The Actions of IGF-1 in the Growth Plate and Its Role in Postnatal Bone Elongation

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Abstract

Purpose of Review Bone elongation is a complex process driven by multiple intrinsic (hormones, growth factors) and extrinsic (nutrition, environment) variables. Bones grow in length by endochondral ossification in cartilaginous growth plates at ends of developing long bones. This review provides an updated overview of the important factors that influence this process.

Recent Findings Insulin-like growth factor-1 (IGF-1) is the major hormone required for growth and a drug for treating pediatric skeletal disorders. Temperature is an underrecognized environmental variable that also impacts linear growth. This paper reviews the current state of knowledge regarding the interaction of IGF-1 and environmental factors on bone elongation.

Summary Understanding how internal and external variables regulate bone lengthening is essential for developing and improving treatments for an array of bone elongation disorders. Future studies may benefit from understanding how these unique relationships could offer realistic new approaches for increasing bone length in different growth-limiting conditions.

Keywords Growth plate · Linear growth · Endochondral ossification · Temperature · Insulin-like growth factor-1 (IGF-1) · Chondrocytes · Vasculature

Introduction

Our understanding of the molecular events that control postnatal bone elongation in cartilage growth plates has burgeoned over the last several decades. Bone growth rate is almost entirely dependent on the dynamics of cartilaginous growth plates [1]. Long bones (such as humerus, radius/ulna, femur, and tibia/fibula) lengthen as chondrocytes of the growth plate divide and expand, establishing a model for bone through the process of

endochondral ossification. This process is regulated by local and systemic growth factors, including those of the somatotropic axis, which is the signaling between growth hormone (GH) and insulin-like growth factor-1 (IGF-1). IGF-1 is the major regulator of growth and controls bone elongation by promoting chondrocyte proliferation and hypertrophy [2, 3••]. Environmental factors, such as temperature, can also surprisingly alter bone and cartilage growth in both natural and laboratory settings [4].

The aim of this review is to critically examine the factors that influence bone elongation with respect to their effects on IGF-1 activity in the growth plate. This review focuses in particular on how further understanding of these factors is beneficial for developing methods to combat an array of bone elongation disorders when the processes of longitudinal bone growth are disrupted.

Processes of Bone Elongation

Endochondral Ossification and Growth Plate Morphology

The skeleton develops by means of two different mechanisms: (1) intramembranous ossification, involving direct differentiation

of mesenchyme cells to bone as with the development of the flat bones of the skull, and (2) endochondral ossification, occurring when mesenchyme cells condense and differentiate into cartilage tissue, which then is replaced by bone forming the vertebrae, ribs, and limbs [5]. Endochondral ossification is initiated during fetal life and continues from infancy through adolescence (transitional period from childhood to adulthood). Cartilaginous growth plates situated at the ends of developing long bones function as the command center for bone lengthening (or linear growth in humans). Growth plate cartilage is composed of cells known as chondrocytes that secrete extracellular matrix and are organized into functional zones (Fig. 1).

Growth plates are anatomically located between the epiphysis and metaphysis of long bones. The end portion of the long bone is referred to as the epiphysis and the shaft is termed the diaphysis. Directly between the epiphysis and the diaphysis is the transitional zone, or metaphysis, which contains newly mineralized bone at the bone-cartilage interface (chondro-osseous junction, COJ). The region adjacent to the epiphysis is the reserve zone (RZ). The RZ consists of round quiescent chondrocytes. The role of the RZ has been debated but is most commonly reported to act as the coordinator for the organization and orientation of the neighboring proliferative zone (PZ) [6]. It has been suggested that the RZ contains stem-like cells that promote the production of proliferative chondrocytes and that this proliferative capacity decreases with age aiding to the

closure of the growth plate associated with skeletal maturity [6–9]. Adjacent to the RZ is the PZ, which contains rapidly dividing chondrocytes that are stacked and flattened in multi-cellular columns. The PZ is a region of actively dividing cells that directs longitudinal growth along the long axis of the bone, in which the cells are aligned in columns.

The proliferating chondrocytes at the base of the columns make the transition from the PZ to the hypertrophic zone (HZ) where chondrocytes behave as terminally differentiated cells that begin to enlarge, secrete extracellular matrix, and were once thought to ultimately undergo physiological death [10–13]. Hypertrophic chondrocytes induce vascular invasion and recruit osteogenic cells in the replacement of cartilage with bone at the chondro-osseous junction (the interface between growth cartilage and newly mineralized metaphyseal bone). A majority of the bone-forming osteoblasts are thought to be differentiated from bone marrow stromal cells. However, current research in the mammalian growth plate suggests that not all hypertrophic chondrocytes undergo apoptosis but instead transdifferentiate into osteoblasts during both bone development and repair [14••, 15, 16••, 17]. Studies by Bahney et al. [14••] used cartilage grafts to promote bone regeneration through endochondral ossification (analogous to long bone development). Their results from lineage tracing experiments showed that chondrocytes directly differentiated into osteoblasts during bone repair [14••]. It is thought that since transdifferentiation occurs adjacent to the vasculature and that the vasculature may have a signaling role in the transformation of chondrocytes to osteoblasts [16••]. Emerging research shows that this chondrocyte-to-osteoblast transdifferentiation is a critical component of bone elongation through the process of endochondral ossification [18].
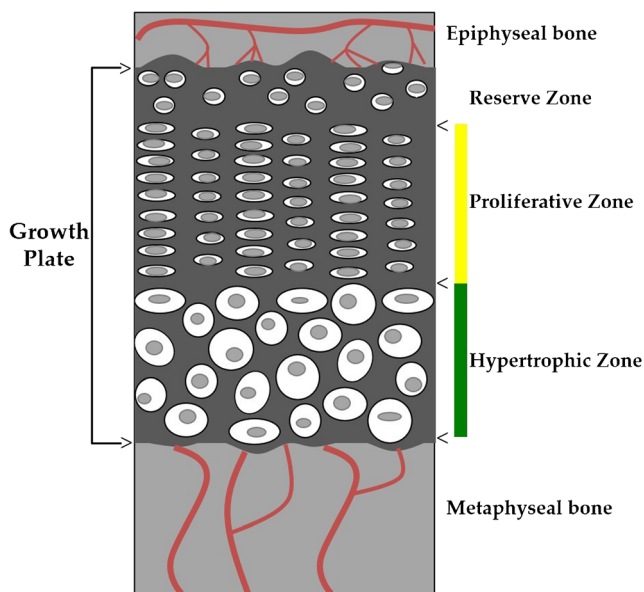
Differential Growth Plate Activity

While the anatomical structure of the growth plate is comparable between the developing long bones, the rate at which these growth plates contributes to bone lengthening differs from bone to bone, and from proximal end to distal end. This phenomenon is referred to as differential growth [19, 20]. The humerus, radius, and ulna are the major long bones of the upper limb (excluding the hand and foot), and the femur and tibia comprise the lower limb. When comparing proximal to distal ends of these bones, the faster-growing sites are located at the proximal humerus (shoulder), distal radius and ulna (wrist), and distal femur and proximal tibia (knee) [1, 8, 21–27]. The most active developing growth plate in humans and rodents is the proximal tibia followed by the distal femur (knee), distal radius (wrist), and proximal humerus (shoulder) [28]. Wilsman et al. [27] have shown that the proximal tibial growth plate of 28-day-old rats (growth rate of 396 μM/day) is nearly nine times faster than the much slower proximal radial growth plate (growth rate of 47 μM/day).

Fig. 1 Diagram of a growth plate. Illustration represents the different cellular zones that comprise cartilaginous growth plates (dark gray). Arrows indicate the different zones. The vertical yellow line (top) denotes the proliferative zone (PZ) and the vertical green line (bottom) denotes the hypertrophic zone (HZ). The PZ is a region of actively dividing chondrocytes that are stacked and flattened in multicellular columns. The HZ is a region of enlarged chondrocytes. Epiphyseal and metaphyseal bone on each end of the growth plate contains blood vessels (red lines) that supply each region of bone.

The main factors contributing to differential growth rate are the number of chondrocytes in the proliferative zone, the rate of proliferation, and the size of expanded cells in the hypertrophic zone [7, 29–32]. Indeed, the faster growing sites are well known to have increased numbers of proliferative chondrocytes, increased rates of proliferation, and hypertrophic chondrocyte expansion [1, 8, 22, 25–28, 33••, 34, 35].

Limb Length Discrepancy: Causes and Treatments

Limb length discrepancy (LLD), or anisomelia, is characterized by asymmetric length of lower extremities [36, 37]. LLD usually emerges during childhood but the etiology varies and may be congenital, acquired, or idiopathic. Prior to the development of the polio vaccine in the 1960s, one of the more common causes of LLD in children was poliomyelitis (viral infection causing paralysis) that resulted in shortening of the paralyzed limb [38, 39]. While polio has been eliminated from the United States, treatments are still sought for adults suffering from long-term LLD as a result of poliomyelitis infection during childhood [40, 41]. Other acquired causes of LLD include infection, tumors, and trauma [38, 39, 42]. LLD caused by trauma most commonly involves epiphyseal fractures, which typically result in shortening on the fractured side, and meta- and diaphyseal fractures, which usually lead to lengthening on the fractured side as a result of increased blood flow to the affected growth plate [38, 39, 42–46]. Regardless of the cause, untreated limb length inequalities underlie many painful musculoskeletal problems in adults [37, 47] including lower back pain [48, 49], gait abnormalities [50–54], scoliosis (abnormal curvature of the spine) [55], and osteoarthritis of the hip and knee [56–59].

Limb length discrepancy can vary in severity depending on the underlying cause. Limb length inequalities of 2 cm or more (about 2.4% of adult height [60–62]) typically involve surgical correction [37, 63–65]. Limb shortening is recommended for limb length inequalities of 2–5 cm using epiphysiodesis procedures [63]. These methods involve shortening the longer limb by either permanently halting growth using a surgically formed bony bridge [66] or temporarily slowing elongation using guided growth techniques [63, 67, 68]. Limb lengthening is often recommended for inequalities over 5 cm [63] and includes methods based off the traditional Ilizarov technique using external fixators [69] or mechanical bone guidance [70]. One drawback to any type of invasive procedure, however, is that these surgeries can be associated with complications including pain, implant failure, angular deformities, and infection [39, 70].

The projected limb length discrepancy at the point of maturity varies based on the initial inequality and how much limb growth remains [71]. There have been numerous methods of predicting final limb length including the Green-Anderson method [72], Menelaus method [73], Moseley method [74], and Paley method [75]. These methods are based on mathematical modeling of growth patterns in the femur and tibia at different age points. While each method takes a slightly different approach, they are all based on growth charts developed by Green and Anderson [72] that show femoral and tibial lengths, along with stature, for boys and girls at different chronological and skeletal ages. The basic idea is to compare femoral and/or tibial length in a child to known growth charts to determine the proportion of adult limb length achieved, and thus the predicted amount of growth remaining. However, these predictions are subject to error because skeletal age can be more or less advanced than chronological age depending on the maturation rate of the child. Therefore, the predicated limb length discrepancy varies between these methods [76] and does not always match the actual limb length difference at maturity. Therefore, determining the most effective timing to start treatment is difficult and can lead to possible over- or under-compensation for unequal limb length. When the correction is not successful, surgical interventions may be repeated [63], putting a child at increased risk of post-surgical complications.

Although surgical interventions are typically only performed for a marked length inequality [37, 77, 78], studies have shown that discrepancies as small as 0.5–1 cm (0.6–1.2% limb length difference based on young adult height) may impact everyday walking [79], leading to problems such as back pain and knee osteoarthritis in adults [58]. Shoe lifts or prosthetics are inexpensive and noninvasive approaches, but they do not actually change bone length [39, 47, 70]. Alternative non-invasive methods for permanently lengthening limbs have been tested. A study by Zhang et al. [65] showed that applying intermittent 0.5 N lateral loads to the knee joints of mice (∼ 8 weeks old) increased femoral (2.3%) and tibial length (3.7%). This increase in limb length is noteworthy because treatment occurred during slower rates of longitudinal bone growth when mice were close to skeletal maturity. Serrat et al. [80] were able to use once daily targeted limb heating (40 °C for 40 min/day) to unilaterally increase femoral (1.3%) and tibial (1.5%) length of growing mice (3–5 weeks of age). At skeletal maturity (12 weeks of age), femoral (1.0%) and tibial (1.0%) lengths remained significantly increased on the heat-treated sides [80], suggesting that heat could be a realistic alternative, or at least supplement, to surgical limb lengthening in certain cases.

Regulation of Postnatal Bone Lengthening

Endocrine and Paracrine Factors

The process of postnatal bone elongation is tightly controlled by (1) systemic endocrine molecules, such as hormones distributed through blood that act on target cells of the growth plate, and (2) locally produced paracrine/autocrine factors expressed in epiphyseal chondrocytes or surrounding perichondrium. Disruption of

these regulators results in dysfunctional chondrocytes, abnormal longitudinal growth, and skeletal dysplasia, including short-limbed dwarfism [81]. Some of the primary endocrine factors that regulate longitudinal growth include growth hormone (GH), insulin-like growth factor 1 (IGF-1), thyroid hormone, estrogen, androgen, glucocorticoids, and vitamin D [2, 81–86], all of which can have both coordinated and independent actions. For example, while circulating thyroid hormone has been shown to increase longitudinal bone growth indirectly by increasing systemic GH secretion [83], it also has been shown to directly interact with the epiphyseal chondrocytes and initiate terminal differentiation [87, 88]. The actions of IGF-1 in the growth plate are discussed in detail below.

Locally acting autocrine/paracrine molecules in the growth plate include Indian hedgehog (Ihh), parathyroid hormone-related protein (PTHrP), fibroblast growth factors (FGFs) (including FGF1, -2, -9, and -18), bone morphogenic proteins (BMPs) (including BMP2–7), vascular endothelial growth factor (VEGF), IGF-1, and Wnt [2, 11, 81, 85, 86, 89, 90]. Each of these factors can regulate different regional zones of the growth plate. For instance, prehypertrophic chondrocytes at the maturational stage between active proliferation and hypertrophic expansion express signaling molecules responsible for the successful and timely transition from proliferative to hypertrophic phenotype. These include FGF receptors [91, 92], BMPs [93, 94], and Ihh/PTHrP receptors [95].

Bone lengthening is the ultimate result of the coordinated signaling of both endocrine and paracrine/autocrine factors that interact to promote chondrocyte proliferation and hypertrophy in the growth plate. In addition to circulating IGF-1, estrogen (endocrine) can regulate local expression of IGF-1 in growth plate chondrocytes through an autocrine/paracrine route [82]. While the impact of some factors, such as IGF-1 discussed below, has a dominant role in postnatal growth, normal bone elongation depends on the interaction of multiple regulatory inputs.

Nutritional Factors

In addition to hormones and growth factors, adequate nutrition is also an important facet of limb elongation. There are strong correlations between undernutrition (inadequate caloric/vitamin intake) and stunted growth [96–98], as well as overnutrition (high-fat/calorie diets) and accelerated linear growth [99–102]. In the absence of catch-up growth, the stunting associated with food restriction can lead to permanently shortened bones [103]. However, in cases of overnutrition (obesity-induced growth acceleration), it appears that an earlier onset of skeletal maturity during puberty counters the accelerated period of linear growth resulting in a final height within a normal range [101]. While inadequate nutrition can have some of the greatest impacts during the infancy phase of postnatal linear growth (the first 2 years of life), proper nutrition is essential

for all stages of linear growth continuing from 2 years of life past the onset of puberty [104–106].

Nutrition can also alter endocrine/paracrine signaling in the growth plate [96]. Particularly, IGF-1, the major growth-stimulating hormone, is commonly involved in mechanisms of under- and overnutrition. Serrat et al. [107] found that a high-fat diet increases the delivery of IGF-1 to the growth plate and accelerates bone elongation in young mice. Others have shown, in both human and animal studies, that nutritional deficiency inhibits IGF-1 signaling in the growth plate and reduces final adult height [96, 108, 109].

Since there are multiple nutrient deficiencies in most food-restricted diets, it is difficult to determine the role of individual nutrients and their actions on longitudinal bone growth. Zinc (Zn), an essential trace mineral for linear growth, has been extensively studied. While the mechanism is unknown, it is thought that zinc reduces circulating IGF-1 levels [110, 111], possibly due to an overall decrease in appetite. However, studies in rats suggest that zinc may also have a direct effect on the local action of IGF-1 in the growth plate [96, 112, 113]. Overall, key pathways for hormonal regulation of linear growth, including IGF-1, are clearly influenced by nutritional status.

Temperature Effects on Linear Growth

In addition to nutrition, other environmental factors such as altitude, climate, and temperature can have a significant, yet often underrecognized, impact on bone elongation [114]. The classic effects of temperature on limb length have been described by Allen's "extremity size rule," which states that endotherms (warm-blooded animals) living in warm climates have relatively longer appendages (ears, limbs, tail) compared to their colder climate counterparts [115]. This correlation between temperature and limb length was long presumed to be a thermoregulatory adaptation by increasing surface area for heat dissipation in warmer environments, while reducing surface area relative to body mass for heat conservation in colder climates [116]. Allen's rule is indeed still applied to a variety of different species in present day studies. In humans, Allen's rule was used to suggest that shorter limbs (including those of Neanderthals, a group of archaic humans) are advantageous for survival in cold climates by reducing the metabolic cost of maintaining body temperature [117].

Other studies, however, have shown that the phenotype described by Allen's rule can be replicated under laboratory conditions, suggesting that it might represent growth plasticity rather than a thermoregulatory adaptation to climate. In reptiles, for example, elevated incubation temperature (32 °C) increased tibial length of embryonic crocodiles [118]. Serrat [4] reviewed the impact of temperature on extremity growth in various mammalian species, with a primary focus on rodents that had been most widely studied. Their group also experimentally demonstrated that mice housed at warm ambient temperature (27 °C) during

post-weaning growth have longer limbs than their cold-reared littermates (7 °C) [24], with the most substantial differences occurring during the active period of postnatal growth. Al-Hilli and Wright [119] studied effects of warm ambient temperature (33 °C) on tail growth in weanling mice and showed that heat-enhanced tail growth occurred during the first 3–4 weeks of exposure. Serrat [120] found that the "critical phase" of temperature sensitive growth occurs in the immediate post-weaning phase, followed by a later "maintenance phase," whereby temperature-induced limb length differences are simply maintained through adulthood. Serrat et al. [80] later showed that targeted intermittent heat exposure (40 °C on one side of the body for 40 min per day) unilaterally increased limb length on the heat-treated side growing mice, demonstrating a direct impact of temperature on bone elongation. While it is difficult to identify one single mechanism responsible for temperature-enhanced limb lengthening, it is likely the effect is driven by multiple direct and indirect mechanisms such as altered cell kinetics, gene expression, vascularization, transport of nutrients and growth factors [121, 122], and systemic hormone concentrations [4].

Potential Function of IGF-1 in Heat-Enhanced Linear Growth

IGF-1 is the main regulator of linear growth and normal skeletal development does not occur without functional IGF-1 activity (see Table 1) [123–126]. In addition to liver-derived serum IGF-1 (endocrine), there is strong evidence that locally expressed IGF-1 (autocrine/paracrine) regulates longitudinal bone growth in the growth plate [87, 127–132]. Therefore, a working hypothesis for heat-enhanced linear growth is that temperature increases activity of IGF-1, which increases rate of chondrocyte proliferation and hypertrophy to subsequently enhance limb growth. Heat-enhanced linear growth may

occur: (1) indirectly by increasing IGF-1 access/delivery to the growth plate with temperature-enhanced blood flow (endocrine actions), (2) directly by increasing IGF-1 activity within the growth plate (autocrine/paracrine actions) by temperature-enhanced expression of local regulators, or (3) a combination of both direct and indirect mechanisms.

While blood flow and vascular transport of nutrients may play a role in heat-enhanced limb elongation (discussed in "Role of Vasculature in Bone Elongation"), temperature may also directly alter growth through changes in IGF-1 expression. For example, eels (an ectotherm with endoskeleton made entirely of bone) reared in warmer temperatures (22 °C) were significantly longer and had increased IGF-1 gene expression compared to those raised at colder temperatures (16 °C) [133]. Broiler chicks had increased IGF-1 expression in skeletal muscle that was associated with increasing rearing temperature [134, 135]. In addition to the suggested relationship between temperature and IGF-1 expression in developing tissues, elevated temperature has also been shown to weaken the affinity of IGF-1 for the acid labile subunit (ALS), which results in increased free circulating IGF-1 [136]. Further research is needed to elucidate the role of temperature in modulating IGF-1 action in bone and cartilage of mammals during development.

Growth Hormone and Insulin-Like Growth Factor Signaling

Evolution of the Somatomedin Hypothesis

GH and IGF-1 are integral for regulating normal growth and development. Our understanding of the means by which GH and IGF-1 regulate growth was set into motion after experiments conducted in 1957 by Salmon and Daughaday showed that a

Table 1 Mouse models with disrupted IGF-1 regulation

Model	Phenotype	Citation
IGF-1 null mice (global IGF-1 disruption)	95% mice died prenatally; undetectable levels of IGF-1 in serum and tissues; significant decrease in tibial length (70%); abnormal chondrocyte proliferation and differentiation	[36, 123, 124, 147]
Igf2 null mice (global IGF-2 disruption)	Mice are viable; reduced body growth (60%) at birth; normal postnatal growth	[148, 149]
LID mice (systemic disruption of liver-derived IGF-1)	75% reduction in serum IGF-1; no significant change in body length; no significant change in tibial length; slight decrease in femoral length (6%)	[149, 150]
ALS knockout mice (systemic disruption ternary complexes (IGF-1/ALS/IGFBP-3)	65% reduction in serum IGF-1; no significant change in body length; slight decrease in femoral length (7.5%)	[151]
LID+ALSKO mice (systemic disruption of liver-derived IGF-1 and ternary complexes (IGF-1/ALS/IGFBP-3))	85–90% reduction in serum IGF-1; significant reduction in body length (30%); significant decrease in femoral length (20%)	[152]
(Col2a1)-driven Cre mice (local disruption of chondrocyte-derived IGF-1)	40% reduction in chondrocyte IGF-1; Significant decrease in postnatal body length; no morphological differences in proliferative and hypertrophic regions	[153]
Cart$IGF-1r^{-/-}$ mice (local disruption of cartilage-specific IGF-1R)	Mice died shortly after birth	[3••]
TamCart$IGF-1r^{-/-}$ mice (local disruption of cartilage-specific IGF-1R induced by tamoxifen injections)	Significant decrease in body length (40%); disorganized growth plates associated with reduced chondrocyte proliferation and differentiation	[3••, 143, 144••]

factor in the serum ("sulfation factor") was able to stimulate sulfate incorporation into cartilage in vitro [137]. Later identified as somatomedin in the early 1970s, the somatomedin hypothesis described an intricate relationship in which pituitary-gland-derived GH stimulated the liver to secrete an intermediate hormone (somatomedin), which in turn caused somatic growth [138]. The intermediates were termed insulin-like growth factor 1 (IGF-1) and insulin-like growth factor 2 (IGF-2) (also referred to as somatomedin C and A) later in the decade [139, 141]. Both factors are important for growth and development at different stages of maturation as abnormal phenotypes result if either is disrupted (Table 1).

The introduction of the dual-effector theory in 1985 disputed the original somatomedin hypothesis and proposed an additional IGF-1-independent role of GH [142]. This revision came after reports of local IGF-1 production in non-hepatic tissues, including bone [143], demonstrating that IGF-1 acts as both an endocrine and autocrine/paracrine growth regulator. In the growth plate, GH directly stimulates cell differentiation in the precursor cells of the RZ, while local IGF-1 production mediates clonal expansion of PZ chondrocytes [87, 127–131]. More recent studies support the direct contribution of GH acting on the growth plate independent of IGF-1 [144••, 145]. Thus, since its inception, the somatomedin hypothesis has evolved to describe a more complex interplay between GH and IGF-1 (commonly referred to as the GH/IGF-1 axis) including negative feedback mechanisms where IGF-1 inhibits further GH production (Fig. 2) [146].
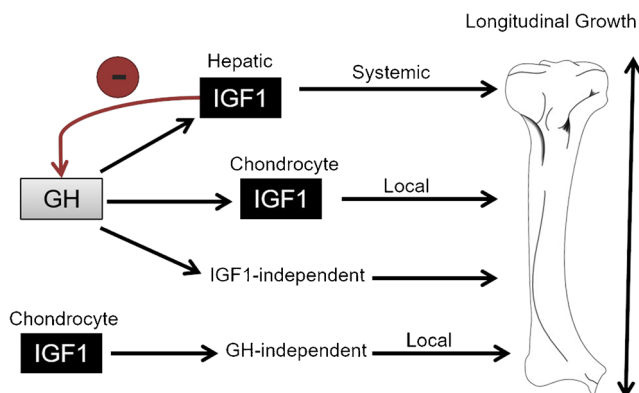

Fig. 2 GH/IGF-1 axis in postnatal limb elongation. Flow diagram illustrates the basic pathways in which GH and IGF-1 interplay to promote longitudinal bone growth. In addition to stimulating IGF-1 production in the liver as originally proposed in the somatomedin hypothesis, GH also stimulates longitudinal growth (1) by promoting local production of IGF-1 in growth plate chondrocytes and (2) independent of IGF-1 as explained by the dual effector theory. IGF-1 also has autocrine/paracrine effects independent of GH by promoting longitudinal growth locally in growth plate chondrocytes. The red arrow demonstrates the negative feedback mechanism of IGF-1 inhibiting further GH synthesis and release

IGF Signaling Pathways

IGF binding protein-3 (IGFBP-3) is the major carrier of IGF-1 in serum. Most circulating IGF-1 (~ 75%) exists in a ternary complex consisting of IGF-1, IGFBP-3, and the acid labile subunit (ALS) [136, 152]. This ternary complex prolongs the half-life of serum IGF-1 and regulates transport from the circulation to the target tissue [136, 154, 155]. Similar to IGF-1, IGF-2 binds to IGFBPs [154, 155]. Upon release at the surface of the target tissue, both IGFs are capable of binding to the receptor tyrosine kinase type 1 IGF receptor (IGF-1R) [155] expressed in all regions of the growth plate [156, 157]. The activation of the IGF-1R leads to a signaling cascade involving the phosphatidylinositide 3-kinase (P13K) and mitogen-activated protein kinase (MAPK) pathways that lead to cell survival and proliferation, which ultimately result in bone elongation (Fig. 3). While IGF-2 is classically thought to function only during prenatal growth [148, 149, 158], IGF-1 is expressed at low levels during embryonic development but is essential for postnatal growth [36, 123, 125]. Evidence supports that IGF-1 is more critical to postnatal longitudinal bone growth compared to IGF-2 because the postnatal phenotype of the IGF-1 null is more severe than Igf2 knockouts (see Table 1) [36, 125, 148, 149].

Distinct and Overlapping Functions of GH and IGF-1 in Linear Growth

The growth hormone receptor knockout (GHR−/−) mouse model developed by the Kopchick laboratory in the 1990s [160] has made important contributions to our understanding of the role of the GH/IGF-1 axis in postnatal bone elongation. The GHR−/− mouse is a model of human Laron syndrome, a recessively inherited inactivating mutation(s) in the GHR. The disease is characterized by GH resistance, high serum GH, and low serum IGF-1 [160–163]. While at birth, these mice are similar in size to their wild-type littermates (suggesting that prenatal growth is not dependent on GH), the significant size difference becomes apparent during postnatal development [160]. GHR−/− mice have a 30–40% reduction in body size [160, 162–164] as well as a 65% reduction in tibial elongation rate [165, 166] and decreased chondrocyte proliferation and hypertrophy [162]. Studies by Lupu et al. [31] found that GH and IGF-1 have distinct, yet overlapping, functions during mammalian linear growth. When comparing the GHR−/− mouse (lacking GH action) to double-Ghr/IGF-1 mutants (lacking GH and IGF action), the observed growth reduction of the double Ghr/IGF-1 mutants was more severe than that of either single mutant model [31]. With advancements in the field of endocrinology, investigators continue to refine the

Fig. 3 IGF-1-induced intracellular signaling pathway. The binding of IGF-1 to its receptor (IGF-1R) triggers downstream signaling cascades, which includes the phosphatidyl inositol-3 kinase (P13K) and mitogen-activated protein kinase (MAPK) pathways that ultimately lead to cell survival and proliferation. Illustration based on Crudden, Girnita, A, and Girnita, L [159]

Cell survival and proliferation
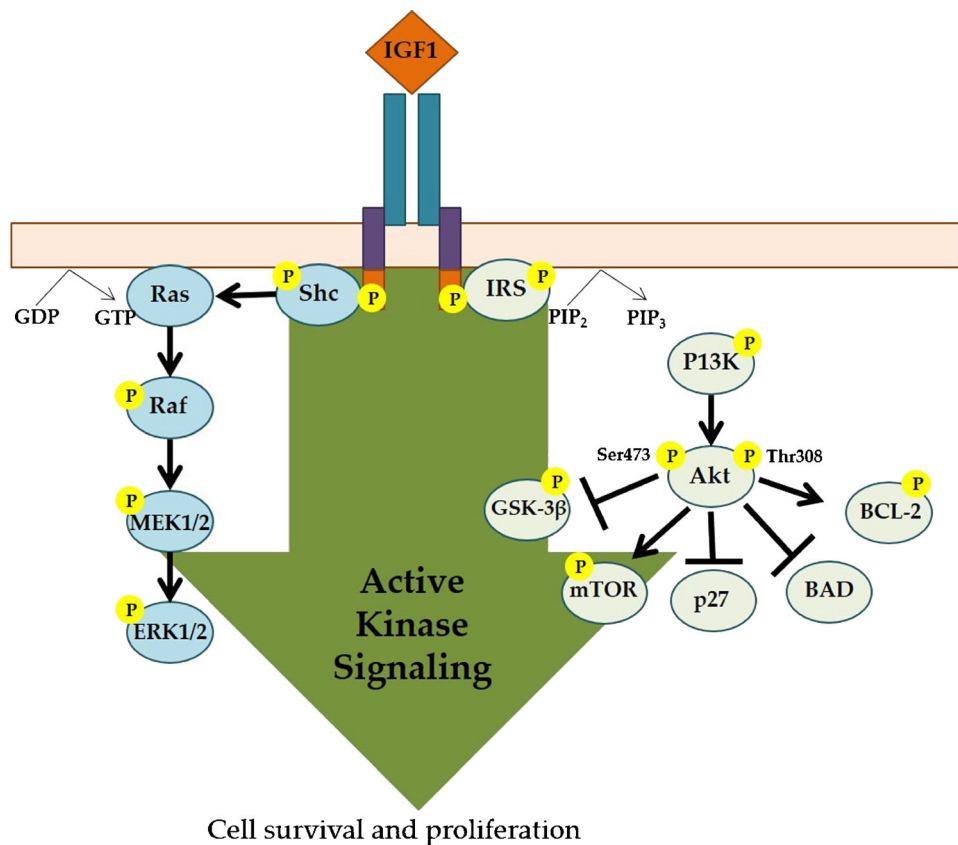
original somatomedin hypothesis to determine how GH and IGF-1 interact to regulate linear growth.

GH and IGF-1 Treatment of Multiple Endocrine Deficiencies

Endocrine disorders involving deficient or excessive production of the important growth-promoting hormones (GH, IGF-1, thyroid hormones, glucocorticoids, and sex steroids) can lead to abnormally short or tall stature. Short stature characterized by stunted linear growth may result from GH deficiency [31, 130, 167], IGF-1 deficiency [123–126, 164, 168], hypothyroidism [169], or hypercortisolism [170, 171]. Thyroid hormone (T3) deficiency in children is associated with growth reduction [172], and studies in mice using knockout models have also shown that linear growth is impaired in thyroid hormone-deficient mice [169, 173] when levels are normal but the thyroid receptor (TRα1 and TRβ) is mutated [174, 175].

A significant problem that results from short stature is the development of behavioral and emotional problems in children [81, 176, 177]. One common treatment option for children with stunted linear growth involves frequent subcutaneous injections of recombinant human GH until adult height is reached [130, 178, 179]. GH has also been used to treat short stature resulting from chromosomal disorders and genetic syndromes including Turner syndrome [167, 178–180], Achondroplasia [181], Prader-Willi syndrome [178, 182], and Noonan syndrome [178, 183]. Other therapies to increase linear bone growth include oxandrolone (synthetic steroid similar to testosterone), estrogens, gonadotropin-releasing hormone (GnRH), and IGF-1 [179]. While GH therapy is more commonly used in clinical settings, IGF-1 can also effectively reverse skeletal growth discrepancies [31, 163, 184–192]. However, IGF-1 is not the optimal first-line of treatment because of adverse effects such as hypoglycemia (abnormally low levels of glucose in the bloodstream), resulting from IGF-1 acting on the insulin receptor [187, 193–196]. In patients that already show symptoms of hypoglycemia, such as those with Laron's syndrome (GH insensitive), IGF-1 therapy can intensify these symptoms and can lead to loss of consciousness or seizures [186, 197, 198]. Other risks with IGF-1 treatment include headaches, intracranial hypertension,

growth of the nasopharyngeal lymphoid tissues, hearing loss, and injection site lipohypertrophy [185, 186, 189, 190]. Researchers continue to seek better delivery mechanisms using a lower dose of IGF-1 to avoid the adverse effects of increased systemic levels [199••].

IGF-1 as a Primary Mediator of Longitudinal Bone Growth

Role of Locally Expressed IGF-1

Numerous studies have supported the importance of both GH and IGF-1 in mediating linear bone growth; however, IGF-1 appears to be the most critical regulator of postnatal growth. In mutant animal models where GH action is impaired [31, 160–163, 166], animals thrived despite being significantly smaller than the wild-type counterparts. In contrast, in mutant animals where local IGF-1 action is impaired, most animals died shortly after birth and those that did survive had severe growth defects [3••, 142, 143] supporting the importance of locally produced IGF-1. The observed growth defects suggest that while a degree of circulating IGF-1 is necessary [152], local action of IGF-1 may be more critical for longitudinal bone growth (see Table 1).

The role of local IGF-1 in chondrocyte proliferation is still somewhat unclear. In the early to mid-1990s, investigators reported the expression of IGF-1 in the PZ of the epiphyseal growth plate measured by in situ hybridization [87, 200, 201], and these findings were replicated in a separate study a decade later [31]. However, these results differed from findings of two other groups that were both unable to detect IGF-1 mRNA in proliferating chondrocytes by in situ hybridization methods, and instead found IGF-2 mRNA [157, 202]. Other investigators have also identified the expression of IGF-2 in the PZ [156, 203]. These inconsistencies could be due to age of the study animals, however, because levels of IGF-2 in the PZ consistently decrease with age, whereas IGF-1 levels increase after birth [156]. Since several studies that did not detect IGF-1 mRNA in proliferating chondrocytes were done during the earliest stages of postnatal development [157, 202], it is possible that these animals had not yet reached the postnatal age at which IGF-1 can be detected in the PZ.

Although once thought to only function during prenatal development, the significance of IGF-2 in early postnatal chondrocyte development has begun to emerge. IGF-2 has not been included in the classic GH-IGF-1 signaling axis [204, 205] but the role of IGF-2 in postnatal bone growth has been revisited after a group discovered that human postnatal growth restriction was associated with nonsense IGF-2 mutations [206]. Uchimura et al. [207] found significantly reduced bone length in IGF2-null mice at postnatal periods prior to weaning (1–3 weeks of age) and their histological

analysis suggests that IGF-2 may have a role in controlling the progression of chondrocytes from proliferation to hypertrophy. The mechanism and extent by which IGF-2 regulates longitudinal bone growth throughout the postnatal period remain unclear and are an important avenue for future investigation.

Regardless of its local versus systemic roles, IGF-1 is undisputedly a significant factor in epiphyseal cartilage development. Local regulation of IGF-1 has been studied in other regions of the growth plate aside from the PZ. Cell kinetic studies using hypophysectomized rats (pituitary gland removed reducing systemic levels of GH and IGF-1) have shown that IGF-1 regulates all phases of chondrocyte differentiation in the growth plate including chondrocyte precursors in the RZ of [208]. IGF-1 expression has also been observed in the HZ of epiphyseal growth plates [200, 201] and local regulation by IGF-1 has been shown to augment chondrocyte hypertrophy [124, 168, 209, 210]. An observed 35% reduction in HZ height was reported in an IGF-1 null mouse model [168]. IGF-1 has also been shown to induce collagen X production (made in the HZ) [168, 211]. When local expression of IGF-1 was blocked by the binding of Wnt induced secreted protein 3 (WISP3), a protein involved in cell differentiation, IGF-1-induced collagen X expression was also reduced [211].

IGF-1 Antagonists

Mouse models designed to control the expression of IGF-1 through genetic manipulation have been critical in our understanding of IGF-1 regulation. Another approach for studying the role of IGF-1 in regulating linear bone growth is by using pharmaceutical inhibitors to block IGF-1 action. The major target of an IGF-1 antagonist is the IGF-1R, which serves as the gateway for IGF-1 action (see Fig. 3). When activation of the IGF-1R is inhibited, the growth-promoting effects of IGF-1 are blocked (Fig. 4). Multiple types of antagonists of IGF-1 have been studied including small-molecule tyrosine kinase inhibitors (TKIs) and competitive antagonists such as monoclonal antibodies directed against the IGF-1R or the use of IGF-1 peptide analogs (Table 2). Since hormones and growth factors, including IGF-1, function to promote growth by inducing cell proliferation and survival, hormone antagonists are often investigated as means for treating abnormal cell growth such as carcinogenesis. However, many of these antagonists face scrutiny because of the reports of failure in clinical trials [212]. Therefore, investigators are continuing to seek a better understanding of these antagonists using animal models [145] and continue to research alternatives for IGF-1 antagonists to improve targeted therapies [212].

Apart from clinical studies using IGF-1 antagonists as anticancer drugs, testing antagonists for their ability to block IGF-1 action in growing bone is an important area of research. Since many of these drugs are administered systemically, the

Fig. 4 Inhibition of IGF-1R signaling. The IGF-1 peptide analog, JB1, competitively binds to the IGF-1R. JB1 thus inhibits IGF-1 binding and prevents IGF-1R activation, ultimately blocking kinase signaling that would otherwise lead to cell survival and proliferation (repressed downstream effects indicated by the red "X"). Illustration based on Crudden, Girnita, A, and Girnita, L. [159]
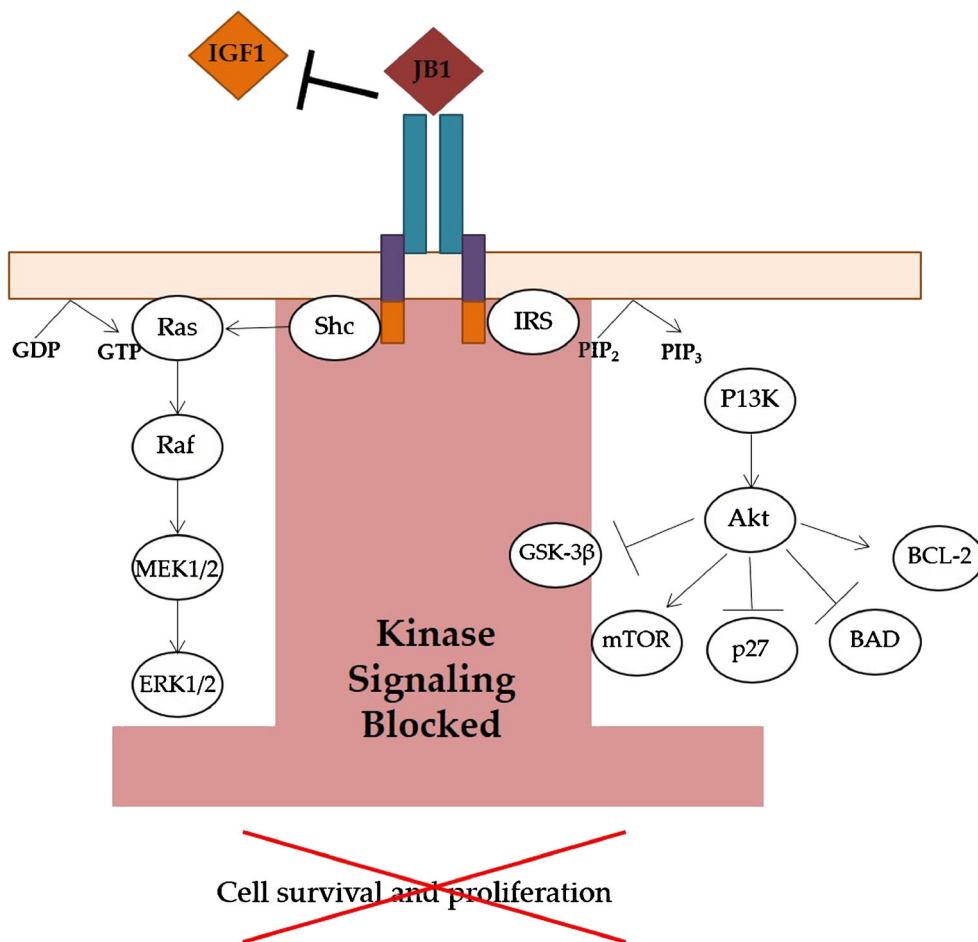
smaller sized antagonists (< 0.9 kDa) are more soluble and better for transport out of the vasculature and especially into dense connective tissue [213, 214]. As highlighted in Table 2, IGF-1 peptide analogs are effective because of their specificity, small molecular size (0.6–1.2 kDa), and low toxicity [215–219]. Multiple IGF-1 peptide analogs have been used to inhibit cellular proliferation including JB1, JB2, and JB3 [218]. JB1 is a commercially-available analog that competitively

binds to the IGF-1R and blocks downstream IGF-1 activity [216, 217, 220–222] (Fig. 4). As with many antagonists, resistance is possible. Haylor et al. [215] reported a bell-shaped curve with the dose response of JB3 and its successful inhibition of kidney growth in rats. In addition to determining an effective range of treatment, challenges still arise in eliciting a tissue-specific response without affecting other systems dependent on IGF-1 for normal growth. Further investigation into

Table 2 Summary of IGF-1 antagonists

Antagonist	Advantage	Disadvantage	Citation
Anti-IGF-1R antibodies (i.e., ganitumab and αIR3)	Highly specific for the IGF-1R	Activates the insulin receptor and promotes unwanted growth; large molecular weight (145–155 kDa); drug resistance	[146, 216]
Small-molecule TKIs (i.e., Linsitinib and BMS-754807)	Targets both IGF-1R and insulin receptor; small molecular weight (0.42–0.46 kDa)	Less specific; reported toxicity (i.e. diarrhea and myelosuppression); drug resistance	[223–225]
IGF-1 peptide analogs (i.e., JB1, JB2, and JB3)	Highly specific for the IGF-1R; small molecular weight (0.6–1.2 kDa); low toxicity	Drug resistance	[215–219]

methods of targeting small molecules will be beneficial in optimizing application of IGF-1 antagonists.

Role of Vasculature in Bone Elongation

Vascular Supply of the Growth Plate

The vasculature is crucial for transporting regulators that support the well-controlled processes of endochondral ossification. Although bone is a highly vascularized tissue, signaling molecules involved in bone elongation must overcome a challenge that is unique to the growth plate: cartilage does not have a penetrating blood supply. Systemic factors essential for bone elongation are delivered from surrounding blood vessels [226–228]. These vascular routes include (1) epiphyseal vessels, (2) metaphyseal vessels, and (3) perichondral vessels [229–232], which arise from a ring vessel found in the encircling groove of Ranvier [229, 233]. Studies have shown that the epiphyseal vasculature is essential for normal growth plate cartilage development [227, 228, 234, 235]. Traditionally, epiphyseal vessels were thought to provide the means for normal chondrocyte proliferation, while chondrocyte hypertrophy was thought to be dependent on the metaphyseal vessels [236, 237]. More recently, however, Farnum and colleagues found that small solutes reach growth plate chondrocytes from all three vascular routes [238], suggesting that both epiphyseal and metaphyseal vessels are crucial in bone elongation.

With the emergence of new imaging modalities, we now have a better understanding of the mechanisms of molecular transport to the growth plate from the surrounding vasculature. Multiphoton microscopy (MPM) as described by Zipfel, Williams, and Webb [140] is a minimally invasive method of in vivo fluorescent imaging that can be used to study solute transport to the growth plate of live anesthetized animals at cellular-level resolution [238]. This imaging approach provides a new approach for tracking systemic fluorescent tracers through the vasculature into the growth plate in real time, in a way not possible using other techniques [121, 122, 235, 238, 239]. For example, dextrans larger than 40 kDa are somewhat size-limited from entering the growth plate [122], but molecules less than 10 kDa enter the growth plate through all three vascular routes [238]. Using these approaches to study the transport of molecules into the growth plate may ultimately provide a better understanding of how larger signaling molecules such as FGFs (FGF2, 18 kDA; FGF18, 23 kDa), PTHrP (9–23 kDa), and BMPs (BMP-2, 26 kDa) expressed in the perichondrium can regulate growth plate chondrogenesis [11].

Transport of Systemic IGF-1 into the Growth Plate

IGF-1 is the major circulating hormone of growth. At a molecular weight of 7.6 kDa, IGF-1 falls within the range of molecules that readily enter the growth plate through the surrounding vasculature (less than 10 kDa) [122]. To study the role of IGF-I uptake in bone elongation, Serrat and Ion [240] developed methods for visualizing the transport of fluorescently labeled, biologically active IGF-1 into the proximal tibial growth plate of live young mice using MPM. They showed that biologically active IGF-488 (IGF-1 conjugated with Alexa Fluor 488) entered the growth plate and localized to chondrocytes [240]. Since IGF-1 is used as a drug to treat short stature in GH insensitive children, such as Laron syndrome patients [31, 161, 163, 180, 184–192], it is essential to understand how IGF-1 is transported through the vasculature. Real-time imaging could be a crucial step forward in developing approaches to better target IGF-1 delivery to the growth plate using mechanisms such as warm temperature [122, 239, 240].

Chondrocyte Expression of Angiogenic Factors

Angiogenesis (development of new blood vessels) is an essential part of normal linear bone growth. During endochondral ossification, hypertrophic chondrocytes secrete angiogenic factors that initiate vascular invasion and recruit bone absorbing and forming cells to replace mineralized cartilage with bone. The key regulator of angiogenesis during both prenatal and postnatal growth and development is vascular endothelial growth factor (VEGF). Survival is dependent on functional VEGF during both embryonic [241, 242] and early postnatal life [243]. Different VEGF isoforms exist but only VEGF-A is expressed in growth plate cartilage and therefore is the most important for regulating longitudinal bone growth [244]. There are also additional splice isoforms of VEGF-A that vary between species. Hypertrophic chondrocytes in both human and murine growth plates secrete VEGF [7, 81, 234, 245–248] to promote vascular invasion from the metaphyseal bone throughout postnatal limb elongation. In human growth plates, the most common splice isoforms are VEGF121, VEGF165, and VEGF189 [234, 244]. The analogous isoforms in mice are VEGF120, VEGF164, and VEGF188 [234, 249]. Interestingly, Maes et al. [234] discovered that different processes of vascularization require specific isoforms of VEGF-A and that the combined action of VEGF120 and VEGF188 is required for both epiphyseal and metaphyseal vascularization. Other local factors expressed by hypertrophic chondrocytes that promote angiogenesis include FGFs [250–252] and matrix metalloproteinase 9 (MMP-9) [81, 246, 249, 253, 254]. Systemic factors including estrogen [244] and IGF-1 [255, 256]) have also been shown to induce vascular invasion by stimulation of VEGF in growth plate chondrocytes.

Normal longitudinal bone growth is dependent on VEGF expression in growth plate chondrocytes. Inhibition of VEGF in young mice (24 days old) suppressed blood vessel invasion, lengthened the hypertrophic zone, and reduced bone growth, all of which was corrected after anti-VEGF treatment ended [246]. These results are clinically relevant to actively growing children that may require therapeutic intervention using angiogenesis inhibitors to prevent unwanted formation of new blood vessels, such as in pediatric cancers. A monoclonal antibody against VEGF, bevacizumab (Avastin®; Genentech, Inc), has been used in adults as an FDA-approved anti-cancer agent [242] and is considered a promising treatment option for children [257, 258]. It is especially important to improve chemotherapy in pediatric patients since children are undergoing an essential stage of bone development requiring growth factors, including IGF-1 and VEGF, for bone elongation. Therefore, as with IGF-1 antagonists, the development of non-invasive methods for targeting drugs directly to a tissue will be essential for preserving linear growth in children.

Conclusions

The goal of this review was to highlight factors that influence postnatal bone elongation with respect to their effects on IGF-1 activity in the growth plate, and in particular, the current state of knowledge regarding effects of IGF-1 and environmental variables, such as temperature, on bone elongation in cartilaginous growth plates. The available evidence demonstrates that environmental variables such as temperature and nutrition impact IGF-1 activity in the growth plate. While there are still gaps in our knowledge, there are many potential avenues of future studies to elucidate the complex mechanisms by which IGF-1 interacts with environmental variables to enhance bone elongation. By understanding the interplay between these important factors during postnatal linear growth, the goal is to ultimately develop better approaches for treating children with a range of bone elongation disorders.

Compliance with Ethical Standards

Abbreviations *ALS*, acid labile subunit; *BMP*, bone morphogenic protein; *COJ*, chondro-osseous junction; *FDA*, Food and Drug Administration; *FGF*, fibroblast growth factor; *GH*, growth hormone; *GHR−/−*, growth hormone receptor knockout; *GnRH*, gonadotropin releasing hormone; *GP*, growth plate; *HZ*, hypertrophic zone of growth plate cartilage; *IGF-1*, insulin-like growth factor 1; *IGF-1R*, insulin-like growth factor 1 receptor; *IGF2*, insulin-like growth factor 2; *IGFBP*, insulin-like growth factor binding protein; *Ihh*, Indian hedgehog; *LID*, liver IGF-1-deficient; *LLD*, limb length discrepancy; *MAPK*, mitogen-activated protein kinase; *MMP-9*, matrix metalloproteinase 9; *MPM*, multiphoton microscopy; *PTHrP*, parathyroid hormone-related protein; *PZ*, proliferative zone of growth plate cartilage; *RZ*, reserve zone of growth plate cartilage; *T3*, triiodothyronine; *TKI*, tyrosine kinase inhibitors; *TRα1*, thyroid hormone receptor alpha 1; *TRβ*, thyroid hormone receptor beta; *VEGF*, vascular endothelial growth factor; *WISP3*, Wnt-induced secreted protein 3; *Wnt*, wingless/integrated; *Zn*, zinc

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