTH/PTHrP receptor expression in the growth plate chondrocytes [43]. This reduction of PTH/PTHrP receptor expression in the rachitic chicks was apparently due to compensatory high plasma PTH, which downregulated PTH/PTHrP receptor expression [43]. In addition to the effect on the growth plate, local vitamin D deficiency also affects vascular invasion [37, 44]. Chondrocyte-specific inactivation of Cyp27b1 which is the $1,25(OH)_2D_3$-synthesizing enzyme in mice led to decreased mRNA expression of vascular endothelial growth factor (VEGF), an essential growth factor for vascular invasion [37, 44]. Such a decrease in angiogenesis may partly contribute to growth plate deformity and stunt growth. Although rickets may also lead to abnormal endosteal and periosteal ossification, low BMD in the patients partly results from impaired endochondral bone formation [39]. It is possible that, regardless of the calcipenic or phosphopenic causes of rickets, failure of apoptosis of the growth plate hypertrophic chondrocytes, which is a common underlying mechanism of rickets, may lead to a reduction in the space of primary spongiosa for calcification of the cartilaginous matrix, thereby resulting in impaired osteogenesis and reduced bone elongation. This might, in turn, result in low bone mass in rachitic individuals.

Vitamin A deficiency can have profound deleterious effects on endochondral bone growth. Deficiency of vitamin A suppresses all stages of growth plate chondrocyte development, resulting in thin bony trabeculae formed

across the face of the growth plate [34]. Thus, the long bones of vitamin A-deficient animals are shorter and thicker than normal. The shortness of long bone is caused by failure of endochondral bone growth, whereas an increase in bone thickness results from an imbalance between bone calcium apposition and remodeling process, the former of which appears to be predominant [34]. An in vitro study in primary growth plate chondrocyte culture revealed that retinoic acid (a metabolite of vitamin A) is important for growth plate maturation by modulating Ihh and PTHrP receptors and increasing Runx2 mRNA expression [45, 46].

Vitamin K deficiency also potentially attenuates endochondral bone growth [47, 48]. Price and colleagues [47] reported that rats treated with vitamin K antagonist warfarin for 8 months exhibited a severe growth plate disorder characterized by complete fusion of the tibial growth plate and cessation of longitudinal bone growth, resembling fetal warfarin syndrome in human. Such a growth plate defect appeared to result from impaired production of vitamin K-dependent proteins, namely osteocalcin and matrix Gla protein (MGP) [48]. In general, osteocalcin, a protein with a binding capacity with calcium, is expressed and secreted not only by mature osteoblasts, but also by hypertrophic chondrocytes, and thus may serve as a scaffold for calcification of cartilaginous matrix [49, 50]. MGP also has a high affinity for calcium, and mutations in MGP gene are responsible for Keutel syndrome, an autosomal recessive disorder with abnormal cartilage calcification [51].

Endochondral bone growth and bone calcium accretion in pregnancy and lactation

Interestingly, an increase in endochondral bone growth is also observed during pregnancy and lactation in humans, sheep, and rodents, of which the growth plate cartilage is intact [52–55]. High BMD is usually observed in pregnant rats, whereas lactating rats have low BMD [54, 56], suggesting that endochondral bone growth is not always positively correlated with BMD. An investigation in female mole rats revealed that the femur and lumbar vertebral lengths were increased during pregnancy [57, 58]. Similarly, femoral and tibial lengths of pregnant and lactating rats were increased from mid-pregnancy until the end of lactation [54, 55]. This elongation of the long bones was inversely correlated with the thickness of the total growth plate and the hypertrophic zone [55]. Although the underlying cellular mechanism of the growth plate changes during lactation remains elusive, an absence of bone elongation and a reduction in the hypertrophic zone height in lactating rats treated with an inhibitor of prolactin release, bromocriptine, suggests that the lactogenic

hormone prolactin from the pituitary gland could be an important regulatory factor responsible for the reduction of the hypertrophic zone height during lactation [55]. Besides prolactin, PTHrP secreted from the mammary gland during lactation and suckling may also contribute to the lactation-induced growth plate changes, since PTHrP can modulate Ihh production [10, 26]. Normally, growth plate height is an outcome of a balance between chondrogenesis and osteogenesis. It is, therefore, possible that prolactin might induce hypertrophic chondrocyte apoptosis to accelerate matrix calcification, which leads to bone elongation [54, 55]. It is noted that, to provide adequate calcium for milk production during lactation, prolactin also stimulates the intestinal calcium absorption and trabecular bone resorption [54, 59], the latter of which culminates in low BMD in lactating mothers [56, 59]. It is reasonable to speculate that, with bone loss from enhanced bone resorption, the process of bone elongation (a product of endochondral bone formation) may help replenish the maternal bone trabeculae so as to retain bone mass and to compensate for the reduction in BMD.

Long-term exposure to prolactin not only regulates maternal bones but, during the neonatal period, may contribute to longitudinal bone growth in the newborn. Several investigators have reported that the serum prolactin levels in the newborn ranging between 140–500 ng/mL are much higher than that in non-pregnant adults (~ 7–10 ng/mL) [60–62]. Hwang and co-workers [61] investigated changes in serum prolactin in pregnancy, postpartum, newborn infants, and children, and found that serum prolactin concentrations in the newborn at term were comparable to the maternal levels. After delivery, prolactin levels in the newborn progressively declined to the adult levels within 6 weeks. However, since ~ 16 % of the ingested prolactin subsequently appeared in the plasma of the rat neonate, the infants still continuously received exogenous prolactin through breast milk [63]. Since an in vitro experiment in human mesenchymal stem cells demonstrated that prolactin stimulated differentiation of these cells into chondrocytes [64], prolactin might act in concert with other hormones to enhance endochondral bone growth in newborns.

Relationship between endochondral bone growth and peak bone mass: does endochondral bone growth determine peak bone mass?

Peak bone mass is defined as BMD during the stable period following growth and accrual of bone mass prior to subsequent age-related bone loss [65]. In normal intrauterine development, a high rate of longitudinal bone growth is observed during the third trimester of fetal life, and bone

growth gradually decelerates until 3 years of age [8, 52]. In early puberty, the rate of longitudinal bone growth is substantially increased again reaching a plateau peak bone mass in adulthood [8, 31]. It is believed that early attainment of high peak bone mass is protective against osteoporosis later in life [7, 30]. However, besides the genetic factor, whether endochondral bone growth determines peak bone mass is controversial.

There are only a few studies on the relationship between endochondral bone growth and peak bone mass. Some investigations suggest that peak bone mass is not directly dependent on endochondral bone growth. Johnston and co-workers [66] performed a 3-year, double blind placebo-controlled trial to see the effect of calcium supplement on bone mineral density in identical twins. After a 3-year follow-up, they found that dietary calcium supplement could induce gain of bone mass in prepubertal twins and potentially resulted in high peak bone mass. However, although bone gain was evident, the heights of the children were not different. In another cohort study, male subjects who experienced short breastfeeding duration ($\leq$3 months) during their infancy had higher peak bone mass in adult life, but the height was not different as compared to subjects with long breastfeeding duration [67].

Gain of bone mass without height change (or endochondral bone growth) and vice versa suggest that endochondral bone growth is not a direct determinant of peak bone mass. Nevertheless, some growth factors or hormones, such as FGF-2 and progesterone, may interact with the genetic background to affect bone mass, rather than endochondral bone growth [68, 69]. FGF-2, a pleiotropic mitogen of the FGF family, is expressed in several cell types, e.g., heart, lung, spleen, and osteoblasts [70, 71]. Previous in vivo investigation revealed that overexpression of FGF-2 caused achondrodysplasia of the growth plate and shortening of the long bones. The growth plate height was increased by hyperplasia of the reserve and proliferative zones, but the hypertrophic zone was nearly absent [70]. Similarly, systemic FGF-2 administration in rats also decreased longitudinal growth rate and growth plate chondrocyte proliferation, but increased bone formation through an increase in osteoprogenitor cell proliferation [68, 72].

Besides FGF-2, progesterone is another hormone that may control both endochondral bone growth and peak bone mass through progesterone receptor in osteoblasts and osteoclasts [69, 73]. Recent investigation in progesterone receptor knockout (PRKO) mice showed increases in total, cancellous, and cortical bone mass, without changes in the tibial longitudinal bone growth [69]. Yao and colleagues [73] performed a longitudinal study in 1- to 12-month-old PRKO mice and found that these mice developed higher peak bone mass at both cancellous and cortical sites.

However, this higher bone mass was not associated with endochondral bone growth, i.e., no difference in femoral lengths of PRKO versus wild-type littermates.

Perspective and concluding remarks

It could be concluded that endochondral bone growth and bone elongation are associated with bone calcium accretion, at least in a subregion of the spongiosa. In most cases, this calcium accretion leads to an increase in BMD. Interestingly, in lactation, trabecular bone resorption results in decreased BMD concurrently with bone elongation. However, the endochondral bone growth and the resultant bone elongation do not directly determine peak bone mass, which is presumably predetermined by genetic factors.

Is there any factor that can increase both endochondral bone growth and peak bone mass? It is reasonable to propose that one of the potential factors could be exercise, which has been known to induce chondrocyte proliferation and osteoblast-mediated bone formation [5, 6]. Although physical activity and exercise are the major lifestyle determinants of BMD and body height [74], types, duration, or intensity of exercise, or the exercise training protocol, that most efficiently improve endochondral bone growth and peak bone mass require more investigation. Weight-bearing impact exercises, e.g., jogging and gymnastics, usually increase bone mass [75–78], whereas non-impact exercises, e.g., swimming, increase bone length and body height [79, 80]. It is also possible that endochondral bone growth as represented by bone length primarily determines the size of the skeletal framework, whereas certain regular exercise may increase calcium accretion and mineral density within the skeletal structure, thereby positively correlating the endochondral bone growth with peak bone mass especially in young growing individuals.

References

1. Mackie EJ, Ahmed YA, Tatarczuch L, Chen KS, Mirams M (2008) Endochondral ossification: how cartilage is converted into bone in the developing skeleton. Int J Biochem Cell Biol 40:46–62

2. Burdan F, Szumiło J, Korobowicz A, Farooquee R, Patel S, Patel A, Dave A, Szumiło M, Solecki M, Klepacz R, Dudka J (2009) Morphology and physiology of the epiphyseal growth plate. Folia Histochem Cytobiol 47:5–16

3. Villemure I, Stokes IAF (2009) Growth plate mechanics and mechanobiology. A survey of present understanding. J Biomech 42:1793–1803

4. Kierszenbaum AL, Tres LL (2012) Osteogenesis. In: Kierszenbaum AL, Tres LL (eds) Histology and cell biology: an introduction to pathology, 3rd edn. Saunders, Philadelphia, pp 151–168

5. Plochocki JH, Riscigno CJ, Garcia M (2006) Functional adaptation of the femoral head to voluntary exercise. Anat Rec A Discov Mol Cell Evol Biol 288:776–781

6. Fujimura R, Ashizawa N, Watanabe M, Mukai N, Amagai H, Fukubayashi T, Hayashi K, Tokuyama K, Suzuki M (1997) Effect of resistance exercise training on bone formation and resorption in young male subjects assessed by biomarkers of bone metabolism. J Bone Miner Res 12:656–662

7. Gafni RI, McCarthy EF, Hatcher T, Meyers JL, Inoue N, Reddy C, Weise M, Barnes KM, Abad V, Baron J (2002) Recovery from osteoporosis through skeletal growth: early bone mass acquisition has little effect on adult bone density. FASEB J 16:736–738

8. van der Eerden BCJ, Karperien M, Wit JM (2003) Systemic and local regulation of the growth plate. Endocr Rev 24:782–801

9. Kronenberg HM (2003) Developmental regulation of the growth plate. Nature 423:332–336

10. Lai LP, Mitchell J (2005) Indian hedgehog: its roles and regulation in endochondral bone development. J Cell Biochem 96:1163–1173

11. Vortkamp A, Lee K, Lanske B, Segre GV, Kronenberg HM, Tabin CJ (1996) Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related protein. Science 273:613–622

12. Lee K, Lanske B, Karaplis AC, Deeds JD, Kohno H, Nissenson RA, Kronenberg HM, Segre GV (1996) Parathyroid hormone-related peptide delays terminal differentiation of chondrocytes during endochondral bone development. Endocrinology 137:5109–5118

13. Nurminskaya M, Linsenmayer TF (1996) Identification and characterization of up-regulated genes during chondrocyte hypertrophy. Dev Dyn 206:260–271

14. Huebner JL, Johnson KA, Kraus VB, Terkeltaub RA (2009) Transglutaminase 2 is a marker of chondrocyte hypertrophy and osteoarthritis severity in the Hartley guinea pig model of knee OA. Osteoarthr Cartil 17:1056–1064

15. Inada M, Yasui T, Nomura S, Miyake S, Deguchi K, Himeno M, Sato M, Yamagiwa H, Kimura T, Yasui N, Ochi T, Endo N, Kitamura Y, Kishimoto T, Komori T (1999) Maturational disturbance of chondrocytes in Cbfa1-deficient mice. Dev Dyn 214:279–290

16. Minina E, Wenzel HM, Kreschel C, Karp S, Gaffield W, McMahon AP, Vortkamp A (2001) BMP and Ihh/PTHrP signaling interact to coordinate chondrocyte proliferation and differentiation. Development 128:4523–4534

17. Minina E, Kreschel C, Naski MC, Ornitz DM, Vortkamp A (2002) Interaction of FGF, Ihh/Pthlh, and BMP signaling integrates chondrocyte proliferation and hypertrophic differentiation. Dev Cell 3:439–449

18. Wuelling M, Vortkamp A (2010) Transcriptional networks controlling chondrocyte proliferation and differentiation during endochondral ossification. Pediatr Nephrol 25:625–631

19. Akiyama H, Chaboissier MC, Martin JF, Schedl A, de Crombrugghe B (2002) The transcription factor Sox9 has essential roles in successive steps of the chondrocyte differentiation pathway and is required for expression of Sox5 and Sox6. Genes Dev 16:2813–2828

20. Fujita T, Azuma Y, Fukuyama R, Hattori Y, Yoshida C, Koida M, Ogita K, Komori T (2004) Runx2 induces osteoblast and chondrocyte differentiation and enhances their migration by coupling with PI3K-Akt signaling. J Cell Biol 166:85–95

21. Yoshida CA, Yamamoto H, Fujita T, Furuichi T, Ito K, Inoue K, Yamana K, Zanma A, Takada K, Ito Y, Komori T (2004) Runx2 and Runx3 are essential for chondrocyte maturation, and Runx2 regulates limb growth through induction of Indian hedgehog. Genes Dev 18:952–963

22. Ducy P, Schinke T, Karsenty G (2000) The osteoblast: a sophisticated fibroblast under central surveillance. Science 289:1501–1504

23. Arnold MA, Kim Y, Czubryt MP, Phan D, McAnally J, Qi X, Shelton JM, Richardson JA, Bassel-Duby R, Olson EN (2007) MEF2C transcription factor controls chondrocyte hypertrophy and bone development. Dev Cell 12:377–389

24. Vega RB, Matsuda K, Oh J, Barbosa AC, Yang X, Meadows E, McAnally J, Pomajzl C, Shelton JM, Richardson JA, Karsenty G, Olson EN (2004) Histone deacetylase 4 controls chondrocyte hypertrophy during skeletogenesis. Cell 119:555–566

25. Himeno M, Enomoto H, Liu W, Ishizeki K, Nomura S, Kitamura Y, Komori T (2002) Impaired vascular invasion of Cbfa1-deficient cartilage engrafted in the spleen. J Bone Miner Res 17:1297–1305

26. St-Jacques B, Hammerschmidt M, McMahon AP (1999) Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. Genes Dev 13:2072–2086

27. Karaplis AC, Luz A, Glowacki J, Bronson RT, Tybulewicz VLJ, Kronenberg HM, Mulligan RC (1994) Lethal skeletal dysplasia from targeted disruption of the parathyroid hormone-related peptide gene. Genes Dev 8:277–289

28. Weir EC, Philbrick WM, Amling M, Neff LA, Baron R, Broadus AE (1996) Targeted overexpression of parathyroid hormone-related peptide in chondrocytes causes chondrodysplasia and delayed endochondral bone formation. Proc Natl Acad Sci USA 93:10240–10245

29. Toribio RE, Brown HA, Novince CM, Marlow B, Hernon K, Lanigan LG, Hildreth BE 3rd, Werbeck JL, Shu ST, Lorch G, Carlton M, Foley J, Boyaka P, McCauley LK, Rosol TJ (2010) The midregion, nuclear localization sequence, and C terminus of PTHrP regulate skeletal development, hematopoiesis, and survival in mice. FASEB J 24:1947–1957

30. Gafni RI, Baron J (2007) Childhood bone mass acquisition and peak bone mass may not be important determinants of bone mass in late adulthood. Pediatrics 119(Suppl 2):S131–S136

31. Heaney RP, Abrams S, Dawson-Hughes B, Looker A, Marcus R, Matkovic V, Weaver C (2000) Peak bone mass. Osteoporos Int 11:985–1009

32. Goldberg G (2006) Nutrition and bone. Women Health Med 3:157–159

33. Itakura C, Yamasaki K, Goto M, Takahashi M (1978) Pathology of experimental vitamin D deficiency rickets in growing chickens. I. Bone. Avian Pathol 7:491–513

34. Wolbach SB (1947) Vitamin-A deficiency and excess in relation to skeletal growth. J Bone Joint Surg Am 29:171–192

35. Miller SC, Miller MA, Omura TH (1988) Dietary lactose improves endochondral growth and bone development and mineralization in rats fed a vitamin D-deficient diet. J Nutr 118:72–77

36. Prentice A, Schoenmakers I, Laskey MA, de Bono S, Ginty F, Goldberg GR (2006) Nutrition and bone growth and development. Proc Nutr Soc 65:348–360

37. St-Arnaud R, Naja RP (2011) Vitamin D metabolism, cartilage and bone fracture repair. Mol Cell Endocrinol 347:48–54

38. Christakos S (2012) Mechanism of action of 1,25-dihydroxyvitamin D_3 on intestinal calcium absorption. Rev Endocr Metab Disord 13:39–44

39. Berry JL, Davies M, Mee AP (2002) Vitamin D metabolism, rickets, and osteomalacia. Semin Musculoskelet Radiol 6:173–181

40. Tiosano D, Hochberg Z (2009) Hypophosphatemia: the common denominator of all rickets. J Bone Miner Metab 27:392–401

41. Mughal MZ (2011) Rickets. Curr Osteoporos Rep 9:291–299

42. Amling M, Priemel M, Holzmann T, Chapin K, Rueger JM, Baron R, Demay MB (1999) Rescue of the skeletal phenotype of vitamin D receptor-ablated mice in the setting of normal mineral ion homeostasis: formal histomorphometric and biomechanical analyses. Endocrinology 140:4982–4987

43. Ben-Bassat S, Genina O, Lavelin I, Leach RM, Pines M (1999) Parathyroid receptor gene expression by epiphyseal growth plates in rickets and tibial dyschondroplasia. Mol Cell Endocrinol 149:185–195

44. Naja RP, Dardenne O, Arabian A, St Arnaud R (2009) Chondrocyte-specific modulation of Cyp27b1 expression supports a role for local synthesis of 1,25-dihydroxyvitamin D_3 in growth plate development. Endocrinology 150:4024–4032

45. Yoshida E, Noshiro M, Kawamoto T, Tsutsumi S, Kuruta Y, Kato Y (2001) Direct inhibition of Indian hedgehog expression by parathyroid hormone (PTH)/PTH-related peptide and up-regulation by retinoic acid in growth plate chondrocyte cultures. Exp Cell Res 265:64–72

46. Kirimoto A, Takagi Y, Ohya K, Shimokawa H (2005) Effects of retinoic acid on the differentiation of chondrogenic progenitor cells, ATDC5. J Med Dent Sci 52:153–162

47. Price PA, Williamson MK, Haba T, Dell RB, Jee WSS (1982) Excessive mineralization with growth plate closure in rats on chronic warfarin treatment. Proc Natl Acad Sci USA 79:7734–7738

48. Feteih R, Tassinari MS, Lian JB (1990) Effect of sodium warfarin on vitamin K-dependent proteins and skeletal development in the rat fetus. J Bone Miner Res 5:885–894

49. Pullig O, Weseloh G, Ronneberger D, Käkönen S, Swoboda B (2000) Chondrocyte differentiation in human osteoarthritis: expression of osteocalcin in normal and osteoarthritic cartilage and bone. Calcif Tissue Int 67:230–240

50. Gerstenfeld LC, Shapiro FD (1996) Expression of bone-specific genes by hypertrophic chondrocytes: implication of the complex functions of the hypertrophic chondrocyte during endochondral bone development. J Cell Biochem 62:1–9

51. Munroe PB, Olgunturk RO, Fryns JP, Van Maldergem L, Ziereisen F, Yuksel B, Gardiner RM, Chung E (1999) Mutations in the gene encoding the human matrix Gla protein cause Keutel syndrome. Nat Genet 21:142–144

52. Bowman BM, Miller SC (2001) Skeletal adaptations during mammalian reproduction. J Musculoskelet Neuronal Interact 1:347–355

53. Dengler-Crish CM, Catania KC (2009) Cessation of reproduction-related spine elongation after multiple breeding cycles in female naked mole-rats. Anat Rec (Hoboken) 292:131–137

54. Suntornsaratoon P, Wongdee K, Goswami S, Krishnamra N, Charoenphandhu N (2010) Bone modeling in bromocriptine-treated pregnant and lactating rats: possible osteoregulatory role of prolactin in lactation. Am J Physiol Endocrinol Metab 299:E426–E436

55. Suntornsaratoon P, Wongdee K, Krishnamra N, Charoenphandhu N (2010) Possible chondroregulatory role of prolactin on the tibial growth plate of lactating rats. Histochem Cell Biol 134:483–491

56. Suntornsaratoon P, Wongdee K, Krishnamra N, Charoenphandhu N (2010) Femoral bone mineral density and bone mineral content in bromocriptine-treated pregnant and lactating rats. J Physiol Sci 60:1–8

57. Henry EC, Dengler-Crish CM, Catania KC (2007) Growing out of a caste—reproduction and the making of the queen mole-rat. J Exp Biol 210:261–268

58. Redd EH, Miller SC, Jee WSS (1984) Changes in endochondral bone elongation rates during pregnancy and lactation in rats. Calcif Tissue Int 36:697–701

59. Nakkrasae LI, Thongon N, Thongbunchoo J, Krishnamra N, Charoenphandhu N (2010) Transepithelial calcium transport in prolactin-exposed intestine-like Caco-2 monolayer after combinatorial knockdown of TRPV5, TRPV6 and $Ca_v1.3$. J Physiol Sci 60:9–17

60. Badawi M, Van Exter C, Delogne-Desnoeck J, Van Meenen F, Robyn C (1978) Cord serum prolactin in relation to the time of the day, the sex of the neonate and the birth weight. Acta Endocrinol (Copenh) 87:241–247

61. Hwang P, Guyda H, Friesen H (1971) A radioimmunoassay for human prolactin. Proc Natl Acad Sci USA 68:1902–1906

62. Smith YF, Mullon DK, Hamosh M, Scanlon JW, Hamosh P (1979) Serum prolactin and respiratory distress syndrome in the newborn. Pediatr Res 14:93–95

63. Whitworth NS, Grosvenor CE (1978) Transfer of milk prolactin to the plasma of neonatal rats by intestinal absorption. J Endocrinol 79:191–199

64. Romero-Prado M, Blázquez C, Rodríguez-Navas C, Muñoz J, Guerrero I, Delgado-Baeza E, García-Ruiz JP (2006) Functional characterization of human mesenchymal stem cells that maintain osteochondral fates. J Cell Biochem 98:1457–1470

65. Berger C, Goltzman D, Langsetmo L, Joseph L, Jackson S, Kreiger N, Tenenhouse A, Davison KS, Josse RG, Prior JC, Hanley DA, CaMos Research Group (2010) Peak bone mass from longitudinal data: implications for the prevalence, pathophysiology, and diagnosis of osteoporosis. J Bone Miner Res 25:1948–1957

66. Johnston CC, Miller JZ, Slemenda CW, Reister TK, Hui S, Christian JC, Peacock M (1992) Calcium supplementation and increases in bone mineral density in children. N Engl J Med 327:82–87

67. Pirilä S, Taskinen M, Viljakainen H, Kajosaari M, Turanlahti M, Saarinen-Pihkala UM, Mäkitie O (2011) Infant milk feeding influences adult bone health: a prospective study from birth to 32 years. PLoS ONE 6:e19068. doi:10.1371/journal.pone.0019068

68. Nagai H, Tsukuda R, Yamasaki H, Mayahara H (1999) Systemic injection of FGF-2 stimulates endocortical bone modelling in SAMP6, a murine model of low turnover osteopenia. J Vet Med Sci 61:869–875

69. Rickard DJ, Iwaniec UT, Evans G, Hefferan TE, Hunter JC, Waters KM, Lydon JP, O'Malley BW, Khosla S, Spelsberg TC, Turner RT (2008) Bone growth and turnover in progesterone receptor knockout mice. Endocrinology 149:2383–2390

70. Coffin JD, Florkiewicz RZ, Neumann J, Mort-Hopkins T, Dorn GW 2nd, Lightfoot P, German R, Howles PN, Kier A, O'Toole BA, Sasse J, Gonzalez AM, Baird A, Doetschman T (1995) Abnormal bone growth and selective translational regulation in basic fibroblast growth factor (FGF-2) transgenic mice. Mol Biol Cell 6:1861–1873

71. Montero A, Okada Y, Tomita M, Ito M, Tsurukami H, Nakamura T, Doetschman T, Coffin JD, Hurley MM (2000) Disruption of the fibroblast growth factor-2 gene results in decreased bone mass and bone formation. J Clin Invest 105:1085–1093

72. Mayahara H, Ito T, Nagai H, Miyajima H, Tsukuda R, Taketomi S, Mizoguchi J, Kato K (1993) In vivo stimulation of endosteal bone formation by basic fibroblast growth factor in rats. Growth Factors 9:73–80

73. Yao W, Dai W, Shahnazari M, Pham A, Chen Z, Chen H, Guan M, Lane NE (2010) Inhibition of the progesterone nuclear receptor during the bone linear growth phase increases peak bone mass in female mice. PLoS ONE 5:e11410. doi:10.1371/journal.pone.0011410

74. Cooper C, Cawley M, Bhalla A, Egger P, Ring F, Morton L, Barker D (1995) Childhood growth, physical activity, and peak bone mass in women. J Bone Miner Res 10:940–947

75. Bauer DC, Browner WS, Cauley JA, Orwoll ES, Scott JC, Black DM, Tao JL, Cummings SR (1993) Factors associated with appendicular bone mass in older women. Ann Intern Med 118:657–665

76. Bradney M, Pearce G, Naughton G, Sullivan C, Bass S, Beck T, Carlson J, Seeman E (1998) Moderate exercise during growth in prepubertal boys: changes in bone mass, size, volumetric density, and bone strength: a controlled prospective study. J Bone Miner Res 13:1814–1821

77. Vainionpää A, Korpelainen R, Leppäluoto J, Jämsä T (2005) Effects of high-impact exercise on bone mineral density: a randomized controlled trial in premenopausal women. Osteoporos Int 16:191–197

78. Welten DC, Kemper HC, Post GB, van Mechelen W, Twisk J, Lips P, Teule GJ (1994) Weight-bearing activity during youth is a more important factor for peak bone mass than calcium intake. J Bone Miner Res 9:1089–1096

79. Taaffe DR, Snow-Harter C, Connolly DA, Robinson TL, Brown MD, Marcus R (1995) Differential effects of swimming versus weight-bearing activity on bone mineral status of eumenorrheic athletes. J Bone Miner Res 10:586–593

80. Swissa-Sivan A, Simkin A, Leichter I, Nyska A, Nyska M, Statter M, Bivas A, Menczel J, Samueloff S (1989) Effect of swimming on bone growth and development in young rats. Bone Miner 7:91–105