Thematically Review

40 Years of IGF1

Insulin-like Growth Factors: Actions on the Skeleton

Shoshana Yakar¹, Haim Werner² and Clifford J Rosen³

¹David B. Kriser Dental Center, Department of Basic Science and Craniofacial Biology, New York University College of Dentistry, New York, New York, USA
²Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel
³Maine Medical Center Research Institute, Scarborough, Maine, USA

Correspondence should be addressed to S Yakar: sy1007@nyu.edu

This paper forms part of a special section on 40 years of IGF1. The guest editors for this section were Derek LeRoith and Emily Gallagher.

Abstract

The discovery of the growth hormone (GH)-mediated somatic factors (somatomedins), insulin-like growth factors I (IGF)-I and -II, has elicited an enormous interest primarily among endocrinologists who study growth and metabolism. The advancement of molecular endocrinology over the past four decades enables investigators to re-examine and refine the established somatomedin hypothesis. Specifically, gene deletions, transgene overexpression or more recently, cell-specific gene-ablations, have enabled investigators to study the effects of the Igf1 and Igf2 genes in temporal and spatial manners. The GH/IGF axis, acting in an endocrine and autocrine/paracrine fashion, is the major axis controlling skeletal growth. Studies in rodents have clearly shown that IGFs regulate bone length of the appendicular skeleton evidenced by changes in chondrocytes of the proliferative and hypertrophic zones of the growth plate. IGFs affect radial bone growth and regulate cortical and trabecular bone properties via their effects on osteoblast, osteocyte and osteoclast function. Interactions of the IGFs with sex steroid hormones and the parathyroid hormone demonstrate the significance and complexity of the IGF axis in the skeleton. Finally, IGFs have been implicated in skeletal aging. Decreases in serum IGFs during aging have been correlated with reductions in bone mineral density and increased fracture risk. This review highlights many of the most relevant studies in the IGF research landscape, focusing in particular on IGFs effects on the skeleton.

Introduction

More than 60 years ago, the seminal discovery of a factor in serum that mediated the effect of growth hormone (GH) in the cartilage of rats led to the isolation of insulin-like growth factors 1 and 2 (IGF-1, -2). Since then, several hypotheses about the complex interactions between GH and IGFs have been generated. In the present review, we attempt to summarize more than 40 years of research with a focus on the specific actions of IGFs in the regulation of skeletal growth (more details on GH actions in bone can be found in Yakar and Isaksson (2016)).
Identification of insulin-like growth factors 1 and 2

In the late 1940s, GH (also called somatotropin) activity was determined based on the thickness of the tibial chondral plate in hypophysectomized rats treated with GH (Greenspan et al. 1949; Daughaday 2006). In the late 1950s, William Daughaday and William D Salmon showed that ^35S-sulfate uptake was increased in normal rat serum upon stimulation with GH, but was absent in sera of hypophysectomized rats (Murphy et al. 1956, Salmon & Daughaday 1957). The ‘sulfation factor’ in serum was purified and named ‘somatomedin’ to better reflect the actions of the isolated peptide(s) on somatic growth (Van Wyk et al. 1971, Daughaday et al. 1972, Hall 1972). At the same time, an independent investigation of insulin-like activities in serum revealed active components that possessed nonsuppressible insulin-like activity (NSILA). Further characterization of NSILA identified two peptides that shared ~50% homology with pro-insulin (Rinderknecht & Humbel 1976) and were later found to be the two somatomedins previously described; these were finally named insulin-like growth factor-1 and -2 (IGF-1, -2) (Rinderknecht & Humbel 1978a,b).

The somatomedin hypothesis

Based on these early studies, investigators proposed the ‘somatomedin hypothesis,’ whereby the effect of GH on the bone and other tissues of the body was not direct but was mediated by the IGFs (somatomedins) (Kaplan & Cohen 2007). Specifically, pituitary-derived GH induces liver production of somatomedins, which in turn promote bone growth in an endocrine manner. However, in the early 1980s, it was shown that GH injection directly into the tibial growth plate stimulated unilateral epiphyseal growth, while injection of the same GH dose systemically was ineffective (Nilsson et al. 1986). Thus, the ‘somatomedin hypothesis’ was revised to indicate that the effects of GH on bone (and other tissues) are mediated by IGFs produced by the liver and secreted into the circulation (endocrine) and by IGFs produced locally by tissues (paracrine/autocrine). In vitro experiments testing the effects of GH on cell lines demonstrated that GH-stimulated cellular differentiation while IGF1 promoted clonal expansion, leading Green et al. (1985) to formulate the ‘dual effector theory of GH action,’ namely that GH has both IGF-1-dependent and -independent effects on cellular growth. In vivo evidence for the dual effector theory came from crossing dwarf GH receptor (GHR)-null mice (GHRKO) with growth-retarded IGF-I-null mice (IGF-IKO). The cross produced mice that were smaller than either single knockout, suggesting that GH and IGF1 have distinct and overlapping effects on body growth and skeletal size (Lupu et al. 2001). The linkage between GH-IGF and its systemic effects became known as the somatotropic axis (also called the GH/IGF axis).

Overview of the somatotropic axis

The somatotropic axis, (GH/IGF axis) regulates body and bone mass, body adiposity, as well as carbohydrate and lipid metabolism. This axis consists of hormonal circuits that regulate somatic growth. These include the hypothalamic growth hormone-releasing hormone (GHRH) and its receptor, pituitary-secreted GH, IGFs, the IGF-binding proteins (IGFBPs) and the acid-labile subunit (ALS). The circuits also include the hypothalamic somatostatin, an inhibitor of GH secretion, and ghrelin an intestinal-derived stimulator of GH secretion. Also part of the axis are the IGF1 receptor (IGF1R), the GH receptor (GHR) and its downstream mediators Janus kinase 2 (JAK2), Signal Transducer and Activator of Transcription 5 (STAT5) and suppressors of cytokine signaling 1–3 (SOCS1–3) (Fig. 1).

Upon binding to its receptor, the hypothalamic GHRH stimulates pituitary secretion of GH in a pulsatile manner. Pituitary GH is the major regulator of liver-produced IGF1, which is transported via the circulation to peripheral tissues where it acts in an endocrine manner. In serum, IGFs are bound by a family of IGF-binding proteins (IGFBPs). There are six well-characterized IGFBPs, which display serum- and tissue-specific patterns of expression. IGFBPs increase the half-life of IGFs while delivering them to extrahepatic tissues (Baxter 2000). However, a significant body of evidence suggests that a few of the IGFBPs also have IGF-independent effects on bone and other tissues (Hoeflich et al. 2007). In serum, ~75% of IGF1 is found in ternary complexes with IGFBP-3 or -5 and the ALS that further increase the half-life of IGF1 (to ~16h). Approximately 20% of IGF1 in serum is bound in binary complexes with IGFBPs, and ~5% of the peptide circulates free with a very short half-life of ~10min.

 Liberation of endocrine IGFs from their binding proteins, in close proximity to the IGF1R in tissues or serum, occurs via activation of specific proteases (Collett-Solberg & Cohen 1996). Pregnancy-associated plasma protein-A (PAPP-A) and prostate-specific antigen (PSA) are circulating serine proteases that upon binding to the IGFBPs change their binding affinities and liberate the IGFs (Cohen et al. 1992, 1994). In tissues, a number of potential IGFBP...
IGFS and bone

The relative contribution of transcription initiation sites, alternative splicing, and two promoters (Ekstrom 1994) dramatically reduce serum and tissue IGF1 levels (et al. 1989). Due to alternative transcription initiation sites, alternative splicing, and different polyadenylation sequences, numerous Igf1 mRNAs are produced. These transcripts differ slightly in their coding sequences but mostly diverge in their 5’ and 3’ un-translated regions (Rotwein 1986, Roberts et al. 1987, Adamo et al. 1991). The relative contribution of transcriptional and post-transcriptional mechanisms to control basal and GH-stimulated Igf1 gene expression is still controversial (Benbassat et al. 1999).

IGF2

Igf2 transcripts are found in all fetal tissues in humans and rodents. In most rodent tissues, expression of Igf2 declines early in the postnatal period (Bondy et al. 1990). In humans, however, IGf2 levels in serum and tissues, specifically in the brain, remain high. The reasons for this species-specific difference are not clear. Mature IGf2 peptide displays 47% sequence homology with insulin (Nielsen 1992, O’Dell & Day 1998) and can bind the IR. Igf2 gene constitutes a classical example of a paternally imprinted gene. The transcription of the Igf2 gene is under the control of four promoters (P1–P4). P1 is not normally imprinted while the other three promoters undergo silencing via DNA methylation (Ekstrom 1994). Imprinting alterations of all four Igf2 promoters have been reported in hepatocellular carcinoma and Wilm’s tumor. Overexpression of Igf2 in cancer or overgrowth syndromes (such as Beckwith–Wiedemann syndrome (Sun et al. 1997b)) may be caused by a number of genetic events, including gene duplication, loss of heterozygosity or loss of imprinting of the Igf2 gene, leading to bi-allelic gene expression (Bentov & Werner 2004).

Molecular characteristics of IGF1 and IGF2 receptors

IGF1 receptor (IGF1R)

The Igf1r promoter lacks canonical TATA and CAAT elements that are required for transcription initiation (Werner et al. 1992) but has a unique ‘initiator’ motif that directs transcription initiation in the absence of a TATA box. The Igf1r promoter is extremely GC-rich (80%) and contains several binding sites for members of the Sp1 family of zinc-finger nuclear proteins. Control of Igf1r expression occurs mainly at the level of transcription (Werner 2012). Comprehensive analyses have established that transcription of the Igf1r promoter is dependent on a number of stimulatory zinc-finger proteins, including Sp1. In addition, the Igf1r promoter was identified as a downstream target for tumor suppressor activity, and

proteases, including kallikreins (serine proteases) (Hekim et al. 2010), cathepsins (cysteine proteases) (Conover et al. 1995) or matrix metalloproteinases (MMP, Zn²⁺-binding endopeptidase) (Fowlkes et al. 1994a,b), have been shown to play important roles in tissue remodeling and wound healing by cleaving the IGFBPs to release IGFS.

Molecular characteristics of IGF1 and IGF2

IGF1

The Igf1 gene expression is regulated in temporal- and tissue-specific manners. Nutritional status is a major determinant of Igf1 gene expression, not only in liver, but also in other tissues; fasting was shown to dramatically reduce serum and tissue IGF1 levels (Lowe et al. 1989). Transcription of Igf1 is governed by at least two promoters (Wang et al. 1997). Due to alternative transcription initiation sites, alternative splicing, and
multiple anti-oncogenes (e.g. p53, breast cancer gene-1 (BRCA1) and von-Hippel Lindau (VHL)) have been shown to inhibit Igf1r transcription (Werner et al. 1996, Yuen et al. 2007, Werner & Bruchim 2012).

IGF1R is synthesized as a single chain 1367-amino acid pre-pro-peptide with a 30-amino acid signal peptide that is cleaved after translation. The pre-peptide is then glycosylated, dimerized and transported to the Golgi apparatus where it is processed at a furin cleavage site to yield α- and β-subunits. A mature tetramer, β-α-α-β, is then formed through disulfide bridges, followed by translocation to the plasma membrane. N-linked glycosylation of IGF1R is necessary for its translocation to the cell surface. The two extracellular α-subunits are involved in ligand binding, and the two transmembrane β-subunits, which contain cytoplasmic tyrosine kinase domains, are involved in signal transduction. The IGF1R has high homology to the insulin receptor (IR/ INSR), which has two isoforms IR-A and IR-B. The heterodimers IGF-IR/IR-A bind IGF2 with higher affinity than IGF1 (Fig. 1).

In humans, chromosomal aberrations involving the 15q26 locus (e.g. ring chromosome 15) are associated with hemizygosity for the Igf1r locus and severe growth failure (Peoples et al. 1995). On the other hand, a patient with three copies of the gene due to partial duplication of the long arm of chromosome 15, presented with height and weight above the 97th percentile and showed accelerated cellular growth (Okubo et al. 2003).

**IGF2 receptor (IGF2R)**

The IGF-II/mannose 6-phosphate (M6P) receptor targets IGF2 for lysosomal degradation and thus reduces the bioavailability of circulating IGF2 (Oates et al. 1998). The IGF-II/M6PR lacks a tyrosine kinase domain and is apparently not involved in signaling events. However, the IGF-II/M6PR is often mutated in breast cancer and its loss of function may lead to reduced IGF2 clearance and further activation of the IGF1R (Ellis et al. 1998). In addition, significant portions of the proliferative activities of IGF2 are mediated by a particular isoform of the IR, IR-A.

**Effects of systemic ablation of IGFs/IGF-IR on the skeleton**

**IGF1R**

IGF-IR-null mice (IGF-IRKO) are not viable and show severe growth retardation in utero, with embryos reaching only ~45% of normal size (Liu et al. 1993, Holzenberger et al. 2000). These mice show embryonic delays in ossification of the cranial and facial bones, as well as in the ossification of the interparietal bone, and bones of the trunk and extremities. Haploinsufficient IGF1R mice (IGF-IR+/−) are viable, but show reductions in serum IGF1 levels and reduced body size (Holzenberger et al. 2001, Castilla-Cortazar et al. 2014). Femurs of the IGF-IR−/− mice are shorter and have decreased mineral density due to lifelong deficits in bone formation and impaired osteoblast activity (Yeh et al. 2015). To avoid the developmental lethality seen in the IGF-IRKO mice, investigators used the Cre-loxP technology. Generation of IGF-IR-null mice using a Cre recombinase that produced a pattern of ‘mosaic-early embryonic-ubiquitous’ Igf1r gene deletion (Meu-IGF-IRKO), revealed that the levels of IGF1R in tissues correlate directly with body size (Holzenberger et al. 2001). Using the inducible ubiquitously (UBI) expressed Cre recombinase, Holzenberger et al. showed that the UBI-IGF-IRKO mouse, in which the Igf1r was deleted in the adult (3 months old) mouse (Francois et al. 2017), exhibited a 12% reduction in femoral dry mass and reduced mid-diaphyseal periosteal circumference, without changes in bone mineral content by DEXA. These findings suggest that systemic IGF1R receptor inactivation compromises radial bone growth even in the adult mouse.

In humans, only two patients carrying homozygous Igf1r mutations have been described (Ganne-Yared et al. 2013, Prontera et al. 2015). A few cases of compound Igf1r heterozygotes have been reported, but the majority of Igf1r defects in humans are heterozygous carriers or deletions of chromosomal region 15q, which includes the Igf1r locus (reviewed in Klammt et al. 2011, Walenkamp et al. 2013). In general, heterozygous deletion or inactivating mutations of the Igf1r gene lead to significant reductions in IGFIR protein levels, resulting in IGF1 resistance. All heterozygous subjects showed postnatal growth failure; however, detailed studies of their skeletal phenotype were not reported.

**IGF1**

Igf-I-null mice (IGF-IKO) showed severe growth retardation (reaching only ~30% of adult size) and high early postnatal mortality (Liu et al. 1993). Furthermore, these mice exhibited reduced linear bone growth due to abnormal chondrocyte proliferation and differentiation, as well as severely decreased bone mineral density (BMD) in the appendicular skeleton (Bikle et al. 2001). Detailed characterization revealed a >25% reduction in size of bones in the axial and appendicular skeleton that was...
associated with a >25% reduction in bone formation rate in IGF-IKO mice when compared to wild-type littermates (Mohan et al. 2003). Haplo-insufficient IGF1 mice (IGF-1+/−) showed moderate reductions in serum and tissue IGF1 levels that correlated with decreased body size and skeletal morphological traits and reduced BMD (Mohan & Baylink 2005, He et al. 2006). Another model of inactive IGF1 is the MIDI mouse, in which site-specific insertion of a targeting construct into the IGF-I-coding region results in mice with very low IGF1 expression (Stabnov et al. 2002). These mice also showed growth retardation, reduced skeletal size and BMD, but were bigger than the global IGF-IKO mice, likely due to residual IGF1 activity. Combined deletion of both IGF1r and the IGF1 genes in mice resulted in a phenotype similar to the IGF-IRKO mice (Liu et al. 1993), suggesting that the IGF1R is the sole mediator of IGF1 actions on cellular growth and differentiation.

Very few inactivating mutations have been described in the human Igf1 gene. A recent genome-wide analysis identified several missense mutations in the coding regions of the IGF gene family; however, the majority of these mutations and polymorphisms are extremely rare (Rotwein 2017). In humans, homozygous deletions or missense mutations in the IGF1 gene result in a complete loss of function (Woods et al. 1996, Walenkamp et al. 2005), severe prenatal and postnatal growth failure and several developmental delays. Subjects with a homozygous hypomorphic mutation (with partial loss of function) (Netchine et al. 2009) or heterozygous mutations (van Duyvenvoorde et al. 2010, Fuqua et al. 2012, Batey et al. 2014), also present with growth failure, although not as severe.

IGF2

Deletion of Igf2 in mice (IGF-IIKO) resulted in intrauterine growth retardation (DeChiara et al. 1991) with pups born ~70% of normal size. However, by 3 months of age, IGF-IIKO mice caught up and reached normal size, suggesting that IGF2 mainly regulates prenatal growth in mice. In humans, however, a nonsense mutation in the Igf2 gene, reported recently in a family of four members, was associated with prenatal and postnatal growth restriction (Begemann et al. 2015), suggesting that in humans, IGF2 also affects postnatal growth. The phenotype affected only subjects who have inherited the variant through paternal transmission, a finding that is consistent with the maternal imprinting status of Igf2. Another condition observed in humans is Silver–Russell syndrome where prenatal and postnatal growth failure is caused by decreased transcription of the paternally inherited Igf2 allele (Gicquel et al. 2005). These findings are in agreement with the earlier report of a patient with paternally transmitted severe intrauterine growth retardation and short stature due to a translocation breakpoint leading to significant reductions in IGF2 levels (Murphy et al. 2008).

Effects of endocrine IGFs on the skeleton

Serum IGF1

With the availability of the Cre/lox P system two independent laboratories generated mice with an Igf1 deletion specifically in the liver. One group expressed the Cre recombinase under the Mx1 promoter to generate the Li-IGF-IKO mice (Sjogren et al. 1999, 2002) and the other group expressed Cre recombinase under the albumin-enhancer promoter sequence to generate the liver-specific Igf1 deleted (LID) mice (Yakar et al. 1999, 2009a). In both models, serum IGF1 levels were decreased by ~75% and GH levels were elevated due to negative feedback loop between these two hormones. Both Li-IGF-IKO and the LID mice showed minimal reductions in linear bone growth (~5–6%), likely due to increases in GH levels. In contrast, marked reductions in radial bone growth (~20%) and BMD were noted throughout life (Yakar et al. 2009a). Detailed characterization of skeletal growth in LID mice revealed inhibition of periosteal bone expansion and impaired bone adaptation to increases in body weight (Yakar et al. 2009a). As a result of compromised morphology, LID bones were more slender and exhibited inferior mechanical properties (Yakar et al. 2009a).

A complementary model to the LID mice is the als gene deletion (ALSKO) model (Ueki et al. 2000). These mice exhibit significant reductions (~65%) in serum IGF1, decreased body weight and compromised skeletal morphology (Courtland et al. 2010). Reduced levels of serum IGF1 in the ALSKO mice was due to instability in the ‘IGF1 delivery system’, namely, rapid degradation of IGFBP-3 and enhanced clearance of IGF1 from the circulation. Mice with combined knockout of LID/ALSKO (Yakar et al. 2002) exhibited further decreased serum IGF1 levels (>85%) and reduced longitudinal growth as reflected by decreased (~20%) height of the growth plate. As in the single knockouts, the double LID/ALSKO-knockout mice had slender bones with decreased femur cross-sectional area (~35%) and significant reductions in BMD (~10%) (Yakar et al. 2002).

As noted previously, GH is the main regulator of liver-derived IGF1 (serum IGF1). Thus, models of
liver-specific deletion of the growth hormone receptor gene (*Ghr*) (Liu et al. 2017) and its mediators, JAK2 (Nordström et al. 2011) or STAT5 (Cui et al. 2007) also led to severe reductions in serum IGF1 (>95%) and compromised skeletal development. Similar to the LID mice, ablation of GHR activity in the liver (Li-GHRKO) resulted in mice with slender bones as evidenced by significantly reduced cortical bone thickness and reduced BMD throughout life (Liu et al. 2017). Micro computed tomography (µCT) analyses of bones from liver-specific JAK2KO mice (Nordström et al. 2011), also showed significantly decreased total cross-sectional area, bone area and cortical bone thickness (S Yakar, personal communication). Overall, serum IGF1 regulates periosteal bone expansion, namely radial bone growth, which plays critical roles in bone mechanical strength and has minor effects on linear bone growth.

There are no human counterparts to the liver-specific GHRKO or the LID mice. However, several families with mutations in the *Igfals* gene, modeled by the ALSKO mice, have been described in the past decade. Subjects with mutated *Igfals* show reduced serum IGF1 levels and manifest skeletal abnormalities including short stature and reduced BMD (Domene et al. 2009, 2010).

Excess in endocrine IGF1 levels also affects radial bone growth but has little effect on linear bone growth. Expression of a hepatic *Igf1* transgene (HIT) resulted in increased serum IGF1 levels (Ellis et al. 2010a,b, 2011b, Wu et al. 2013) and significantly increased radial bone growth in male and female mice. Using a quantitative trait locus approach (QTL), one can generate a congenic line where only one chromosomal locus is replaced by the same locus from a different inbred line. C57BL/6J congenic mice carrying a QTL from chromosome 10 of C3H/HeJ mice showed ~20% increase in serum IGF1. However, that increase did not lead to enhancement in cortical bone parameters (Delahunty et al. 2006), suggesting that enhancement in radial bone growth requires super-physiological levels of IGF1 (as seen in the HIT mice). Earlier reports of mice with ubiquitous overexpression of *Igf1* (under the metallothionein promoter) showed that despite a ~20–30% increase in body weight and organomegaly, the femur, tibia and tail lengths were similar to controls (Mathews et al. 1988, Behringer et al. 1990, D’Ercole 1993). The lack of increase in linear bone growth in response to excess serum IGF-1 levels is likely due to inhibition of endogenous GH secretion and its direct actions on the growth plate.

In humans, excess IGF1 in tissues and serum are seen in acromegaly. This pathological condition is caused by pituitary adenomas that secrete excess of GH, which stimulates liver as well as extrahepatic tissues to produce IGF1 (Capatina & Wass 2015). Skeletal abnormalities in patients with acromegaly are largely attributed to elevations in IGF1, although GH-mediated changes in vitamin D metabolism in the kidney, and its secondary effects on PTH production, also contribute to the abnormal phenotype. Patients with acromegaly show site-specific increases in bone size and shape, however, overall they exhibit increases in bone turnover associated with a higher vertebral fracture risk (Ueland et al. 2002, Bonadonna et al. 2005, Mazziotti et al. 2008, Wassenaar et al. 2011, Madeira et al. 2013).

Excess endocrine IGF1 can compensate for the growth retardation resulting from congenital *Igf1* ablation. Crossing of the haploinsufficient IGF-I+/− with HIT mice resulted in IGF-IKO-HIT mice (Ellis et al. 2010b), which displayed only the endocrine actions of IGF1, while the autocrine/paracrine actions of IGF1 were deleted. Owing to the *Igf1* transgene, IGF-IKO-HIT mice showed a 2-fold increase in serum IGF1 throughout life. Endocrine IGF1 rescued the growth retardation caused by *Igf1* deletion, such that by 8–16 weeks of age, the IGF-IKO-HIT mice had caught up and their bones were indistinguishable from controls both in morphology and mechanical properties. A similar strategy was undertaken to generate IGF-IKO-IGF-II+/− mice (by crossing IGF-I+/− mice to mice overexpressing the *Igf2* under the PEPCK promoter, mainly in liver) (Moerth et al. 2007). However, despite the high levels of IGF2 in tissues and serum, postnatal growth of IGF-IKO-IGF-II+/− mice was not rescued, indicating that only endocrine IGF1 can compensate for the postnatal growth retardation of IGF-IKO in mice.

The same strategy was used to generate *Ghr*-knockout (GHRKO) mice with restored endocrine IGF1 levels (GHRKO-HIT) (Wu et al. 2013). The GHRKO-HIT mice have no GH in any tissues: thus, *Igf1* expression levels in tissues are very low. Serum IGF1 in GHRKO-HIT mice reached control levels due to *Igf1* transgene (HIT) expression in the liver. However, the skeletal size of the GHRKO-HIT mice was intermediate between GHRKO and controls. Therefore, the same *Igf1* transgene that rescued the growth retardation in IGF-IKO mice could not rescue the impaired skeletal growth of GHRKO mice. This was due to a compromised IGF1 delivery system, as IGFBP-3, the main carrier of IGF1 in serum, and the GH-regulated ALS that stabilizes the IGF1/IGFBP-3 complex were almost undetectable in GHRKO-HIT mice. Notably, there are no human counterparts to the GHRKO-HIT mouse model, but a similar scenario occurred when Laron syndrome

Serum IGF2

The endocrine roles of IGF2 in regulating skeletal growth and integrity in humans and animal models have not been studied in detail. In a mouse model where the Igf2 expression was driven by the PEPCK promoter (IGF-II<Tg>), serum IGF2 levels were increased and correlated with increased body weight (~15%); however, changes in skeletal morphology or BMD were not apparent (Moerth et al. 2007). In humans, increase in serum IGF2 was described in subjects with Beckwith–Wiedemann syndrome (Sun et al. 1997b) who show prenatal and postnatal overgrowth, multiple organ overgrowth and increased risk of tumor development.

In summary, serum IGF1 is the main regulator of postnatal growth in rodents, while IGF2 does not play a significant role. Specifically, in mice, serum IGF1 deficiency leads to slender bones, while excess serum IGF1 results in more robust bones. Overall, endocrine IGF1 is a major regulator of radial bone growth, particularly of the appendicular skeleton.

Modulations of IGF-bioavailability

IGF1 bioavailability is controlled by IGFBPs and IGFBP proteases. The notion that IGFBPs sequester IGFs from its receptor and inhibit its action is supported by data from mouse models with overexpression of Igfbp(s), which exhibit decreased body weight and skeletal growth. IGFBP-1 transgenic (IGFBP-1<Tg>) mice showed intrauterine and postnatal growth retardation with delayed bone mineralization and reduced BMD (Rajkumar et al. 1995, Ben Lagha et al. 2002, 2006, Watson et al. 2006). Likewise, IGFBP-3<Tg> (Modric et al. 2001, Silha et al. 2003), IGFBP-4<Tg> (Zhang et al. 2003), and IGFBP-5<Tg> mice (Salih et al. 2005) show reductions in body weight, bone length and reduced BMD. However, IGFBP-2<Tg> mice show only 5–10% reduced body weight and minor decreases (~3%) in bone length, with no effect on BMD (Hoeflich et al. 1999).

Studies of Igfbp(s)-knockout mice have yielded conflicting results, suggesting IGF-independent functions and redundancy of the IGFBPs. For example, IGFBP-2 knockout (IGFBP-2KO) in mice was associated with a 10% increase in serum IGF1 levels and sexually dimorphic phenotype in young adults (DeMambro et al. 2008). While male IGFBP-2KO mice showed reduced linear growth with thinner cortices and reduced trabecular volume (due to reduced bone formation), female mice showed increased radial bone growth and increased cortical thickness without effects on trabecular bone. In humans, IGFBP-2 binds mostly IGF2. The IGFBP2/IGF-II complex has high affinity for bone matrix and can stimulate osteoblasts proliferation (Ishibe et al. 1998). Administration of IGFBP2/IGF2 complexes into adult rats prevented disuse- and ovariectomy-induced bone loss (Conover et al. 2002). Note that in humans, IGF2 actions have been linked to mitogenesis and tumorigenesis; thus, in order to use IGFBP2/IGF2 complex as a treatment for bone loss, it would be necessary to direct the complex specifically to bone.

IGFBP-3KO mice had minor reductions in serum IGF1 (~30%), with normal cortical bone morphology, but a 30% decrease in trabecular bone volume (Yakar et al. 2009b). Ablation of IGFBP4, the most abundant IGFBP in bone, led to 10% prenatal growth retardation that persisted postnataally (Ning et al. 2006, 2008), suggesting that IGFBP4 regulates the bone-tissue pool of IGF1 (protecting it from degradation) and controls IGF1 bioavailability. Liberation of IGF1 from IGFBP4 is controlled by PAPP-A. PAPP-AKO mice showed significantly reduced IGF1 bioavailability (Miller et al. 2007), which resulted in decreased linear growth, cortical bone thickness, trabecular bone volume and BMD, all associated with low-turnover osteopenia. Interestingly, elevations in IGF2 rescued the growth retardation and delayed ossification that occurs during fetal development in PAPP-AKO mice during embryogenesis (Mason et al. 2011), but not during postnatal growth (Bale & Conover 2005). These findings are consistent with the notion that IGF2 is a fetal growth factor in mice. A combined knockout of IGFBP-3, -4, and -5 in mice resulted in a 50% reductions in serum IGF1 levels that was associated with a 25% reductions in body weight (Ning et al. 2006); however, full skeletal characterization was not reported.

To our knowledge, mutations in the Igfbp(s) have not been described in humans. However, a homozygous mutation of the gene encoding the protease PAPPA2 (PAPPA2)-encoding gene was recently described in two families. The associated short stature was attributed to insufficient IGF1 bioavailability (Dauber et al. 2016, Munoz-Calvo et al. 2016). PAPPA2 mutations in humans...
caused skeletal dysplasia, thin long bones (via inhibition of radial bone expansion) and reduced BMD in the lumbar spine. These human cases clearly demonstrate that control of IGFs bioactivity in serum and tissues is essential for developing a healthy skeleton.

The effects of IGF1 bioavailability on skeletal growth also were studied in mice with mutated Igf1, which resulted in reduced affinity for the IGFBPs and thus increased bioavailability in tissues. Using a gene-targeting approach two knock-in (KI) models were generated where the endogenous Igf1 was replaced by des1-3-Igf1 (KID) or LR3-Igf1 (KIR). These two Igf1 analogues were previously shown to have reduced stability in serum but increased activity in vitro (Francis et al. 1990, Ballard et al. 1991, Bastian et al. 1993). In particular, recombinant des1-3-Igf1 injected into nude mice was rapidly cleared from serum and distributed into tissues (Ballard et al. 1991, Sun et al. 1997a). KID and KIR mice showed very low levels of Igf1 in serum (Elis et al. 2011a). Surprisingly, however, both KID and KIR mice exhibited enhanced growth and skeletal properties with more robust, stronger bones when compared to controls. The KID and KIR models provide in vivo evidence for the potency of des1-3-Igf1 (KID) and LR3-Igf1 (KIR) in increasing somatic growth, independent of serum Igf1 levels (Elis et al. 2011a), suggesting that bioavailability of the autocrine/paracrine IGF1 in tissues plays critical roles in stimulating growth.

Overall, IGFBPs have IGF-dependent and -independent effects on the skeleton. There are overlapping functions between the different IGFBPs, and certainly, sex-specific effects that have not been studied in detail. Data from mouse studies suggest that IGFBP-4 and the proteinase PAPP-A are significant determinants of IGFs bioavailability.

### Bone cell-specific effects of IGFs

Bone modeling is a multicellular process during which linear and appositional bone growth occur and peak mineral density is achieved. Linear bone growth is regulated by chondrocytes of the growth plates, while appositional growth is regulated by osteoprogenitors at the periosteal or endocortical bone surfaces. Bone remodeling, on the other hand, occurs in the adult and aging skeleton and its primary functions are to maintain mechanically competent skeleton and to preserve mineral homeostasis. Bone modeling and remodeling involve several phases and involves interactions between osteoblasts, osteoclasts and osteocytes. The first phase requires conversion of a resting bone surface into a remodeling surface. During that phase, mononuclear osteoclast progenitors are recruited to the surface. Marrow stromal cells (MSCs)- and osteoblast-secreted receptor activator of nuclear factor-kappa B (NF-κB) ligand (RANKL) stimulate osteoclasts differentiation. In the second phase, osteoclasts secrete hydrochloric acid and acidic proteases such as cathepsin-K, tartrate-resistant acid phosphatase (TRAP) and MMPs that degrade/resorb the organic matrix. Resorption of the organic matrix liberates growth factors among them are IGFs (Xian et al. 2012), bone morphogenetic proteins (BMPs) and transforming growth factor-β (TGF-β). These growth factors stimulate osteoblasts to form bone. The third phase involves attraction of osteoblast precursors to the bone surface and osteoblasts differentiation to couple the bone resorption phase with the bone formation phase. The bone formation phase involves deposition of osteoid (predominantly type I collagen) and its mineralization, both of which are stimulated by IGFs. At the end of mineralization osteoblasts that are trapped in the bone matrix become osteocytes. Osteocytes in the mineralized matrix contact with each other through dendritic processes called canaliculi. These cells integrate/summate hormonal and mechanical influences, and directly influence bone material properties.

Understanding bone cell-specific effects of IGFs has been advanced with the use of cre-lox/P technology. Several Cre-derived mouse lines have been used along with the floxed gh, Igf1 and the Igfr mouse lines to inactivate IGFs in bone (Fig. 2), as will be described in the following paragraphs. However, despite the valuable advantages of this technology, several issues should be taken into account when using cre-driver lines. These include tissue non-specificity and inconsistent recombination efficiency resulting in high variability between littermates.

### Chondrocytes of the growth plate

Chondrocytes are of mesoderm origin and produce a matrix rich in collagen, proteoglycans and glycosaminoglycans. Chondrocytes at the growth plate proliferate, undergo hypertrophy and control bone linear growth (length). The growth plate includes three zones: the germinal zone (containing chondrocyte progenitors), the proliferation zone (containing replicating chondrocytes) and the hypertrophic zone (containing apoptotic chondrocytes). IGFs, produced mainly in proliferative chondrocytes, are regulated by GH (Nilsson et al. 1986, 1990, Wroblewski et al. 1987). Analysis of the growth plate of IGF-IKO mice revealed that chondrocyte proliferation and cell numbers in the growth plate were preserved despite marked (~35%) reductions in bone length (Wang et al. 1999),
with growth defects attributed to decreased chondrocyte hypertrophy. Studies of chondrocytes in the growth plate of GH-deficient or fasting mice and rats have shown that GH stimulates IGF production in pre and early chondrocytes, but has minimal effects on differentiated chondrocytes or chondrocytes of the hypertrophic zone (Gevers et al. 2009).

Mechanistically, upon binding to the tyrosine kinase IGF1R on chondrocytes, IGFs induce expression of the type II collagen Ia (Col2a1) likely via activation of SOX9/SOX5/SOX6 and SP1 transcription factors (Renard et al. 2012), and aggrecan via activation of the phosphatidylinositol 3-kinase (PI3K)/phosphoinositide-dependent kinase-1 (PDK-1)/Akt (AKT) pathway (Ciarmatori et al. 2007). With progression of chondrocytes to terminal differentiation, IGF1R signaling is shut down.

Chondrocyte-specific Igf1 gene deletion in mice was achieved using the Col2a1-driven Cre recombinase (Col2a1-IGF-IKO), which is expressed in proliferating chondrocytes (Govoni et al. 2007). Col2a1-IGF-IKO mice showed decreased body and bone lengths, and decreased bone diameter and BMD, which were attributed to reductions in IGF1 production by perichondrial cells. A tamoxifen-inducible Col2a1-driven Cre recombinase (Col2a1Tam-cre) was also used to delete the IGF1R in chondrocytes (Col2a1Tam-IGF-IRKO) at postnatal day 5. Col2a1Tam-IGF-IRKO mice showed a marked decrease in bone length, disorganized chondrocyte columns in the growth plate, delayed ossification in utero and neonates (Wang et al. 2011) and under-mineralized skeletons at 3 weeks of age (Wang et al. 2014). Another study showed that Col2a1Tam-IGF-IRKO mice had reduced chondrogenesis and chondrocyte proliferation, as well as increased chondrocyte apoptosis, resulting in a shorter hypertrophic zone and reduced bone length (Wu et al. 2015).

Lastly, early studies showed that IGF2 was strongly expressed in chondrocytes of the proliferating zone of the growth plate (Shinar et al. 1993, Tsang et al. 2007). A recent study, using ex vivo bone organ culture, reported that IGF2 controls longitudinal and appositional bone growth in early postnatal cartilage development by inducing progression of chondrocytes from the proliferating to the hypertrophic phase and increased perichondrial cell proliferation and differentiation (Uchimura et al. 2017). Nonetheless, in vivo overexpression of IGF2 failed to stimulate growth in the absence of IGF1 (Moerth et al. 2007). Clearly, more studies are needed to determine the actions of IGF2 in chondrocytes.

Collectively, IGF signaling in the perichondrium and the growth plate drives proliferation, clonal expansion and chondrocyte hypertrophy. Despite the limitations of the Cre system, ablations of IGF1 or IGF1R using Cre-driven expression of promoters of the chondrocyte lineage (Col2a1Tam and Col2a1) show the importance of the IGF axis in the growth plate.

Osteoblasts

Lineage commitment of MSC-derived osteoprogenitors to osteoblasts consists of proliferation, matrix maturation and matrix mineralization. Mechanistically, upon activation of the IGF1R on osteoblasts, IGFs initiate signaling cascades to stimulate the expression of the runt-related transcription factor 2 (Runx2) downstream of PI3K/AKT or extracellular signal-regulated kinase (ERK). Runx2 is required for the transition of mesenchymal stem cells into osteoprogenitors. Downstream of runx2 is osterix (osx), which controls the fate from progenitor...
to mature osteoblast. Osteoblasts are responsible for the production and secretion of the extracellular matrix proteins as well as expression of genes involved in extracellular matrix mineralization. In vitro studies have shown that both IGF1 and IGF2 have mitogenic effects on osteoblasts (Canalis & Lian 1988, Mohan et al. 1988, 1989). Activation of the IGF1R in preosteoblasts in vitro increased the expression of the proto-oncogene, c-fos (Merriman et al. 1990) and stimulated type I collagen synthesis, alkaline phosphatase activity and osteocalcin secretion (Schmid et al. 1984, Mohan et al. 1989), partially via induction of osx. Finally, IGfs were also shown to be required for Indian hedgehog (Ihh) regulation of osteoblasts differentiation via activation of the mammalian target of rapamycin complex 2 (mTORC2, defined by Rictor)/AKT pathway and induction of Gli2-mediated transcription (Shi et al. 2015).

In vivo targeting of IGF1 overexpression in late-differentiated osteoblasts via the Col1a1(3.6) promoter (Col1a1(3.6)-IGF-I(Tg)) increased bone remodeling where both bone formation and resorption were enhanced (Jiang et al. 2006, Brennan-Speranza et al. 2011). Col1a1(3.6)-IGF-I(Tg) mice showed thickening of calvarial bones, increased, femoral length and circumference, but no change in trabecular bone volume. Likewise, overexpression of the PAPP-A protease (which liberates IGF1 and increases its bioavailability) under the Col1a1(2.3) promoter (Col1a1(2.3)-PAPP-A(Tg) mice) resulted in increased longitudinal and radial growth of the appendicular skeleton; PAPP-A overexpression was associated with increased osteoid surface and bone formation rate (Qin et al. 2006), indicating that IGF1 stimulates matrix deposition. Similarly, targeting of IGF1 expression in late-differentiated osteoblasts using the osteocalcin promoter (OC-IGF-I(Tg) mice), resulted in increased bone formation rate, trabecular bone volume and BMD. These effects were not associated with increased osteoblast cell number but rather with increased osteoblast activity (Zhao et al. 2000).

In vivo ablation of IGF1R in osteoprogenitors using the osx promoter-driven Cre recombinase (Osx-IGF-IRKO) resulted in impaired growth plate chondrogenesis, secondary ossification and trabecular bone formation (Wang et al. 2015). We note that osx promoter is also expressed in the olfactory glomerular cells and in a subset of the gastric and intestinal epithelium (Chen et al. 2014). Thus, the contribution of IGF1R deletion in non-osteoprogenitor cells to skeletal abnormalities cannot be ruled out. However, ablation of the IGF1R in differentiated osteoblasts using the osteocalcin promoter-derived Cre recombinase (OC-IGF-IRKO mice) resulted in marked reductions in trabecular bone volume and significant reductions in trabecular mineralization, as well as reduced cortical BMD, despite normal numbers of osteoblasts (Zhang et al. 2002, Wang et al. 2007). The latter study has established a significant role for IGF1 in skeletal mineralization. Inhibition of IGF signaling via overexpression of osteocalcin promoter-derived IGFBP-4 (Zhang et al. 2003) or IGFBP-5 (Devlin et al. 2002) in late-differentiated osteoblasts, resulted in decreased bone turnover and BMD.

The roles of IGF2 in osteoblastogenesis are less clear. IGF2 expression in mouse MSCs is very low. However, exogenous IGF2 added to MSCs-induced alkaline phosphatase, osteocalcin and osteopontin expression. IGF2 also augmented BMP-9-induced ectopic bone formation and enhanced bone regeneration (Chen et al. 2010). However, more studies are required to elucidate the roles of IGF2 in the adult skeleton.

Osteoclasts

Osteoclast progenitors originate from the hematopoietic lineage when monocyte-macrophages fuse to form multinucleated cells. Differentiation of osteoclasts is regulated by the macrophage colony-stimulating factor (M-CSF) and the RANKL. M-CSF regulates the survival and proliferation of osteoclast precursors and induces the expression of receptor activator of NF-kB (RANK). Once activated, RANK stimulates the expression of nuclear factor-activated T cells c1 (NFATc1), the master regulator of osteoclast differentiation (Takayanagi et al. 2002).

Ex vivo experiments showed that IGF1 increased resorption in fetal mouse metacarpals or metatarsals cultures (Slootweg et al. 1992). When added to preexistent osteoclasts, IGF1 stimulated bone resorption in a dose-dependent manner (Mochizuki et al. 1992). Another study showed that both IGF1 and IGF2 could stimulate osteoclast bone resorption only if co-cultured with mesenchymal stem cells (MSCs) (Hill et al. 1995). Together, these studies indicate that IGF1 or IGF2 act through an osteoblastic-derived mediator to stimulate osteoclast activity (Hill et al. 1995). The IGF-IKO mice showed reduced numbers of osteoclasts progenitors, inhibition of fusion-induced multinucleation and reduced osteoclast resorption activity (Wang et al. 2006). However, using the global IGF-IKO mice, the contribution of other cells to the phenotype could not be excluded. In an in vitro model of hypoxia-induced osteoclastogenesis, upregulation of IGF2 and stromal cell-derived factor-1 (SDF-1) gene expression was found...
to be crucial for osteoclastogenesis (Fukuoka et al. 2005). Further investigation revealed that IGF2 acts upstream of SDF-1 and CXC chemokine ligand 7 (CXCL7), which have synergistic effects on osteoclastogenesis (Nakao et al. 2009). Overall, these findings suggested that neither IGF1 nor IGF2 is not detrimental for osteoclastogenesis but rather can stimulate osteoclast activity.

**Osteocytes**

Osteocytes are terminally differentiated osteoblasts that reside within the bone matrix and regulate bone matrix integrity and mineral metabolism. The roles of GH or IGF-1 in osteocyte function were studied recently using the dentin matrix acidic phosphoprotein 1 (DMP-1) promoter-driven Cre recombinase to ablate the Ghr, Igf1r or the Igf1. Surprisingly, Ghr gene recombination in DMP-1-expressing cells resulted in slender bones with reduced cortical bone area in mice of both sexes (Liu et al. 2016a). Likewise, Dmp-1-mediated Igf1r deletion (DMP-1-IGF-IRKO) resulted in significant reductions in cortical bone area and a reduced cortical bone thickness with 40–50% increase in marrow area, indicating enhanced endosteal bone resorption in both sexes (Liu et al. 2016b). Finally, Igf1 was also deleted in DMP-1-expressing cells (DMP-1-IGF-IKO), which resulted in slender bones with reduced cortical bone area (Sheng et al. 2013). Collectively, inactivation of the IGF axis in osteocytes compromises cortical bone morphology. However, the DMP-1 promoter-driven Cre is also expressed in late osteoblasts and reported to be expressed in other cells (Lim et al. 2017), which may contribute to the ‘thinner cortex’ phenotype.

Osteocytes sense and respond to mechanical stimuli, which play key roles in bone remodeling. Exposure of osteocytes to fluid shear stress rapidly activates the Wnt signaling pathway via translocation of β-catenin to the nucleus (Norvell et al. 2004, Huesa et al. 2010, Santos et al. 2010). A large body of evidence suggests that IGF1 also mediates bone cell responses to mechanical stimuli, as Igf-1 expression has been shown to increase following mechanical stimulation (Lean et al. 1995, Lau et al. 2006, Reijnders et al. 2007, Sunters et al. 2010). Additionally, DMP-1-IGF-IKO mice showed blunted responses to 4-point bending, significant reductions in the expression of the early mecano-sensitive genes Cox2, C fos and Igf1 and reduced Wnt signaling (Lau et al. 2013). Loss of mechanical stimulation, seen in disuse or zero gravity, is associated with bone loss (Lang et al. 2004, Sievanen 2010). Several studies have shown that unloading-induced bone loss is IGF1 dependent. Hind limb suspension model in rats inhibits the IGFR signaling pathways, which is accompanied by reduced number of osteoblast progenitors, with reduced differentiation and reduced bone formation (Bikle et al. 1994, Sakata et al. 2003, 2004). However, responsiveness to IGF1 could be restored with reloading (Sakata et al. 2003, Boudignon et al. 2007). Unloading in mature osteoblast-specific IGF-IRKO mice (OC-IGF-IRKO), which also show loss of the IGFR in osteocytes, resulted in bone loss that could not be restored by reloading (specifically at the periosteal bone surface) (Kubota et al. 2013), suggesting that IGF1 signaling is necessary for periosteal bone formation following disuse or zero gravity.

**Interactions between IGFs and sex steroids in the skeleton**

Sex steroids can modulate the secretion of GH/IGF1 or their actions in their target tissues. GH, secreted in a sex-specific pattern, contributes to sexual dimorphic gene expression patterns in the liver and other tissues (Flores-Morales et al. 2001, Clodfelter et al. 2006). GH and estrogen (E2) levels show positive correlations in pre-pubertal girls and boys (Kerrigan & Rogol 1992, Veldhuis et al. 2000); in pre-pubertal boys, testosterone (T) induces GH release (Mauras et al. 1987), partially via its aromatization into estrogen (Keenan et al. 1993, Eakman et al. 1996, Veldhuis et al. 1997). The interactions between sex steroids and the GH/IGF1 axis in males occur mainly during puberty (Martha et al. 1989), while in girls, the effects of E2 on the GH/IGF1 axis depend on the administration route, dose and menstrual status (Mauras et al. 1989).

Numerous studies with rodents have been performed to understand the interactions between sex steroids and GH/IGF1 axis in the skeleton. Sexual dimorphism of the skeleton is lost in IGF-IKO mice, indicating blunted effects of sex steroids on bones in this model (Bikle et al. 2001). On the other hand, E2 given to orchiectomized (ORX) GHRKO mice increased serum IGF1 levels, which was associated with increased radial bone growth and thickening of the cortex (Venken et al. 2005), suggesting that IGF1 interacts with E2, independent of GH. The effects of E2 on IGF-1-mediated actions on bone are dose dependent. High doses of E2 decreased serum IGF1 levels, which was associated with suppression of longitudinal and radial bone growth in male rats and lower rate of trabecular bone loss (Wakley et al. 1997). The effects of T on IGF1 levels, on the other hand, can be explained by tissue conversion of T to E2. Treatment of male ORX rats with the combination of T and GH resulted in increased...
cortical bone area, periosteal bone formation rate and femoral BMD. These effects could be partially attributed to the E2-mediated prevention of intracortical bone porosity (Prakash et al. 1999). GH replacement in ovariectomized (OVX) and hypophysectomized rats (where both the sex steroid and the GH/IGF axes are ablated) increased growth, serum levels of osteocalcin and cortical bone formation. Replacement of GH or E2 alone only partially prevented trabecular bone loss, while combined treatment (GH+E2) resulted in an additive increase in trabecular bone volume, indicating that the suppressive effect of E2 on bone resorption and the anabolic effect of GH on bone formation are needed to achieve these effects (Yeh et al. 1997). Likewise, administration of GH to aged OVX rats increased vertebral and femoral bone mass by inducing periosteal bone formation in a dose-dependent manner (Mosekilde et al. 1998). Finally, elevations of GH in LID mice (that have decreased serum IGF1 levels) protected mice from OVX-induced bone loss (Fritton et al. 2010). Overall, the GH/IGF and sex steroid axes are interconnected. During pubertal growth, the interactions between these axes are synergistic, but also are dose and site dependent. These interactions are more complicated in the aging skeleton due to changes in body composition and overall metabolic homeostasis.

Interactions between IGFs and PTH in bone

Parathyroid hormone (PTH) stimulates both bone formation and resorption. When given intermittently PTH is anabolic, while its chronic administration enhances bone resorption. Mature osteoblasts express the PTH receptor (PTH-R). When activated, PTH-R induces RANKL expression in osteoblasts to initiate osteoclastogenesis (Lee & Lorenzo 1999, Ma et al. 2001, Iida-Klein et al. 2002, Huang et al. 2004), thus coupling bone formation with bone resorption. PTH induces a cAMP-dependent cascade mediated mainly by the protein kinase A (PKA) and PKC signaling pathways, which in turn activate runx2 and several other transcription factors.

In vitro (Canalis et al. 1989, Linkhart & Mohan 1989, Centrella et al. 2004) and in vivo (Pfeilschifter et al. 1995, Watson et al. 1995) studies show that IGF1 is an essential mediator of PTH activity. IGF-1KO mice did not respond to anabolic PTH treatment (Bikle et al. 2002), and mice with selective deletion of IGFIR from mature osteoblasts (OC-IGF-IRKO) showed blunted responses to PTH (Wang et al. 2007, Babey et al. 2015). Likewise, mice lacking the insulin receptor substrate-1 (IRS-1), which is a key target for the IGFIR signaling pathway, failed to respond to intermittent PTH administration (Yamaguchi et al. 2005).

The anabolic actions of PTH were significantly reduced in the femoral cortex of the LID and the LID/ALSKO mice, which also had 75% and 90% reductions in serum IGF1, respectively. In the trabecular bone compartment, PTH increased femoral and vertebral trabecular bone volume in LID, but not in LID/ALSKO mice (Yakar et al. 2006). On the other hand, the skeletal response to PTH in the HIT mice, which exhibited a 2-fold increase in IGF1 levels in serum, was significantly higher than that of control mice, suggesting synergy between serum IGF1 and PTH in bone (Elis et al. 2010a). However, 2-fold increases in serum IGF1 did not enhance PTH action on bone when local production of IGF1 was blunted, in the IGF-IKO-HIT mice (Elis et al. 2010a). Thus, enhancement of PTH anabolic action also depends on tissue IGF1. These data are supported by a study in which mice deficient in PAPP-A, the main regulator of IGF1 bioavailability in bone, had an attenuated response to intermittent PTH administration (Clifton & Conover 2015).

Interestingly, mice with ablation of the Ghr gene in mature osteoblasts/osteocytes (DMP-1-GHRKO mice) showed reduced serum inorganic phosphate and PTH levels during pubertal growth (4- to 8-week-old mice). These reductions were associated with decreased bone formation indices, and an impaired response to intermittent PTH treatment (Liu et al. 2016a). In vitro studies with an osteocyte cell line showed that PTH sensitized the response of cells to GH via increased Janus kinase-2 and IGF1R protein levels, suggesting that PTH and GHR signaling in bone is necessary to establish normal radial bone growth and optimize mineral acquisition during puberty; however, in this study, it was unclear if the effects were mediated by IGF1 (Liu et al. 2016a).

Interestingly, a recent study showed that PTH stimulated aerobic glycolysis in MC3T3-E1, an osteoblast-like cell line, via the IGF-I-induced PI3K-mTORC2 cascade, which led to the upregulation of glycolytic enzyme activity (Esen et al. 2015). This is of particular importance because differentiated osteoblasts are mostly glycolytic, implying that PTH regulates osteoblast function via IGF1.

In humans, puberty marks a critical period when longitudinal growth and BMD peak. A few studies with healthy adolescents have shown that PTH levels peak during puberty, which is positively associated with gains in bone mineral content and increased height velocity (DeBoer et al. 2015, Stagi et al. 2015, Matar et al. 2016). Further, treatment of premenopausal women with a recombinant human PTH (teriparatide) resulted in increased IGF1R expression on circulating osteoblast
progenitors, which correlated directly with BMD (Cohen et al. 2017). Overall, the mouse data and the limited clinical studies suggest that the anabolic effects of PTH are partially mediated by IGF1.

**IGFs in the aging skeleton**

A few clinical studies have correlated the reductions in serum IGF1 during aging with decreases in BMD. The Framingham Osteoporosis Study of men and women aged 72–94 indicated that higher IGF1 levels were associated with greater BMD in very old women (Langlois et al. 1998). Additional studies have shown that reduced serum IGF1 levels were associated with increased fracture risk at several skeletal sites (Garnero et al. 2000, Szulc et al. 2004). However, other studies showed no relationship between serum IGF1 and BMD (Kassem et al. 1994, Lloyd et al. 1996). Correlations between serum levels of IGF2 and BMD have been inconclusive. Several studies reported lack of association between IGF2 and BMD (Kim et al. 1999, Ormarsdottir et al. 2001, Amin et al. 2004, Riggs et al. 2008), while others reported a positive relationship (Boonen et al. 1999, Gillberg et al. 2002, Ilangovan et al. 2009). Clearly, more studies are needed to determine the roles of IGF2 in skeletal integrity in the adult and aging skeleton. It has been well established that young or adult subjects with GH deficiency (GHD) have reduced areal BMD and an increased risk for fracture (Rosen et al. 1997, Wuster et al. 2001). GH treatment of these subjects (GHD) effectively increased serum IGF1 and BMD measured at 24 or 36 months after treatment (Baum et al. 1996, Janssen et al. 1998). In addition, a randomized double-blinded control trial of post-menopausal women showed that 3 years of GH treatment was beneficial for bone and fracture outcomes even after 10 years (Krantz et al. 2015). The coincidence between the declines in IGFs and age-related bone loss in humans implies that GH or IGF treatment of the elderly should also resolve the declines in BMD. However, trials with rhGH or rhIGF-1 in elderly patients either with primary osteoporosis or frailty yielded conflicting results (Marcus et al. 1990, Rudman et al. 1990, Ghiron et al. 1995, Thompson et al. 1995, Holloway et al. 1997). Thus, more studies are needed to understand the effects of the IGF system on aging bone.

*Ex vivo* studies of human bone specimens showed that skeletal IGF1 content decline by ~60% between the ages of 20 and 60 years (Nicolas et al. 1994). This was confirmed by another study showing an age-related decrease in IGFBP-5, which maintains the IGF pool in the bone matrix (Nicolas et al. 1995, Mohan & Baylink 1997). *In vitro* studies of primary osteoblasts from elderly subjects exhibited IGF1 resistance, as evidenced by the requirement of ~10-fold higher concentrations of IGF1 to stimulate DNA synthesis compared to cells from younger controls (Pfeilschifter et al. 1993).

Mouse models with congenital disruptions of components of the GH/IGF axis cannot distinguish developmental- and age-related effects of IGF1 on the skeleton. Thus, to understand the relationship between GH/IGF levels and skeletal integrity in the mouse, a model with inducible (adult-onset) inactivation of the GH/IGF axis is needed. Using the inducible liver-specific IGF1 deletion (iLID) mouse model, it was shown that reductions in serum IGF1 during aging (at 12 months of age) led to significant reductions in cortical bone thickness, BMD and mechanical properties, suggesting that IGF1 has considerable effects on maintaining a healthy skeleton during aging (Courtland et al. 2011, Ashpole et al. 2016a,b). Further studies are needed to explore which cells and what molecular mechanisms are involved. During aging, bone remodeling slows significantly and osteoprogenitor numbers decline. Loss of osteocytes from bone matrix is perhaps the best characterized example of how these cells are required to maintain bone composition, and how their loss diminishes bone quality (Tatsumi et al. 2007). Given the importance of IGF1 signaling in protecting cells from apoptosis, as well as in metabolism, it is conceivable that IGF1 regulates the viability and function of aging osteocytes and, by inference, bone integrity.

**Summary and concluding remarks**

- The IGF system does not control skeletal morphogenesis but regulates skeletal size and integrity.
- The actions of the IGFs on the skeleton are mediated by the IGF-IR. Defects in the IGF1R or the IGFs in humans are rare and often present with growth retardation and skeletal abnormalities.
- In mice, the predominant IGF that promotes postnatal growth is IGF1, while prenatal growth is regulated by IGF2. Likewise, in humans IGF1 regulates postnatal growth although, IGF2 is expressed both pre- and postnatally. In humans, excess in IGF2 is associated with overgrowth, while deficiency in IGF2 is associated with growth retardation. More studies are needed to understand the roles of IGF2 in the human skeleton.
- IGFs act in an endocrine and autocrine/paracrine fashion.
- IGFs are bound to IGFBPs; the bioavailability of IGFs is controlled by the IGFBPs and the IGFBP proteases.
• Reductions in endocrine IGF1 compromise radial bone growth, but have little effect on linear bone growth. The effects of endocrine IGF2 on the skeleton are yet to be defined.
• All osteogenic cells produce IGFs that act in an autocrine/paracrine fashions.
• IGFs play important roles in differentiation and maturation of chondrocytes of the growth plate. They stimulate osteoblastogenesis and enhance collagen secretion and bone matrix mineralization. In terminally differentiated osteoblasts, namely osteocytes, IGF1 play roles in traducing mechanical stimuli and promoting the anabolic response.
• The IGF system interacts with other hormones, such as PTH and sex steroids, to induce bone anabolism.

Open questions
• During aging, serum IGFs levels decline and associate with reduced BMD. However, the direct effects of IGFs on the aging skeleton are not clear and require further investigation.
• The effects of IGF2 on the adult and aged human skeleton are not yet established.
• The IGF system regulates substrate metabolism (carbohydrate, lipid and protein metabolism) in many tissues including fat, muscle, liver and kidney. However, its effects on substrate metabolism in the skeleton during growth and aging are not clear.
• It is yet unclear how epigenetic modulations (including methylation, acetylation, phosphorylation, ubiquitylation and SUMOylation) or environmental interventions (diet, exercise), which are partially regulated by IGFs, affect skeletal integrity.

References

Declaration of interest
All authors concur with the submission. The material submitted for publication has not previously been reported and is not under consideration for publication elsewhere. Dr Yakar is the guarantor of this review and, as such, had full access to all reported studies and takes responsibility for the integrity and accuracy of the review article.

Funding
This study was supported by funding from the National Institutes of Health (Grant #DK100246 to S Y) and Bi-national Science Foundation (Grant #2013282 to S Y and H W).

Author contribution statement
All authors participated in MS writing and editing.


Clarmatiot S, Kiepe D, Haarmann A, Huegel U & Tonshoff B 2007 Signaling mechanisms leading to regulation of proliferation and differentiation of the mesenchymal chondrogenic cell line RCE3.1CS.18 in response to IGF-I. Molecular Endocrinology 38 493–508. (https://doi.org/10.1677/jme.1.02179)

Clifton KB & Conover CA 2015 Pregnancy-associated plasma protein-A modulates the anabolic effects of parathyroid hormone in mouse bone. Bone 81 413–416. (https://doi.org/10.1016/j.bone.2015.08.015)

Clodfelter KH, Holloway MG, Hodor P, Park SH, Ray WJ & Waxman DJ 2006 Sex-dependent liver gene expression is extensive and largely dependent upon signal transducer and activator of transcription 5b (STAT5b): STAT5b-dependent activation of male genes and repression of female genes revealed by microarray analysis. Molecular Endocrinology 20 1333–1351. (https://doi.org/10.1210/me.2005-0489)


Conover CA, Perry JE & Tindall DJ 1995 Endogenous cathepsin D-mediated hydrolysis of insulin-like growth factor-binding proteins in cultured human prostatic carcinoma cells. Journal of Clinical...
Endocrinology and Metabolism **80** 987–993. (https://doi.org/10.1210/ jcem.80.3.7533776)


Daughaday WH 2006 Endocrinology – the way we were: a personal history of somatomedin. *Growth Hormone and IGF Research* **16** (Supplement A) S3–S5. (https://doi.org/10.1016/j.ygih.2006.03.005)


Greenspan FS, Li CH, Simpson ME & Evans HM 1949 Bioassay of hypophysial growth hormone; the tibia test. *Endocrinology* **45** 455–463. (https://doi.org/10.1210/endo-45-5-455)


Holloway L, Kohlmeier L, Kent K & Marcus R 1997 Skeletal effects of cyclic recombinant human growth hormone and salmon calcitonin in osteopenic postmenopausal women. *Journal of Clinical Endocrinology and Metabolism* **82** 1111–1117. (https://doi.org/10.1210/jcem.82.4.3901)


Lee SK & Lorenzo JA 1999 Parathyroid hormone stimulates TRANCE and inhibits osteoprotegerin messenger ribonucleic acid expression in murine bone marrow cultures: correlation with osteoclast-like cell formation. *Endocrinology* **140** 3552–3561. (https://doi.org/10.1210/endo.140.8.6887)


Liu JP, Baker J, Perkins AS, Robertson EJ & Efstatiadis A 1993 Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell* **75** 59–72. (https://doi.org/10.1016/S0092-8674(05)80084-4)


Lupu F, Terwilliger JD, Lee K, Segre GV & Efstatiadis A 2001 Roles of *Igf-1* and *Igf1r* in skeletal response to intermittent PTH. *Endocrinology* **142** 4047–4054. (https://doi.org/10.1210/endo.142.8.8356)


Clinical Endocrinology and Metabolism 70 519–527. (https://doi.org/10.1210/jcem-70-2-519)


Murphy WR, Daughaday WH & Hartnett C 1956 The effect of hypophysectomy and growth hormone on the incorporation of labeled sulfate into tibial epiphyseal and nasal cartilage of the rat. Journal of Laboratory and Clinical Medicine 47 715–722.


Ning Y, Schuller AG, Conover CA & Pintar JE 2008 Insulin-like growth factor (IGF) binding protein-4 is both a positive and negative regulator of IGF-II bioavailability in vivo. *Molecular Endocrinology* 22 1213–1225. (https://doi.org/10.1210/me.2007-0536)


Rotwein P 1986 Two insulin-like growth factor I messenger RNAs are expressed in human liver. *PNAS* 83 77–81. (https://doi.org/10.1073/pnas.83.1.77)


heterozygous mutation in the insulin-like growth factor I gene. 
Journal of Clinical Endocrinology and Metabolism 95  E363–E367. (https://doi.org/10.1210/jc.2010-0511)


Veldhuis JD, Roemmich JN & Rogol AD 2000 Gender and sexual maturation-dependent contrasts in the neuroregulation of growth hormone secretion in prepubertal and late adolescent males and females – a general clinical research center-based study. Journal of Clinical Endocrinology and Metabolism 85  2385–2394. (https://doi.org/10.1210/jcem.85.5.6697)


Wakley GK, Evans GI & Turner RT 1997 Short-term effects of high dose estrogen on tibiae of growing male rats. Calcified Tissue International 60  37–42. (https://doi.org/10.1007/1002239900158)


Walenkamp MJ, Losekoot M & Wit JM 2013 Molecular IGF-1 and IGF-1 receptor defects: from genetics to clinical management. Endocrine Reviews 34  259–299. (https://doi.org/10.1210/endo-rv-34-4-0022)


Werner H & Bruchim I 2012 IGF-1 and BRCA1 signaling pathways in familial cancer. Lancet Oncology 13  e537–e544. (https://doi.org/10.1016/S1470-2241(12)70362-5)


Received in final form 29 March 2018
Accepted 6 April 2018
Accepted Preprint published online 6 April 2018