THEMATIC REVIEW

40 YEARS OF IGF1

Insulin-like growth factors: actions on the skeleton

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Abstract

The discovery of the growth hormone (GH)-mediated somatic factors (somatomedins), insulin-like growth factor (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.

Key Words

- ▶ insulin-like
- ▶ osteoblast
- ▶ osteoclast
- ▶ osteocyte
- ▶ chondrocyte

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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-I-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 somatotrophic axis (also called the GH/IGF axis).

Overview of the somatotrophic axis

The somatotrophic 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 ~16 h). 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 ~10 min.

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

Figure 1

The somatotrophic axis. Hypothalamic GHRH and somatostatin as well as intestine-secreted ghrelin regulate pituitary secretion of GH. Once released to circulation, GH stimulates liver production of IGFs and a few of the IGFBPs. In the vasculature, IGFs are found in binary and ternary complexes, which increase their half-lives. Serum and tissue proteases act upon the IGFBPs to liberate IGFs and increase their bioavailability. Target cells expressing the IGF-IR, IR or hybrid receptors bind the IGFs and initiate phosphorylation cascades to enhance cellular proliferation, differentiation and function. GHR is found on almost all cells. Upon binding to its receptor, GH initiates signaling cascades to promote cellular function that may be IGF dependent or independent.

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

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 Wilms' 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

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 pro-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 hemizyosity 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 mice, 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 (Gannage-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-I^{+/-}) 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* (Nordstrom *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 (μ CT) analyses of bones from liver-specific JAK2KO mice (Nordstrom *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 (Elis *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% increases 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 (Elis *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^{tg} 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^{tg} 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 GHR 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 IGF-I/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

subjects (with dysfunctional GHR) were treated with recombinant human IGF1 (rhIGF-I). Most studies (Laron *et al.* 1992, Guevara-Aguirre *et al.* 1995, 1997, Klinger & Laron 1995, Ranke *et al.* 1995, Backeljauw & Underwood 1996, 2001, Mauras *et al.* 2000, Tonella *et al.* 2010, El Kholly & Elsedfy 2011) indicated increases in linear growth and height velocity; however, rhIGF-I treatment did not normalize skeletal growth.

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 postnatally (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 PAPP2 (PAPP2)-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). PAPP2 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 *des₍₁₋₃₎-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 *des₍₁₋₃₎-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 *des₍₁₋₃₎-Igf1* (KID) and *LR3-Igf1* (KIR) in increasing somatic growth, independent of serum IGF-1 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- κ B (NF- κ B) 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 morphogenic 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 *ghr*, *Igf1* and the *Igf1r* 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),

Figure 2

Bone cell-specific effects of IGFs. IGFs regulate the mesenchymal and hematopoietic osteoprogenitor pools and participate in lineage commitment. In the growth plate IGFs promote clonal expansion of chondrocytes at the proliferation zone, and cellular maturation of pre-hypertrophic chondrocytes. During bone modeling and remodeling IGFs enhance osteoblastogenesis and promote matrix deposition and mineralization. Once embedded in the matrix, osteoblasts undergo terminal differentiation to osteocytes that play important roles in keeping the bone matrix integrity. Osteocytes are the bone mechanosensors that mediate their response to loads partially via secretion of IGFs. The roles of IGFs in osteoclastogenesis are yet to be defined. IGFs can stimulate fusion of osteoclast progenitors and enhance osteoclasts resorption activity.

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 (Col2a1^{Tam-cre}) was also used to delete the IGF1R in chondrocytes (Col2a1^{Tam}-IGF-IRKO) at postnatal day 5. Col2a1^{Tam}-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 Col2a1^{Tam}-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 (Col2a1^{Tam} 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-IT⁸) increased bone remodeling where both bone formation and resorption were enhanced (Jiang *et al.* 2006, Brennan-Speranza *et al.* 2011). *Col1a1*_(3,6)-IGF-IT⁸ 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^{T8} 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-IT⁸ 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- κ B (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-IRKO 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-IRKO 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, Sinters *et al.* 2010). Additionally, DMP-1-IGF-IKO mice showed blunted responses to 4-point bending, significant reductions in the expression of the early mechano-sensitive genes *Cox2*, *Cfos* 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 IGF1R 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-1RKO), which also show loss of the IGF1R 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 orchietomized (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-I-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 (Prakasam *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 IGF1R from mature osteoblasts (OC-IGF-1RKO) 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 IGF1R 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-1KO-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-I 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.

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.

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References

- Adamo ML, Ben-Hur H, Roberts CT Jr & LeRoith D 1991 Regulation of start site usage in the leader exons of the rat insulin-like growth factor-I gene by development, fasting, and diabetes. *Molecular Endocrinology* **5** 1677–1686. (<https://doi.org/10.1210/mend-5-11-1677>)
- Amin S, Riggs BL, Atkinson EJ, Oberg AL, Melton LJ 3rd & Khosla S 2004 A potentially deleterious role of IGFBP-2 on bone density in aging men and women. *Journal of Bone and Mineral Research* **19** 1075–1083. (<https://doi.org/10.1359/JBMR.040301>)
- Ashpole NM, Herron JC, Estep PN, Logan S, Hodges EL, Yabluchanskiy A, Humphrey MB & Sonntag WE 2016a Differential effects of IGF-1 deficiency during the life span on structural and biomechanical properties in the tibia of aged mice. *Age* **38** 38. (<https://doi.org/10.1007/s11357-016-9902-5>)
- Ashpole NM, Herron JC, Mitschelen MC, Farley JA, Logan S, Yan H, Ungvari Z, Hodges EL, Csiszar A, Ikeno Y, et al. 2016b IGF-1 regulates vertebral bone aging through sex-specific and time-dependent mechanisms. *Journal of Bone and Mineral Research* **31** 443–454. (<https://doi.org/10.1002/jbmr.2689>)
- Babey M, Wang Y, Kubota T, Fong C, Menendez A, ElAlieh HZ & Bikle DD 2015 Gender-specific differences in the skeletal response to continuous PTH in mice lacking the IGF1 receptor in mature osteoblasts. *Journal of Bone and Mineral Research* **30** 1064–1076. (<https://doi.org/10.1002/jbmr.2433>)
- Backeljauw PF & Underwood LE 1996 Prolonged treatment with recombinant insulin-like growth factor-I in children with growth hormone insensitivity syndrome – a clinical research center study. GHIS Collaborative Group. *Journal of Clinical Endocrinology and Metabolism* **81** 3312–3317. (<https://doi.org/10.1210/jcem.81.9.8784089>)
- Backeljauw PF & Underwood LE 2001 Therapy for 6.5–7.5 years with recombinant insulin-like growth factor I in children with growth hormone insensitivity syndrome: a clinical research center study. *Journal of Clinical Endocrinology and Metabolism* **86** 1504–1510. (<https://doi.org/10.1210/jcem.86.4.7381>)
- Bale LK & Conover CA 2005 Disruption of insulin-like growth factor-II imprinting during embryonic development rescues the dwarf phenotype of mice null for pregnancy-associated plasma protein-A. *Journal of Endocrinology* **186** 325–331. (<https://doi.org/10.1677/joe.1.06259>)
- Ballard FJ, Knowles SE, Walton PE, Edson K, Owens PC, Mohler MA & Ferraiolo BL 1991 Plasma clearance and tissue distribution of labelled insulin-like growth factor-I (IGF-I), IGF-II and des(1–3)IGF-I in rats. *Journal of Endocrinology* **128** 197–204. (<https://doi.org/10.1677/joe.0.1280197>)
- Bastian SE, Walton PE, Wallace JC & Ballard FJ 1993 Plasma clearance and tissue distribution of labelled insulin-like growth factor-I (IGF-I) and an analogue LR3IGF-I in pregnant rats. *Journal of Endocrinology* **138** 327–336. (<https://doi.org/10.1677/joe.0.1380327>)
- Batey L, Moon JE, Yu Y, Wu B, Hirschhorn JN, Shen Y & Dauber A 2014 A novel deletion of IGF1 in a patient with idiopathic short stature provides insight into IGF1 haploinsufficiency. *Journal of Clinical Endocrinology and Metabolism* **99** E153–E159. (<https://doi.org/10.1210/jc.2013-3106>)
- Baum HB, Biller BM, Finkelstein JS, Cannistraro KB, Oppenheim DS, Schoenfeld DA, Michel TH, Wittink H & Klibanski A 1996 Effects of physiologic growth hormone therapy on bone density and body composition in patients with adult-onset growth hormone deficiency. A randomized, placebo-controlled trial. *Annals of Internal Medicine* **125** 883–890. (<https://doi.org/10.7326/0003-4819-125-11-199612010-00003>)
- Baxter RC 2000 Insulin-like growth factor (IGF)-binding proteins: interactions with IGFs and intrinsic bioactivities. *American Journal of Physiology: Endocrinology and Metabolism* **278** E967–E976. (<https://doi.org/10.1152/ajpendo.2000.278.6.E967>)

- Begemann M, Zirn B, Santen G, Wirthgen E, Soellner L, Buttell HM, Schweizer R, van Workum W, Binder G & Eggermann T 2015 Paternally inherited IGF2 mutation and growth restriction. *New England Journal of Medicine* **373** 349–356. (<https://doi.org/10.1056/NEJMoa1415227>)
- Behringer RR, Lewin TM, Quaife CJ, Palmiter RD, Brinster RL & D'Ercole AJ 1990 Expression of insulin-like growth factor I stimulates normal somatic growth in growth hormone-deficient transgenic mice. *Endocrinology* **127** 1033–1040. (<https://doi.org/10.1210/endo-127-3-1033>)
- Ben Lagha N, Menuelle P, Seurin D, Binoux M, Lebouc Y & Berald A 2002 Bone formation in the context of growth retardation induced by hIGFBP-1 overexpression in transgenic mice. *Connective Tissue Research* **43** 515–519. (<https://doi.org/10.1080/03008200290000998>)
- Ben Lagha N, Seurin D, Le Bouc Y, Binoux M, Berald A, Menuelle P & Babajko S 2006 Insulin-like growth factor binding protein (IGFBP-1) involvement in intrauterine growth retardation: study on IGFBP-1 overexpressing transgenic mice. *Endocrinology* **147** 4730–4737. (<https://doi.org/10.1210/en.2006-0171>)
- Benbassat C, Shoba LN, Newman M, Adamo ML, Frank SJ & Lowe WL Jr 1999 Growth hormone-mediated regulation of insulin-like growth factor I promoter activity in C6 glioma cells. *Endocrinology* **140** 3073–3081. (<https://doi.org/10.1210/endo.140.7.6762>)
- Bentov I & Werner H 2004 IGF, IGF receptor and overgrowth syndromes. *Pediatric Endocrinology Reviews* **1** 352–360.
- Bikle DD, Harris J, Halloran BP & Morey-Holton ER 1994 Skeletal unloading induces resistance to insulin-like growth factor I. *Journal of Bone and Mineral Research* **9** 1789–1796. (<https://doi.org/10.1002/jbmr.5650091116>)
- Bikle D, Majumdar S, Laib A, Powell-Braxton L, Rosen C, Beamer W, Nauman E, Leary C & Halloran B 2001 The skeletal structure of insulin-like growth factor I-deficient mice. *Journal of Bone and Mineral Research* **16** 2320–2329. (<https://doi.org/10.1359/jbmr.2001.16.12.2320>)
- Bikle DD, Sakata T, Leary C, Elalieh H, Ginzinger D, Rosen CJ, Beamer W, Majumdar S & Halloran BP 2002 Insulin-like growth factor I is required for the anabolic actions of parathyroid hormone on mouse bone. *Journal of Bone and Mineral Research* **17** 1570–1578. (<https://doi.org/10.1359/jbmr.2002.17.9.1570>)
- Bonadonna S, Mazziotti G, Nuzzo M, Bianchi A, Fusco A, De Marinis L & Giustina A 2005 Increased prevalence of radiological spinal deformities in active acromegaly: a cross-sectional study in postmenopausal women. *Journal of Bone and Mineral Research* **20** 1837–1844. (<https://doi.org/10.1359/JBMR.050603>)
- Bondy CA, Werner H, Roberts CT Jr & LeRoith D 1990 Cellular pattern of insulin-like growth factor-I (IGF-I) and type I IGF receptor gene expression in early organogenesis: comparison with IGF-II gene expression. *Molecular Endocrinology* **4** 1386–1398. (<https://doi.org/10.1210/mend-4-9-1386>)
- Boonen S, Mohan S, Dequeker J, Aerssens J, Vanderschueren D, Verbeke G, Broos P, Bouillon R & Baylink DJ 1999 Down-regulation of the serum stimulatory components of the insulin-like growth factor (IGF) system (IGF-I, IGF-II, IGF binding protein (BP)-3, and IGFBP-5) in age-related (type II) femoral neck osteoporosis. *Journal of Bone and Mineral Research* **14** 2150–2158. (<https://doi.org/10.1359/jbmr.1999.14.12.2150>)
- Boudignon BM, Bikle DD, Kurimoto P, Elalieh H, Nishida S, Wang Y, Burghardt A, Majumdar S, Orwoll BE, Rosen C, et al. 2007 Insulin-like growth factor I stimulates recovery of bone lost after a period of skeletal unloading. *Journal of Applied Physiology* (1985) **103** 125–131. (<https://doi.org/10.1152/jappphysiol.00111.2007>)
- Brennan-Speranza TC, Rizzoli R, Kream BE, Rosen C & Ammann P 2011 Selective osteoblast overexpression of IGF-I in mice prevents low protein-induced deterioration of bone strength and material level properties. *Bone* **49** 1073–1079. (<https://doi.org/10.1016/j.bone.2011.07.039>)
- Canalis E & Lian JB 1988 Effects of bone associated growth factors on DNA, collagen and osteocalcin synthesis in cultured fetal rat calvariae. *Bone* **9** 243–246. ([https://doi.org/10.1016/8756-3282\(88\)90037-3](https://doi.org/10.1016/8756-3282(88)90037-3))
- Canalis E, Centrella M, Burch W & McCarthy TL 1989 Insulin-like growth factor I mediates selective anabolic effects of parathyroid hormone in bone cultures. *Journal of Clinical Investigation* **83** 60–65. (<https://doi.org/10.1172/JCI113885>)
- Capatina C & Wass JA 2015 60 years of neuroendocrinology: acromegaly. *Journal of Endocrinology* **226** T141–T160. (<https://doi.org/10.1530/JOE-15-0109>)
- Castilla-Cortazar I, Guerra L, Puche JE, Munoz U, Barhoum R, Escudero E & Lavandera JL 2014 An experimental model of partial insulin-like growth factor-1 deficiency in mice. *Journal of Physiology and Biochemistry* **70** 129–139. (<https://doi.org/10.1007/s13105-013-0287-y>)
- Centrella M, Christakos S & McCarthy TL 2004 Skeletal hormones and the C/EBP and Runx transcription factors: interactions that integrate and redefine gene expression. *Gene* **342** 13–24. (<https://doi.org/10.1016/j.gene.2004.06.036>)
- Chen L, Jiang W, Huang J, He BC, Zuo GW, Zhang W, Luo Q, Shi Q, Zhang BQ, Wagner ER, et al. 2010 Insulin-like growth factor 2 (IGF-2) potentiates BMP-9-induced osteogenic differentiation and bone formation. *Journal of Bone and Mineral Research* **25** 2447–2459. (<https://doi.org/10.1002/jbmr.133>)
- Chen J, Shi Y, Regan J, Karuppaiah K, Ornitz DM & Long F 2014 Osx-Cre targets multiple cell types besides osteoblast lineage in postnatal mice. *PLoS ONE* **9** e85161. (<https://doi.org/10.1371/journal.pone.0085161>)
- Ciarmatori 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 RCJ3.1C5.18 in response to IGF-I. *Journal of 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>)
- Cohen P, Graves HC, Peehl DM, Kamarei M, Giudice LC & Rosenfeld RG 1992 Prostate-specific antigen (PSA) is an insulin-like growth factor binding protein-3 protease found in seminal plasma. *Journal of Clinical Endocrinology and Metabolism* **75** 1046–1053. (<https://doi.org/10.1210/jcem.75.4.1383255>)
- Cohen P, Peehl DM, Graves HC & Rosenfeld RG 1994 Biological effects of prostate specific antigen as an insulin-like growth factor binding protein-3 protease. *Journal of Endocrinology* **142** 407–415. (<https://doi.org/10.1677/joe.0.1420407>)
- Cohen A, Kousteni S, Bisikirska B, Shah JG, Manavalan JS, Recker RR, Lappe J, Dempster DW, Zhou H, McMahon DJ, et al. 2017 IGF-1 receptor expression on circulating osteoblast progenitor cells predicts tissue-based bone formation rate and response to teriparatide in premenopausal women with idiopathic osteoporosis. *Journal of Bone and Mineral Research* **32** 1267–1273. (<https://doi.org/10.1002/jbmr.3109>)
- Collett-Solberg PF & Cohen P 1996 The role of the insulin-like growth factor binding proteins and the IGFBP proteases in modulating IGF action. *Endocrinology Metabolism Clinics of North America* **25** 591–614. ([https://doi.org/10.1016/S0889-8529\(05\)70342-X](https://doi.org/10.1016/S0889-8529(05)70342-X))
- 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>)
- Conover CA, Johnstone EW, Turner RT, Evans GL, John Ballard FJ, Doran PM & Khosla S 2002 Subcutaneous administration of insulin-like growth factor (IGF)-II/IGF binding protein-2 complex stimulates bone formation and prevents loss of bone mineral density in a rat model of disuse osteoporosis. *Growth Hormone and IGF Research* **12** 178–183. ([https://doi.org/10.1016/S1096-6374\(02\)00044-8](https://doi.org/10.1016/S1096-6374(02)00044-8))
- Courtland HW, DeMambro V, Maynard J, Sun H, Elis S, Rosen C & Yakar S 2010 Sex-specific regulation of body size and bone slenderness by the acid labile subunit. *Journal of Bone and Mineral Research* **25** 2059–2068. (<https://doi.org/10.1002/jbmr.94>)
- Courtland HW, Elis S, Wu Y, Sun H, Rosen CJ, Jepsen KJ & Yakar S 2011 Serum IGF-1 affects skeletal acquisition in a temporal and compartment-specific manner. *PLoS ONE* **6** e14762. (<https://doi.org/10.1371/journal.pone.0014762>)
- Cui Y, Hosui A, Sun R, Shen K, Gavrilova O, Chen W, Cam MC, Gao B, Robinson GW & Hennighausen L 2007 Loss of signal transducer and activator of transcription 5 leads to hepatosteatosis and impaired liver regeneration. *Hepatology* **46** 504–513. (<https://doi.org/10.1002/hep.21713>)
- D'Ercole AJ 1993 Expression of insulin-like growth factor-I in transgenic mice. *Annals of the New York Academy of Sciences* **692** 149–160. (<https://doi.org/10.1111/j.1749-6632.1993.tb26213.x>)
- Dauber A, Munoz-Calvo MT, Barrios V, Domene HM, Kloverpris S, Serra-Juhe C, Desikan V, Pozo J, Muzumdar R, Martos-Moreno GA, et al. 2016 Mutations in pregnancy-associated plasma protein A2 cause short stature due to low IGF-I availability. *EMBO Molecular Medicine* **8** 363–374. (<https://doi.org/10.15252/emmm.201506106>)
- 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.ghir.2006.03.005>)
- Daughaday WH, Hall K, Raben MS, Salmon WD Jr, van den Brande JL & van Wyk JJ 1972 Somatomedin: proposed designation for sulphation factor. *Nature* **235** 107. (<https://doi.org/10.1038/235107a0>)
- DeBoer MD, Weber DR, Zemel BS, Denburg MR, Herskovitz R, Long J & Leonard MB 2015 Bone mineral accrual is associated with parathyroid hormone and 1,25-dihydroxyvitamin D levels in children and adolescents. *Journal of Clinical Endocrinology and Metabolism* **100** 3814–3821. (<https://doi.org/10.1210/jc.2015-1637>)
- DeChiara TM, Robertson EJ & Efstratiadis A 1991 Parental imprinting of the mouse insulin-like growth factor II gene. *Cell* **64** 849–859. ([https://doi.org/10.1016/0092-8674\(91\)90513-X](https://doi.org/10.1016/0092-8674(91)90513-X))
- Delahunty KM, Shultz KL, Gronowicz GA, Koczon-Jaremko B, Adamo ML, Horton LG, Lorenzo J, Donahue LR, Ackert-Bicknell C, Kream BE, et al. 2006 Congenic mice provide in vivo evidence for a genetic locus that modulates serum insulin-like growth factor-I and bone acquisition. *Endocrinology* **147** 3915–3923. (<https://doi.org/10.1210/en.2006-0277>)
- DeMambro VE, Clemmons DR, Horton LG, Bouxsein ML, Wood TL, Beamer WG, Canalis E & Rosen CJ 2008 Gender-specific changes in bone turnover and skeletal architecture in igfbp-2-null mice. *Endocrinology* **149** 2051–2061. (<https://doi.org/10.1210/en.2007-1068>)
- Devlin RD, Du Z, Buccilli V, Jorgetti V & Canalis E 2002 Transgenic mice overexpressing insulin-like growth factor binding protein-5 display transiently decreased osteoblastic function and osteopenia. *Endocrinology* **143** 3955–3962. (<https://doi.org/10.1210/en.2002-220129>)
- Domene HM, Hwa V, Argente J, Wit JM, Camacho-Hubner C, Jasper HG, Pozo J, van Duyvenvoorde HA, Yakar S, Fofanova-Gambetti OV, et al. 2009 Human acid-labile subunit deficiency: clinical, endocrine and metabolic consequences. *Hormone Research* **72** 129–141. (<https://doi.org/10.1159/000232486>)
- Domene HM, Scaglia PA & Jasper HG 2010 Deficiency of the insulin-like growth factor-binding protein acid-labile subunit (ALS) of the circulating ternary complex in children with short stature. *Pediatric Endocrinology Reviews* **7** 339–346.
- Eakman GD, Dallas JS, Ponder SW & Keenan BS 1996 The effects of testosterone and dihydrotestosterone on hypothalamic regulation of growth hormone secretion. *Journal of Clinical Endocrinology and Metabolism* **81** 1217–1223. (<https://doi.org/10.1210/jcem.81.3.8772602>)
- Ekstrom TJ 1994 Parental imprinting and the IGF2 gene. *Hormone Research* **42** 176–181. (<https://doi.org/10.1159/000184190>)
- El Kholy M & Elsedfy HH 2011 Effect of GnRH analogue on height potential in patients with severe growth hormone insensitivity syndrome treated with IGF-I. *Journal of Pediatric Endocrinology and Metabolism* **24** 983–988.
- Elis S, Courtland HW, Wu Y, Fritton JC, Sun H, Rosen CJ & Yakar S 2010a Elevated serum IGF-1 levels synergize PTH action on the skeleton only when the tissue IGF-1 axis is intact. *Journal of Bone and Mineral Research* **25** 2051–2058. (<https://doi.org/10.1002/jbmr.1000>)
- Elis S, Courtland HW, Wu Y, Rosen CJ, Sun H, Jepsen KJ, Majeska RJ & Yakar S 2010b Elevated serum levels of IGF-1 are sufficient to establish normal body size and skeletal properties even in the absence of tissue IGF-1. *Journal of Bone and Mineral Research* **25** 1257–1266. (<https://doi.org/10.1002/jbmr.20>)
- Elis S, Wu Y, Courtland HW, Cannata D, Sun H, Beth-On M, Liu C, Jasper H, Domene H, Karabatas L, et al. 2011a Unbound (bioavailable) IGF1 enhances somatic growth. *Disease Models and Mechanisms* **4** 649–658. (<https://doi.org/10.1242/dmm.006775>)
- Elis S, Wu Y, Courtland HW, Sun H, Rosen CJ, Adamo ML & Yakar S 2011b Increased serum IGF-1 levels protect the musculoskeletal system but are associated with elevated oxidative stress markers and increased mortality independent of tissue igf1 gene expression. *Aging Cell* **10** 547–550. (<https://doi.org/10.1111/j.1474-9726.2011.00683.x>)
- Ellis MJ, Jenkins S, Hanfelt J, Redington ME, Taylor M, Leek R, Siddle K & Harris A 1998 Insulin-like growth factors in human breast cancer. *Breast Cancer Research and Treatment* **52** 175–184. (<https://doi.org/10.1023/A:1006127621512>)
- Esen E, Lee SY, Wice BM & Long F 2015 PTH promotes bone anabolism by stimulating aerobic glycolysis via IGF signaling. *Journal of Bone and Mineral Research* **30** 1959–1968. (<https://doi.org/10.1002/jbmr.2556>)
- Flores-Morales A, Stahlberg N, Tollet-Egnell P, Lundeberg J, Malek RL, Quackenbush J, Lee NH & Norstedt G 2001 Microarray analysis of the in vivo effects of hypophysectomy and growth hormone treatment on gene expression in the rat. *Endocrinology* **142** 3163–3176. (<https://doi.org/10.1210/endo.142.7.8235>)
- Fowlkes JL, Enghild JJ, Suzuki K & Nagase H 1994a Matrix metalloproteinases degrade insulin-like growth factor-binding protein-3 in dermal fibroblast cultures. *Journal of Biological Chemistry* **269** 25742–25746.
- Fowlkes JL, Suzuki K, Nagase H & Thraill KM 1994b Proteolysis of insulin-like growth factor binding protein-3 during rat pregnancy: a role for matrix metalloproteinases. *Endocrinology* **135** 2810–2813. (<https://doi.org/10.1210/endo.135.6.7527335>)
- Francis GL, McMurtry JP, Johnson RJ & Ballard FJ 1990 Plasma clearance of chicken and human insulin-like growth factor-I and their association with circulating binding proteins in chickens. *Journal of Endocrinology* **124** 361–370. (<https://doi.org/10.1677/joe.0.1240361>)
- Francois JC, Aid S, Chaker Z, Lacube P, Xu J, Fayad R, Cote F, Even P & Holzenberger M 2017 Disrupting IGF signaling in adult mice conditions leanness, resilient energy metabolism, and high growth hormone pulses. *Endocrinology* **158** 2269–2283. (<https://doi.org/10.1210/en.2017-00261>)
- Fritton JC, Emerton KB, Sun H, Kawashima Y, Mejia W, Wu Y, Rosen CJ, Panus D, Bouxsein M, Majeska RJ, et al. 2010 Growth hormone protects against ovariectomy-induced bone loss in states of low circulating insulin-like growth factor (IGF-1). *Journal of Bone and Mineral Research* **25** 235–246. (<https://doi.org/10.1359/jbmr.090723>)

- Fukuoka H, Aoyama M, Miyazawa K, Asai K & Goto S 2005 Hypoxic stress enhances osteoclast differentiation via increasing IGF2 production by non-osteoclastic cells. *Biochemical and Biophysical Research Communications* **328** 885–894. (<https://doi.org/10.1016/j.bbrc.2005.01.042>)
- Fuqua JS, Derr M, Rosenfeld RG & Hwa V 2012 Identification of a novel heterozygous IGF1 splicing mutation in a large kindred with familial short stature. *Hormone Research in Paediatrics* **78** 59–66. (<https://doi.org/10.1159/000337249>)
- Gannage-Yared MH, Klammt J, Chouery E, Corbani S, Megarbane H, Abou Ghoch J, Choucair N, Pfaffle R & Megarbane A 2013 Homozygous mutation of the IGF1 receptor gene in a patient with severe pre- and postnatal growth failure and congenital malformations. *European Journal of Endocrinology* **168** K1–K7. (<https://doi.org/10.1530/EJEndo-12-0701>)
- Garnero P, Sornay-Rendu E, Claustat B & Delmas PD 2000 Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the OFELY study. *Journal of Bone and Mineral Research* **15** 1526–1536. (<https://doi.org/10.1359/jbmr.2000.15.8.1526>)
- Gevers EF, Hannah MJ, Waters MJ & Robinson IC 2009 Regulation of rapid signal transducer and activator of transcription-5 phosphorylation in the resting cells of the growth plate and in the liver by growth hormone and feeding. *Endocrinology* **150** 3627–3636. (<https://doi.org/10.1210/en.2008-0985>)
- Ghiron LJ, Thompson JL, Holloway L, Hintz RL, Butterfield GE, Hoffman AR & Marcus R 1995 Effects of recombinant insulin-like growth factor-I and growth hormone on bone turnover in elderly women. *Journal of Bone and Mineral Research* **10** 1844–1852. (<https://doi.org/10.1002/jbmr.5650101203>)
- Gicquel C, Rossignol S, Cabrol S, Houang M, Steunou V, Barbu V, Danton F, Thibaud N, Le Merrer M, Burglen L, et al. 2005 Epimutation of the telomeric imprinting center region on chromosome 11p15 in Silver-Russell syndrome. *Nature Genetics* **37** 1003–1007. (<https://doi.org/10.1038/ng1629>)
- Gillberg P, Olofsson H, Mallmin H, Blum WF, Ljunghall S & Nilsson AG 2002 Bone mineral density in femoral neck is positively correlated to circulating insulin-like growth factor (IGF)-I and IGF-binding protein (IGFBP)-3 in Swedish men. *Calcified Tissue International* **70** 22–29. (<https://doi.org/10.1007/s002230020048>)
- Govoni KE, Lee SK, Chung YS, Behringer RR, Wergedal JE, Baylink DJ & Mohan S 2007 Disruption of insulin-like growth factor-I expression in type II alfa1 collagen-expressing cells reduces bone length and width in mice. *Physiological Genomics* **30** 354–362. (<https://doi.org/10.1152/physiolgenomics.00022.2007>)
- Green H, Morikawa M & Nixon T 1985 A dual effector theory of growth-hormone action. *Differentiation* **29** 195–198. (<https://doi.org/10.1111/j.1432-0436.1985.tb00316.x>)
- Greenspan FS, Li CH, Simpson ME & Evans HM 1949 Bioassay of hypophyseal growth hormone; the tibia test. *Endocrinology* **45** 455–463, illust. (<https://doi.org/10.1210/endo-45-5-455>)
- Guevara-Aguirre J, Vasconez O, Martinez V, Martinez AL, Rosenbloom AL, Diamond FB Jr, Gargosky SE, Nonoshita L & Rosenfeld RG 1995 A randomized, double blind, placebo-controlled trial on safety and efficacy of recombinant human insulin-like growth factor-I in children with growth hormone receptor deficiency. *Journal of Clinical Endocrinology and Metabolism* **80** 1393–1398. (<https://doi.org/10.1210/jcem.80.4.7536209>)
- Guevara-Aguirre J, Rosenbloom AL, Vasconez O, Martinez V, Gargosky SE, Allen L & Rosenfeld RG 1997 Two-year treatment of growth hormone (GH) receptor deficiency with recombinant insulin-like growth factor I in 22 children: comparison of two dosage levels and to GH-treated GH deficiency. *Journal of Clinical Endocrinology and Metabolism* **82** 629–633. (<https://doi.org/10.1210/jcem.82.2.3743>)
- Hall K 1972 Human somatomedin. Determination, occurrence, biological activity and purification. *Acta Endocrinologica Supplementum* **163** 1052.
- He J, Rosen CJ, Adams DJ & Kream BE 2006 Postnatal growth and bone mass in mice with IGF-I haploinsufficiency. *Bone* **38** 826–835. (<https://doi.org/10.1016/j.bone.2005.11.021>)
- Hekim C, Riipi T, Weisell J, Narvanen A, Koistinen R, Stenman UH & Koistinen H 2010 Identification of IGFBP-3 fragments generated by KLK2 and prevention of fragmentation by KLK2-inhibiting peptides. *Biological Chemistry* **391** 475–479. (<https://doi.org/10.1515/BC.2010.039>)
- Hill PA, Reynolds JJ & Meikle MC 1995 Osteoblasts mediate insulin-like growth factor-I and -II stimulation of osteoclast formation and function. *Endocrinology* **136** 124–131. (<https://doi.org/10.1210/endo.136.1.7828521>)
- Hoeflich A, Wu M, Mohan S, Foll J, Wanke R, Froehlich T, Arnold GJ, Lahm H, Kolb HJ & Wolf E 1999 Overexpression of insulin-like growth factor-binding protein-2 in transgenic mice reduces postnatal body weight gain. *Endocrinology* **140** 5488–5496. (<https://doi.org/10.1210/endo.140.12.7169>)
- Hoeflich A, Gotz W, Lichanska AM, Bielohuby M, Tonshoff B & Kiepe D 2007 Effects of insulin-like growth factor binding proteins in bone – a matter of cell and site. *Archives of Physiology and Biochemistry* **113** 142–153. (<https://doi.org/10.1080/13813450701531193>)
- 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>)
- Holzenberger M, Leneuve P, Hamard G, Ducos B, Perin L, Binoux M & Le Bouc Y 2000 A targeted partial invalidation of the insulin-like growth factor I receptor gene in mice causes a postnatal growth deficit. *Endocrinology* **141** 2557–2566. (<https://doi.org/10.1210/endo.141.7.7550>)
- Holzenberger M, Hamard G, Zaoui R, Leneuve P, Ducos B, Beccavin C, Perin L & Le Bouc Y 2001 Experimental IGF-I receptor deficiency generates a sexually dimorphic pattern of organ-specific growth deficits in mice, affecting fat tissue in particular. *Endocrinology* **142** 4469–4478. (<https://doi.org/10.1210/endo.142.10.8461>)
- Huang JC, Sakata T, Pfleger LL, Bencsik M, Halloran BP, Bikle DD & Nissenson RA 2004 PTH differentially regulates expression of RANKL and OPG. *Journal of Bone and Mineral Research* **19** 235–244. (<https://doi.org/10.1359/JBMR.0301226>)
- Huesa C, Helfrich MH & Aspden RM 2010 Parallel-plate fluid flow systems for bone cell stimulation. *Journal of Biomechanics* **43** 1182–1189. (<https://doi.org/10.1016/j.jbiomech.2009.11.029>)
- Iida-Klein A, Zhou H, Lu SS, Levine LR, Ducayen-Knowles M, Dempster DW, Nieves J & Lindsay R 2002 Anabolic action of parathyroid hormone is skeletal site specific at the tissue and cellular levels in mice. *Journal of Bone and Mineral Research* **17** 808–816. (<https://doi.org/10.1359/jbmr.2002.17.5.808>)
- Ilangovan R, Sittadjody S, Balaganesh M, Sivakumar R, Ravi Sankar B, Balasubramanian K, Srinivasan S, Subramanian C, Thompson DM, Queimado L, et al. 2009 Dihydrotestosterone is a determinant of calcaneal bone mineral density in men. *Journal of Steroid Biochemistry and Molecular Biology* **117** 132–138. (<https://doi.org/10.1016/j.jsbmb.2009.08.004>)
- Ishibe M, Ishibashi T, Kaneda K, Koda T, Rosier RN & Puzas JE 1998 Stimulation of bone formation in vivo by insulin-like growth factor-II in rats. *Calcified Tissue International* **63** 36–38. (<https://doi.org/10.1007/s002239900486>)
- Janssen YJ, Hamdy NA, Frolich M & Roelfsema F 1998 Skeletal effects of two years of treatment with low physiological doses of recombinant human growth hormone (GH) in patients with adult-onset GH deficiency. *Journal of Clinical Endocrinology and Metabolism* **83** 2143–2148. (<https://doi.org/10.1210/jcem.83.6.4851>)

- Jiang J, Lichtler AC, Gronowicz GA, Adams DJ, Clark SH, Rosen CJ & Kream BE 2006 Transgenic mice with osteoblast-targeted insulin-like growth factor-I show increased bone remodeling. *Bone* **39** 494–504. (<https://doi.org/10.1016/j.bone.2006.02.068>)
- Kaplan SA & Cohen P 2007 The somatomedin hypothesis 2007: 50 years later. *Journal of Clinical Endocrinology and Metabolism* **92** 4529–4535. (<https://doi.org/10.1210/jc.2007-0526>)
- Kassem M, Brixen K, Blum W, Mosekilde L & Eriksen EF 1994 No evidence for reduced spontaneous or growth-hormone-stimulated serum levels of insulin-like growth factor (IGF)-I, IGF-II or IGF binding protein 3 in women with spinal osteoporosis. *European Journal of Endocrinology* **131** 150–155. (<https://doi.org/10.1530/eje.0.1310150>)
- Keenan BS, Richards GE, Ponder SW, Dallas JS, Nagamani M & Smith ER 1993 Androgen-stimulated pubertal growth: the effects of testosterone and dihydrotestosterone on growth hormone and insulin-like growth factor-I in the treatment of short stature and delayed puberty. *Journal of Clinical Endocrinology and Metabolism* **76** 996–1001. (<https://doi.org/10.1210/jcem.76.4.8473416>)
- Kerrigan JR & Rogol AD 1992 The impact of gonadal steroid hormone action on growth hormone secretion during childhood and adolescence. *Endocrine Reviews* **13** 281–298. (<https://doi.org/10.1210/edrv.13-2-281>)
- Kim JG, Shin CS, Choi YM, Moon SY, Kim SY & Lee JY 1999 The relationship among circulating insulin-like growth factor components, biochemical markers of bone turnover and bone mineral density in postmenopausal women under the age of 60. *Clinical Endocrinology* **51** 301–307. (<https://doi.org/10.1046/j.1365-2265.1999.00769.x>)
- Klammt J, Kiess W & Pfaffle R 2011 IGF1R mutations as cause of SGA. *Best Practice and Research: Clinical Endocrinology and Metabolism* **25** 191–206. (<https://doi.org/10.1016/j.beem.2010.09.012>)
- Klinger B & Laron Z 1995 Three year IGF-I treatment of children with Laron syndrome. *Journal of Pediatric Endocrinology and Metabolism* **8** 149–158.
- Krantz E, Trimpou P & Landin-Wilhelmsen K 2015 Effect of growth hormone treatment on fractures and quality of life in postmenopausal osteoporosis: a 10-year follow-up study. *Journal of Clinical Endocrinology and Metabolism* **100** 3251–3259. (<https://doi.org/10.1210/jc.2015-1757>)
- Kubota T, Elalieh HZ, Saleh N, Fong C, Wang Y, Babey M, Cheng Z & Bikle DD 2013 Insulin-like growth factor-1 receptor in mature osteoblasts is required for periosteal bone formation induced by reloading. *Acta Astronautica* **92** 73–78. (<https://doi.org/10.1016/j.actaastro.2012.08.007>)
- Lang T, LeBlanc A, Evans H, Lu Y, Genant H & Yu A 2004 Cortical and trabecular bone mineral loss from the spine and hip in long-duration spaceflight. *Journal of Bone and Mineral Research* **19** 1006–1012. (<https://doi.org/10.1359/JBMR.040307>)
- Langlois JA, Rosen CJ, Visser M, Hannan MT, Harris T, Wilson PW & Kiel DP 1998 Association between insulin-like growth factor I and bone mineral density in older women and men: the Framingham Heart Study. *Journal of Clinical Endocrinology and Metabolism* **83** 4257–4262. (<https://doi.org/10.1210/jcem.83.12.5308>)
- Laron Z, Anin S, Klipper-Aurbach Y & Klinger B 1992 Effects of insulin-like growth factor on linear growth, head circumference, and body fat in patients with Laron-type dwarfism. *Lancet* **339** 1258–1261. ([https://doi.org/10.1016/0140-6736\(92\)91594-X](https://doi.org/10.1016/0140-6736(92)91594-X))
- Lau KH, Kapur S, Kesavan C & Baylink DJ 2006 Up-regulation of the Wnt, estrogen receptor, insulin-like growth factor-I, and bone morphogenetic protein pathways in C57BL/6J osteoblasts as opposed to C3H/HeJ osteoblasts in part contributes to the differential anabolic response to fluid shear. *Journal of Biological Chemistry* **281** 9576–9588. (<https://doi.org/10.1074/jbc.M509205200>)
- Lau KH, Baylink DJ, Zhou XD, Rodriguez D, Bonewald LF, Li Z, Ruffoni D, Muller R, Kesavan C & Sheng MH 2013 Osteocyte-derived insulin-like growth factor I is essential for determining bone mechanosensitivity. *American Journal of Physiology: Endocrinology and Metabolism* **305** E271–E281. (<https://doi.org/10.1152/ajpendo.00445.2013>)
- Lean JM, Jagger CJ, Chambers TJ & Chow JW 1995 Increased insulin-like growth factor I mRNA expression in rat osteocytes in response to mechanical stimulation. *American Journal of Physiology* **268** E318–E327. (<https://doi.org/10.1152/ajpendo.1995.268.2.E318>)
- 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>)
- Lim J, Burclaff J, He G, Mills JC & Long F 2017 Unintended targeting of Dmp1-Cre reveals a critical role for Bmpr1a signaling in the gastrointestinal mesenchyme of adult mice. *Bone Research* **5** 16049. (<https://doi.org/10.1038/boneres.2016.49>)
- Linkhart TA & Mohan S 1989 Parathyroid hormone stimulates release of insulin-like growth factor-I (IGF-I) and IGF-II from neonatal mouse calvaria in organ culture. *Endocrinology* **125** 1484–1491. (<https://doi.org/10.1210/endo-125-3-1484>)
- Liu JP, Baker J, Perkins AS, Robertson EJ & Efstratiadis A 1993 Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type I IGF receptor (Igf1r). *Cell* **75** 59–72. ([https://doi.org/10.1016/S0092-8674\(05\)80084-4](https://doi.org/10.1016/S0092-8674(05)80084-4))
- Liu Z, Kennedy OD, Cardoso L, Basta-Pljakic J, Partridge NC, Schaffler MB, Rosen CJ & Yakar S 2016a DMP-1-mediated Ghr gene recombination compromises skeletal development and impairs skeletal response to intermittent PTH. *FASEB Journal* **30** 635–652. (<https://doi.org/10.1096/fj.15-275859>)
- Liu Z, Mohan S & Yakar S 2016b Does the GH/IGF-1 axis contribute to skeletal sexual dimorphism? Evidence from mouse studies. *Growth Hormone and IGF Research* **27** 7–17. (<https://doi.org/10.1016/j.ghir.2015.12.004>)
- Liu Z, Han T, Werner H, Rosen CJ, Schaffler MB & Yakar S 2017 Reduced serum IGF-1 associated with hepatic osteodystrophy is a main determinant of low cortical but not trabecular bone mass. *Journal of Bone and Mineral Research* **33** 123–136. (<https://doi.org/10.1002/jbmr.3290>)
- Lloyd ME, Hart DJ, Nandra D, McAlindon TE, Wheeler M, Doyle DV & Spector TD 1996 Relation between insulin-like growth factor-I concentrations, osteoarthritis, bone density, and fractures in the general population: the Chingford study. *Annals of the Rheumatic Diseases* **55** 870–874. (<https://doi.org/10.1136/ard.55.12.870>)
- Lowe WL Jr, Adamo M, Werner H, Roberts CT Jr & LeRoith D 1989 Regulation by fasting of rat insulin-like growth factor I and its receptor. Effects on gene expression and binding. *Journal of Clinical Investigation* **84** 619–626. (<https://doi.org/10.1172/JCI114207>)
- Lupu F, Terwilliger JD, Lee K, Segre GV & Efstratiadis A 2001 Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. *Developmental Biology* **229** 141–162. (<https://doi.org/10.1006/dbio.2000.9975>)
- Ma YL, Cain RL, Halladay DL, Yang X, Zeng Q, Miles RR, Chandrasekhar S, Martin TJ & Onyia JE 2001 Catabolic effects of continuous human PTH (1–38) in vivo is associated with sustained stimulation of RANKL and inhibition of osteoprotegerin and gene-associated bone formation. *Endocrinology* **142** 4047–4054. (<https://doi.org/10.1210/endo.142.9.8356>)
- Madeira M, Neto LV, Torres CH, de Mendonca LM, Gadelha MR & de Farias ML 2013 Vertebral fracture assessment in acromegaly. *Journal of Clinical Densitometry* **16** 238–243. (<https://doi.org/10.1016/j.jocd.2012.06.002>)
- Marcus R, Butterfield G, Holloway L, Gilliland L, Baylink DJ, Hintz RL & Sherman BM 1990 Effects of short term administration of recombinant human growth hormone to elderly people. *Journal of*

- Clinical Endocrinology and Metabolism* **70** 519–527. (<https://doi.org/10.1210/jcem-70-2-519>)
- Martha PM Jr, Rogol AD, Veldhuis JD, Kerrigan JR, Goodman DW & Blizzard RM 1989 Alterations in the pulsatile properties of circulating growth hormone concentrations during puberty in boys. *Journal of Clinical Endocrinology and Metabolism* **69** 563–570. (<https://doi.org/10.1210/jcem-69-3-563>)
- Mason EJ, Grell JA, Wan J, Cohen P & Conover CA 2011 Insulin-like growth factor (IGF)-I and IGF-II contribute differentially to the phenotype of pregnancy associated plasma protein-A knock-out mice. *Growth Hormone and IGF Research* **21** 243–247. (<https://doi.org/10.1016/j.ghir.2011.06.002>)
- Matar M, Al-Shaar L, Maalouf J, Nabulsi M, Arabi A, Choucair M, Tamim H & El-Hajj Fuleihan G 2016 The relationship between calcitropic hormones, IGF-1, and bone mass across pubertal stages. *Journal of Clinical Endocrinology and Metabolism* **101** 4860–4870. (<https://doi.org/10.1210/jc.2016-3071>)
- Mathews LS, Hammer RE, Behringer RR, D'Ercole AJ, Bell GI, Brinster RL & Palmiter RD 1988 Growth enhancement of transgenic mice expressing human insulin-like growth factor I. *Endocrinology* **123** 2827–2833. (<https://doi.org/10.1210/endo-123-6-2827>)
- Mauras N, Blizzard RM, Link K, Johnson ML, Rogol AD & Veldhuis JD 1987 Augmentation of growth hormone secretion during puberty: evidence for a pulse amplitude-modulated phenomenon. *Journal of Clinical Endocrinology and Metabolism* **64** 596–601. (<https://doi.org/10.1210/jcem-64-3-596>)
- Mauras N, Rogol AD & Veldhuis JD 1989 Specific, time-dependent actions of low-dose ethinyl estradiol administration on the episodic release of growth hormone, follicle-stimulating hormone, and luteinizing hormone in prepubertal girls with Turner's syndrome. *Journal of Clinical Endocrinology and Metabolism* **69** 1053–1058. (<https://doi.org/10.1210/jcem-69-5-1053>)
- Mauras N, Martinez V, Rini A & Guevara-Aguirre J 2000 Recombinant human insulin-like growth factor I has significant anabolic effects in adults with growth hormone receptor deficiency: studies on protein, glucose, and lipid metabolism. *Journal of Clinical Endocrinology and Metabolism* **85** 3036–3042. (<https://doi.org/10.1210/jcem.85.9.6772>)
- Mazzitelli G, Bianchi A, Bonadonna S, Cimino V, Patelli I, Fusco A, Pontecorvi A, De Marinis L & Giustina A 2008 Prevalence of vertebral fractures in men with acromegaly. *Journal of Clinical Endocrinology and Metabolism* **93** 4649–4655. (<https://doi.org/10.1210/jc.2008-0791>)
- Merriman HL, La Tour D, Linkhart TA, Mohan S, Baylink DJ & Strong DD 1990 Insulin-like growth factor-I and insulin-like growth factor-II induce c-fos in mouse osteoblastic cells. *Calcified Tissue International* **46** 258–262. (<https://doi.org/10.1007/BF02555005>)
- Miller BS, Bronk JT, Nishiyama T, Yamagiwa H, Srivastava A, Bolander ME & Conover CA 2007 Pregnancy associated plasma protein-A is necessary for expeditious fracture healing in mice. *Journal of Endocrinology* **192** 505–513. (<https://doi.org/10.1677/JOE-06-0011>)
- Mochizuki H, Hakeda Y, Wakatsuki N, Usui N, Akashi S, Sato T, Tanaka K & Kumegawa M 1992 Insulin-like growth factor-I supports formation and activation of osteoclasts. *Endocrinology* **131** 1075–1080. (<https://doi.org/10.1210/endo.131.3.1505451>)
- Modric T, Silha JV, Shi Z, Gui Y, Suwanichkul A, Durham SK, Powell DR & Murphy LJ 2001 Phenotypic manifestations of insulin-like growth factor-binding protein-3 overexpression in transgenic mice. *Endocrinology* **142** 1958–1967. (<https://doi.org/10.1210/endo.142.5.8165>)
- Moerth C, Schneider MR, Renner-Mueller I, Blutke A, Elmlinger MW, Erben RG, Camacho-Hubner C, Hoefflich A & Wolf E 2007 Postnatally elevated levels of insulin-like growth factor (IGF)-II fail to rescue the dwarfism of IGF-I-deficient mice except kidney weight. *Endocrinology* **148** 441–451. (<https://doi.org/10.1210/en.2006-0385>)
- Mohan S & Baylink DJ 1997 Serum insulin-like growth factor binding protein (IGFBP)-4 and IGFBP-5 levels in aging and age-associated diseases. *Endocrine* **7** 87–91. (<https://doi.org/10.1007/BF02778070>)
- Mohan S & Baylink DJ 2005 Impaired skeletal growth in mice with haploinsufficiency of IGF-I: genetic evidence that differences in IGF-I expression could contribute to peak bone mineral density differences. *Journal of Endocrinology* **185** 415–420. (<https://doi.org/10.1677/joe.1.06141>)
- Mohan S, Jennings JC, Linkhart TA & Baylink DJ 1988 Primary structure of human skeletal growth factor: homology with human insulin-like growth factor-II. *Biochimica et Biophysica Acta* **966** 44–55. ([https://doi.org/10.1016/0304-4165\(88\)90127-4](https://doi.org/10.1016/0304-4165(88)90127-4))
- Mohan S, Linkhart T, Rosenfeld R & Baylink D 1989 Characterization of the receptor for insulin-like growth factor II in bone cells. *Journal of Cellular Physiology* **140** 169–176. (<https://doi.org/10.1002/jcp.1041400120>)
- Mohan S, Richman C, Guo R, Amaar Y, Donahue LR, Wergedal J & Baylink DJ 2003 Insulin-like growth factor regulates peak bone mineral density in mice by both growth hormone-dependent and -independent mechanisms. *Endocrinology* **144** 929–936. (<https://doi.org/10.1210/en.2002-220948>)
- Mosekilde L, Thomsen JS, Orhii PB & Kalu DN 1998 Growth hormone increases vertebral and femoral bone strength in osteopenic, ovariectomized, aged rats in a dose-dependent and site-specific manner. *Bone* **23** 343–352. ([https://doi.org/10.1016/S8756-3282\(98\)00107-0](https://doi.org/10.1016/S8756-3282(98)00107-0))
- Munoz-Calvo MT, Barrios V, Pozo J, Chowen JA, Martos-Moreno GA, Hawkins F, Dauber A, Domene HM, Yakar S, Rosenfeld RG, et al. 2016 Treatment with recombinant human insulin-like growth factor-1 improves growth in patients with PAPP-A2 deficiency. *Journal of Clinical Endocrinology and Metabolism* **101** 3879–3883. (<https://doi.org/10.1210/jc.2016-2751>)
- 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.
- Murphy R, Baptista J, Holly J, Umpleby AM, Ellard S, Harries LW, Crolla J, Cundy T & Hattersley AT 2008 Severe intrauterine growth retardation and atypical diabetes associated with a translocation breakpoint disrupting regulation of the insulin-like growth factor 2 gene. *Journal of Clinical Endocrinology and Metabolism* **93** 4373–4380. (<https://doi.org/10.1210/jc.2008-0819>)
- Nakao K, Aoyama M, Fukuoka H, Fujita M, Miyazawa K, Asai K & Goto S 2009 IGF2 modulates the microenvironment for osteoclastogenesis. *Biochemical and Biophysical Research Communications* **378** 462–466. (<https://doi.org/10.1016/j.bbrc.2008.11.083>)
- Netchine I, Azzi S, Houang M, Seurin D, Perin L, Ricort JM, Daubas C, Legay C, Mester J, Herich R, et al. 2009 Partial primary deficiency of insulin-like growth factor (IGF)-I activity associated with IGF1 mutation demonstrates its critical role in growth and brain development. *Journal of Clinical Endocrinology and Metabolism* **94** 3913–3921. (<https://doi.org/10.1210/jc.2009-0452>)
- Nicolas V, Prewett A, Bettica P, Mohan S, Finkelman RD, Baylink DJ & Farley JR 1994 Age-related decreases in insulin-like growth factor-I and transforming growth factor-beta in femoral cortical bone from both men and women: implications for bone loss with aging. *Journal of Clinical Endocrinology and Metabolism* **78** 1011–1016. (<https://doi.org/10.1210/jcem.78.5.8175953>)
- Nicolas V, Mohan S, Honda Y, Prewett A, Finkelman RD, Baylink DJ & Farley JR 1995 An age-related decrease in the concentration of insulin-like growth factor binding protein-5 in human cortical bone. *Calcified Tissue International* **57** 206–212. (<https://doi.org/10.1007/BF00310260>)

- Nielsen FC 1992 The molecular and cellular biology of insulin-like growth factor II. *Progress in Growth Factor Research* **4** 257–290. ([https://doi.org/10.1016/0955-2235\(92\)90023-B](https://doi.org/10.1016/0955-2235(92)90023-B))
- Nilsson A, Isgaard J, Lindahl A, Dahlstrom A, Skottner A & Isaksson OG 1986 Regulation by growth hormone of number of chondrocytes containing IGF-I in rat growth plate. *Science* **233** 571–574. (<https://doi.org/10.1126/science.3523759>)
- Nilsson A, Carlsson B, Isgaard J, Isaksson OG & Rymo L 1990 Regulation by GH of insulin-like growth factor-I mRNA expression in rat epiphyseal growth plate as studied with in-situ hybridization. *Journal of Endocrinology* **125** 67–74. (<https://doi.org/10.1677/joe.0.1250067>)
- Ning Y, Schuller AG, Bradshaw S, Rotwein P, Ludwig T, Frystyk J & Pintar JE 2006 Diminished growth and enhanced glucose metabolism in triple knockout mice containing mutations of insulin-like growth factor binding protein-3, -4, and -5. *Molecular Endocrinology* **20** 2173–2186. (<https://doi.org/10.1210/me.2005-0196>)
- 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 activity in vivo. *Molecular Endocrinology* **22** 1213–1225. (<https://doi.org/10.1210/me.2007-0536>)
- Nordstrom SM, Tran JL, Sos BC, Wagner KU & Weiss EJ 2011 Liver-derived IGF-I contributes to GH-dependent increases in lean mass and bone mineral density in mice with comparable levels of circulating GH. *Molecular Endocrinology* **25** 1223–1230. (<https://doi.org/10.1210/me.2011-0047>)
- Norvell SM, Alvarez M, Bidwell JP & Pavalko FM 2004 Fluid shear stress induces beta-catenin signaling in osteoblasts. *Calcified Tissue International* **75** 396–404. (<https://doi.org/10.1007/s00223-004-0213-y>)
- O'Dell SD & Day IN 1998 Insulin-like growth factor II (IGF-II). *International Journal of Biochemistry and Cell Biology* **30** 767–771. ([https://doi.org/10.1016/S1357-2725\(98\)00048-X](https://doi.org/10.1016/S1357-2725(98)00048-X))
- Oates AJ, Schumaker LM, Jenkins SB, Pearce AA, DaCosta SA, Arun B & Ellis MJ 1998 The mannose 6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2R), a putative breast tumor suppressor gene. *Breast Cancer Research and Treatment* **47** 269–281. (<https://doi.org/10.1023/A:1005959218524>)
- Okubo Y, Siddle K, Firth H, O'Rahilly S, Wilson LC, Willatt L, Fukushima T, Takahashi S, Petry CJ, Saukkonen T, et al. 2003 Cell proliferation activities on skin fibroblasts from a short child with absence of one copy of the type 1 insulin-like growth factor receptor (IGF1R) gene and a tall child with three copies of the IGF1R gene. *Journal of Clinical Endocrinology and Metabolism* **88** 5981–5988. (<https://doi.org/10.1210/jc.2002-021080>)
- Ormarsdottir S, Ljunggren O, Mallmin H, Olofsson H, Blum WF & Loof L 2001 Circulating levels of insulin-like growth factors and their binding proteins in patients with chronic liver disease: lack of correlation with bone mineral density. *Liver* **21** 123–128. (<https://doi.org/10.1034/j.1600-0676.2001.021002123.x>)
- Peoples R, Milatovich A & Francke U 1995 Hemizygosity at the insulin-like growth factor I receptor (IGF1R) locus and growth failure in the ring chromosome 15 syndrome. *Cytogenetics and Cell Genetics* **70** 228–234. (<https://doi.org/10.1159/000134040>)
- Pfeilschifter J, Diel I, Pilz U, Brunotte K, Naumann A & Ziegler R 1993 Mitogenic responsiveness of human bone cells in vitro to hormones and growth factors decreases with age. *Journal of Bone and Mineral Research* **8** 707–717. (<https://doi.org/10.1002/jbmr.5650080609>)
- Pfeilschifter J, Laukhuf F, Muller-Beckmann B, Blum WF, Pfister T & Ziegler R 1995 Parathyroid hormone increases the concentration of insulin-like growth factor-I and transforming growth factor beta 1 in rat bone. *Journal of Clinical Investigation* **96** 767–774. (<https://doi.org/10.1172/JCI118121>)
- Prakasam G, Yeh JK, Chen MM, Castro-Magana M, Liang CT & Aloia JF 1999 Effects of growth hormone and testosterone on cortical bone formation and bone density in aged ovariectomized rats. *Bone* **24** 491–497. ([https://doi.org/10.1016/S8756-3282\(99\)00018-6](https://doi.org/10.1016/S8756-3282(99)00018-6))
- Prontera P, Micale L, Verrotti A, Napolioni V, Stangoni G & Merla G 2015 A new homozygous IGF1R variant defines a clinically recognizable incomplete dominant form of SHORT syndrome. *Human Mutation* **36** 1043–1047. (<https://doi.org/10.1002/humu.22853>)
- Qin X, Wergedal JE, Rehage M, Tran K, Newton J, Lam P, Baylink DJ & Mohan S 2006 Pregnancy-associated plasma protein-A increases osteoblast proliferation in vitro and bone formation in vivo. *Endocrinology* **147** 5653–5661. (<https://doi.org/10.1210/en.2006-1055>)
- Rajkumar K, Barron D, Lewitt MS & Murphy LJ 1995 Growth retardation and hyperglycemia in insulin-like growth factor binding protein-1 transgenic mice. *Endocrinology* **136** 4029–4034. (<https://doi.org/10.1210/endo.136.9.7544274>)
- Ranke MB, Savage MO, Chatelain PG, Preece MA, Rosenfeld RG, Blum WF & Wilton P 1995 Insulin-like growth factor I improves height in growth hormone insensitivity: two years' results. *Hormone Research* **44** 253–264. (<https://doi.org/10.1159/000184637>)
- Reijnders CM, Bravenboer N, Tromp AM, Blankenstein MA & Lips P 2007 Effect of mechanical loading on insulin-like growth factor-I gene expression in rat tibia. *Journal of Endocrinology* **192** 131–140. (<https://doi.org/10.1677/joe.1.06880>)
- Renard E, Poree B, Chadjiachristos C, Kyprioutou M, Maneix L, Bigot N, Legendre F, Ollitrault D, De Crombrughe B, Mallein-Gerin F, et al. 2012 Sox9/Sox6 and Sp1 are involved in the insulin-like growth factor-I-mediated upregulation of human type II collagen gene expression in articular chondrocytes. *Journal of Molecular Medicine* **90** 649–666. (<https://doi.org/10.1007/s00109-011-0842-3>)
- Riggs BL, Melton LJ, Robb RA, Camp JJ, Atkinson EJ, McDaniel L, Amin S, Rouleau PA & Khosla S 2008 A population-based assessment of rates of bone loss at multiple skeletal sites: evidence for substantial trabecular bone loss in young adult women and men. *Journal of Bone and Mineral Research* **23** 205–214. (<https://doi.org/10.1359/jbmr.071020>)
- Rinderknecht E & Humbel RE 1976 Amino-terminal sequences of two polypeptides from human serum with nonsuppressible insulin-like and cell-growth-promoting activities: evidence for structural homology with insulin B chain. *PNAS* **73** 4379–4381. (<https://doi.org/10.1073/pnas.73.12.4379>)
- Rinderknecht E & Humbel RE 1978a The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. *Journal of Biological Chemistry* **253** 2769–2776.
- Rinderknecht E & Humbel RE 1978b Primary structure of human insulin-like growth factor II. *FEBS Letters* **89** 283–286. ([https://doi.org/10.1016/0014-5793\(78\)80237-3](https://doi.org/10.1016/0014-5793(78)80237-3))
- Roberts CT Jr, Lasky SR, Lowe WL Jr, Seaman WT & LeRoith D 1987 Molecular cloning of rat insulin-like growth factor I complementary deoxyribonucleic acids: differential messenger ribonucleic acid processing and regulation by growth hormone in extrahepatic tissues. *Molecular Endocrinology* **1** 243–248. (<https://doi.org/10.1210/mend-1-3-243>)
- Rosen T, Wilhelmsen L, Landin-Wilhelmsen K, Lappas G & Bengtsson BA 1997 Increased fracture frequency in adult patients with hypopituitarism and GH deficiency. *European Journal of Endocrinology* **137** 240–245. (<https://doi.org/10.1530/eje.0.1370240>)
- 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>)
- Rotwein P 2017 Large-scale analysis of variation in the insulin-like growth factor family in humans reveals rare disease links and common polymorphisms. *Journal of Biological Chemistry* **292** 9252–9261. (<https://doi.org/10.1074/jbc.M117.783639>)
- Rudman D, Feller AG, Nagraj HS, Gergans GA, Lalitha PY, Goldberg AF, Schlenker RA, Cohn L, Rudman IW & Mattson DE 1990 Effects of human growth hormone in men over 60 years old. *New England Journal of Medicine* **323** 1–6. (<https://doi.org/10.1056/NEJM199007053230101>)

- Sakata T, Halloran BP, Elalieh HZ, Munson SJ, Rudner L, Venton L, Ginzinger D, Rosen CJ & Bikle DD 2003 Skeletal unloading induces resistance to insulin-like growth factor I on bone formation. *Bone* **32** 669–680. ([https://doi.org/10.1016/S8756-3282\(03\)00088-7](https://doi.org/10.1016/S8756-3282(03)00088-7))
- Sakata T, Wang Y, Halloran BP, Elalieh HZ, Cao J & Bikle DD 2004 Skeletal unloading induces resistance to insulin-like growth factor-I (IGF-I) by inhibiting activation of the IGF-I signaling pathways. *Journal of Bone and Mineral Research* **19** 436–446. (<https://doi.org/10.1359/JBMR.0301241>)
- Salih DA, Mohan S, Kasukawa Y, Tripathi G, Lovett FA, Anderson NF, Carter EJ, Wergedal JE, Baylink DJ & Pell JM 2005 Insulin-like growth factor-binding protein-5 induces a gender-related decrease in bone mineral density in transgenic mice. *Endocrinology* **146** 931–940. (<https://doi.org/10.1210/en.2004-0816>)
- Salmon WD Jr & Daughaday WH 1957 A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. *Journal of Laboratory and Clinical Medicine* **49** 825–836.
- Santos A, Bakker AD, Zandieh-Doulabi B, de Blieck-Hogervorst JM & Klein-Nulend J 2010 Early activation of the beta-catenin pathway in osteocytes is mediated by nitric oxide, phosphatidyl inositol-3 kinase/Akt, and focal adhesion kinase. *Biochemical and Biophysical Research Communications* **391** 364–369. (<https://doi.org/10.1016/j.bbrc.2009.11.064>)
- Schmid C, Steiner T & Froesch ER 1984 Insulin-like growth factor I supports differentiation of cultured osteoblast-like cells. *FEBS Letters* **173** 48–52. ([https://doi.org/10.1016/0014-5793\(84\)81015-7](https://doi.org/10.1016/0014-5793(84)81015-7))
- Sheng MH, Zhou XD, Bonewald LF, Baylink DJ & Lau KH 2013 Disruption of the insulin-like growth factor-1 gene in osteocytes impairs developmental bone growth in mice. *Bone* **52** 133–144. (<https://doi.org/10.1016/j.bone.2012.09.027>)
- Shi Y, Chen J, Karner CM & Long F 2015 Hedgehog signaling activates a positive feedback mechanism involving insulin-like growth factors to induce osteoblast differentiation. *PNAS* **112** 4678–4683. (<https://doi.org/10.1073/pnas.1502301112>)
- Shinar DM, Endo N, Halperin D, Rodan GA & Weinreb M 1993 Differential expression of insulin-like growth factor-I (IGF-I) and IGF-II messenger ribonucleic acid in growing rat bone. *Endocrinology* **132** 1158–1167. (<https://doi.org/10.1210/endo.132.3.8440176>)
- Sievanen H 2010 Immobilization and bone structure in humans. *Archives of Biochemistry and Biophysics* **503** 146–152. (<https://doi.org/10.1016/j.abb.2010.07.008>)
- Silha JV, Mishra S, Rosen CJ, Beamer WG, Turner RT, Powell DR & Murphy LJ 2003 Perturbations in bone formation and resorption in insulin-like growth factor binding protein-3 transgenic mice. *Journal of Bone and Mineral Research* **18** 1834–1841. (<https://doi.org/10.1359/jbmr.2003.18.10.1834>)
- Sjogren K, Liu JL, Blad K, Skrtic S, Vidal O, Wallenius V, LeRoith D, Tornell J, Isaksson OG, Jansson JO, et al. 1999 Liver-derived insulin-like growth factor I (IGF-I) is the principal source of IGF-I in blood but is not required for postnatal body growth in mice. *PNAS* **96** 7088–7092. (<https://doi.org/10.1073/pnas.96.12.7088>)
- Sjogren K, Sheng M, Moverare S, Liu JL, Wallenius K, Tornell J, Isaksson O, Jansson JO, Mohan S & Ohlsson C 2002 Effects of liver-derived insulin-like growth factor I on bone metabolism in mice. *Journal of Bone and Mineral Research* **17** 1977–1987. (<https://doi.org/10.1359/jbmr.2002.17.11.1977>)
- Slootweg MC, Most WW, van Beek E, Schot LP, Papapoulos SE & Lowik CW 1992 Osteoclast formation together with interleukin-6 production in mouse long bones is increased by insulin-like growth factor-I. *Journal of Endocrinology* **132** 433–438. (<https://doi.org/10.1677/joe.0.1320433>)
- Stabnov L, Kasukawa Y, Guo R, Amaar Y, Wergedal JE, Baylink DJ & Mohan S 2002 Effect of insulin-like growth factor-1 (IGF-1) plus alendronate on bone density during puberty in IGF-1-deficient MIDL mice. *Bone* **30** 909–916. ([https://doi.org/10.1016/S8756-3282\(02\)00738-X](https://doi.org/10.1016/S8756-3282(02)00738-X))
- Stagi S, Cavalli L, Ricci S, Mola M, Marchi C, Seminara S, Brandi ML & de Martino M 2015 Parathyroid hormone levels in healthy children and adolescents. *Hormone Research in Paediatrics* **84** 124–129. (<https://doi.org/10.1159/000432399>)
- Sun BF, Kobayashi H, Le N, Yoo TM, Drumm D, Paik CH, McAfee JG & Carrasquillo JA 1997a Biodistribution of 125I-labeled des(1–3) insulin-like growth factor I in tumor-bearing nude mice and its in vitro catabolism. *Cancer Research* **57** 2754–2759.
- Sun FL, Dean WL, Kelsey G, Allen ND & Reik W 1997b Transactivation of Igf2 in a mouse model of Beckwith-Wiedemann syndrome. *Nature* **389** 809–815. (<https://doi.org/10.1038/39797>)
- Sunters A, Armstrong VJ, Zaman G, Kypta RM, Kawano Y, Lanyon LE & Price JS 2010 Mechano-transduction in osteoblastic cells involves strain-regulated estrogen receptor alpha-mediated control of insulin-like growth factor (IGF) I receptor sensitivity to Ambient IGF, leading to phosphatidylinositol 3-kinase/AKT-dependent Wnt/LRP5 receptor-independent activation of beta-catenin signaling. *Journal of Biological Chemistry* **285** 8743–8758. (<https://doi.org/10.1074/jbc.M109.027086>)
- Szulc P, Joly-Pharaboz MO, Marchand F & Delmas PD 2004 Insulin-like growth factor I is a determinant of hip bone mineral density in men less than 60 years of age: MINOS study. *Calcified Tissue International* **74** 322–329. (<https://doi.org/10.1007/s00223-003-0090-9>)
- Takayanagi H, Kim S, Koga T, Nishina H, Ishiki M, Yoshida H, Saiura A, Isobe M, Yokochi T, Inoue J, et al. 2002 Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. *Developmental Cell* **3** 889–901. ([https://doi.org/10.1016/S1534-5807\(02\)00369-6](https://doi.org/10.1016/S1534-5807(02)00369-6))
- Tatsumi S, Ishii K, Amizuka N, Li M, Kobayashi T, Kohno K, Ito M, Takeshita S & Ikeda K 2007 Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. *Cell Metabolism* **5** 464–475. (<https://doi.org/10.1016/j.cmet.2007.05.001>)
- Thompson JL, Butterfield GE, Marcus R, Hintz RL, Van Loan M, Ghiron L & Hoffman AR 1995 The effects of recombinant human insulin-like growth factor-I and growth hormone on body composition in elderly women. *Journal of Clinical Endocrinology and Metabolism* **80** 1845–1852. (<https://doi.org/10.1210/jcem.80.6.7539817>)
- Tonella P, Fluck CE & Mullis PE 2010 Insulin-like growth factor-I treatment in primary growth hormone insensitivity: effect of recombinant human IGF-I (rhIGF-I) and rhIGF-I/rhIGF-binding protein-3 complex. *Hormone Research in Paediatrics* **73** 140–147. (<https://doi.org/10.1159/000277660>)
- Tsang KY, Chan D, Cheslett D, Chan WC, So CL, Melhado IG, Chan TW, Kwan KM, Hunziker EB, Yamada Y, et al. 2007 Surviving endoplasmic reticulum stress is coupled to altered chondrocyte differentiation and function. *PLoS Biology* **5** e44. (<https://doi.org/10.1371/journal.pbio.0050044>)
- Uchimura T, Hollander JM, Nakamura DS, Liu Z, Rosen CJ, Georgakoudi I & Zeng L 2017 An essential role for IGF2 in cartilage development and glucose metabolism during postnatal long bone growth. *Development* **144** 3533–3546. (<https://doi.org/10.1242/dev.155598>)
- Ueki I, Ooi GT, Tremblay ML, Hurst KR, Bach LA & Boisclair YR 2000 Inactivation of the acid labile subunit gene in mice results in mild retardation of postnatal growth despite profound disruptions in the circulating insulin-like growth factor system. *PNAS* **97** 6868–6873. (<https://doi.org/10.1073/pnas.120172697>)
- Ueland T, Ebbesen EN, Thomsen JS, Mosekilde L, Brixen K, Flyvbjerg A & Bollerslev J 2002 Decreased trabecular bone biomechanical competence, apparent density, IGF-II and IGFBP-5 content in acromegaly. *European Journal of Clinical Investigation* **32** 122–128. (<https://doi.org/10.1046/j.1365-2362.2002.00944.x>)
- van Duyvenvoorde HA, van Setten PA, Walenkamp MJ, van Doorn J, Koenig J, Gauguin L, Oostdijk W, Ruivenkamp CA, Losekoot M, Wade JD, et al. 2010 Short stature associated with a novel

- heterozygous mutation in the insulin-like growth factor 1 gene. *Journal of Clinical Endocrinology and Metabolism* **95** E363–E367. (<https://doi.org/10.1210/jc.2010-0511>)
- Van Wyk JJ, Hall K, Van den Brande JL & Weaver RP 1971 Further purification and characterization of sulfation factor and thymidine factor from acromegalic plasma. *Journal of Clinical Endocrinology and Metabolism* **32** 389–403. (<https://doi.org/10.1210/jcem-32-3-389>)
- Veldhuis JD, Metzger DL, Martha PM Jr, Mauras N, Kerrigan JR, Keenan B, Rogol AD & Pincus SM 1997 Estrogen and testosterone, but not a nonaromatizable androgen, direct network integration of the hypothalamo-somatotrope (growth hormone)-insulin-like growth factor I axis in the human: evidence from pubertal pathophysiology and sex-steroid hormone replacement. *Journal of Clinical Endocrinology and Metabolism* **82** 3414–3420. (<https://doi.org/10.1210/jcem.82.10.4317>)
- 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.85.7.6697>)
- Venken K, Schuit F, Van Lommel L, Tsukamoto K, Kopchick JJ, Coschigano K, Ohlsson C, Moverare S, Boonen S, Bouillon R, et al. 2005 Growth without growth hormone receptor: estradiol is a major growth hormone-independent regulator of hepatic IGF-I synthesis. *Journal of Bone and Mineral Research* **20** 2138–2149. (<https://doi.org/10.1359/JBMR.050811>)
- Wakley GK, Evans GL & 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/s002239900183>)
- Walenkamp MJ, Karperien M, Pereira AM, Hilhorst-Hofstee Y, van Doorn J, Chen JW, Mohan S, Denley A, Forbes B, van Duyvenvoorde HA, et al. 2005 Homozygous and heterozygous expression of a novel insulin-like growth factor-I mutation. *Journal of Clinical Endocrinology and Metabolism* **90** 2855–2864. (<https://doi.org/10.1210/jc.2004-1254>)
- Walenkamp MJ, Losekoot M & Wit JM 2013 Molecular IGF-1 and IGF-1 receptor defects: from genetics to clinical management. *Endocrine Development* **24** 128–137. (<https://doi.org/10.1159/000342841>)
- Wang X, Yang Y & Adamo ML 1997 Characterization of the rat insulin-like growth factor I gene promoters and identification of a minimal exon 2 promoter. *Endocrinology* **138** 1528–1536. (<https://doi.org/10.1210/endo.138.4.5061>)
- Wang J, Zhou J & Bondy CA 1999 Igf1 promotes longitudinal bone growth by insulin-like actions augmenting chondrocyte hypertrophy. *FASEB Journal* **13** 1985–1990. (<https://doi.org/10.1096/fasebj.13.14.1985>)
- Wang Y, Nishida S, Elalieh HZ, Long RK, Halloran BP & Bikle DD 2006 Role of IGF-I signaling in regulating osteoclastogenesis. *Journal of Bone and Mineral Research* **21** 1350–1358. (<https://doi.org/10.1359/jbmr.060610>)
- Wang Y, Nishida S, Boudignon BM, Burghardt A, Elalieh HZ, Hamilton MM, Majumdar S, Halloran BP, Clemens TL & Bikle DD 2007 IGF-I receptor is required for the anabolic actions of parathyroid hormone on bone. *Journal of Bone and Mineral Research* **22** 1329–1337. (<https://doi.org/10.1359/jbmr.070517>)
- Wassenaar MJ, Biermasz NR, Hamdy NA, Zillikens MC, van Meurs JB, Rivadeneira F, Hofman A, Uitterlinden AG, Stokkel MP, Roelfsema F, et al. 2011 High prevalence of vertebral fractures despite normal bone mineral density in patients with long-term controlled acromegaly. *European Journal of Endocrinology* **164** 475–483. (<https://doi.org/10.1530/EJE-10-1005>)
- Wang Y, Cheng Z, Elalieh HZ, Nakamura E, Nguyen MT, Mackem S, Clemens TL, Bikle DD & Chang W 2011 IGF-1R signaling in chondrocytes modulates growth plate development by interacting with the PTHrP/Ihh pathway. *Journal of Bone and Mineral Research* **26** 1437–1446. (<https://doi.org/10.1002/jbmr.359>)
- Wang Y, Menendez A, Fong C, ElAlieh HZ, Chang W & Bikle DD 2014 Ephrin B2/EphB4 mediates the actions of IGF-I signaling in regulating endochondral bone formation. *Journal of Bone and Mineral Research* **29** 1900–1913. (<https://doi.org/10.1002/jbmr.2196>)
- Wang Y, Menendez A, Fong C, ElAlieh HZ, Kubota T, Long R & Bikle DD 2015 IGF-I signaling in osterix-expressing cells regulates secondary ossification center formation, growth plate maturation, and metaphyseal formation during postnatal bone development. *Journal of Bone and Mineral Research* **30** 2239–2248. (<https://doi.org/10.1002/jbmr.2563>)
- Watson P, Lazowski D, Han V, Fraher L, Steer B & Hodsman A 1995 Parathyroid hormone restores bone mass and enhances osteoblast insulin-like growth factor I gene expression in ovariectomized rats. *Bone* **16** 357–365. ([https://doi.org/10.1016/8756-3282\(94\)00051-4](https://doi.org/10.1016/8756-3282(94)00051-4))
- Watson CS, Bialek P, Anzo M, Khosravi J, Yee SP & Han VK 2006 Elevated circulating insulin-like growth factor binding protein-1 is sufficient to cause fetal growth restriction. *Endocrinology* **147** 1175–1186. (<https://doi.org/10.1210/en.2005-0606>)
- Werner H 2012 Tumor suppressors govern insulin-like growth factor signaling pathways: implications in metabolism and cancer. *Oncogene* **31** 2703–2714. (<https://doi.org/10.1038/onc.2011.447>)
- Werner H & Bruchim I 2012 IGF-1 and BRCA1 signalling pathways in familial cancer. *Lancet Oncology* **13** e537–e544. ([https://doi.org/10.1016/S1470-2045\(12\)70362-5](https://doi.org/10.1016/S1470-2045(12)70362-5))
- Werner H, Bach MA, Stannard B, Roberts CT Jr & LeRoith D 1992 Structural and functional analysis of the insulin-like growth factor I receptor gene promoter. *Molecular Endocrinology* **6** 1545–1558. (<https://doi.org/10.1210/mend.6.10.1448110>)
- Werner H, Karnieli E, Rauscher FJ & LeRoith D 1996 Wild-type and mutant p53 differentially regulate transcription of the insulin-like growth factor I receptor gene. *PNAS* **93** 8318–8323. (<https://doi.org/10.1073/pnas.93.16.8318>)
- Woods KA, Camacho-Hubner C, Savage MO & Clark AJ 1996 Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. *New England Journal of Medicine* **335** 1363–1367. (<https://doi.org/10.1056/NEJM199610313351805>)
- Wroblewski J, Engstrom M, Skottner A, Madsen K & Friberg U 1987 Subcellular location of IGF-I in chondrocytes from rat rib growth plate. *Acta Endocrinologica* **115** 37–43. (<https://doi.org/10.1530/acta.0.1150037>)
- Wu Y, Sun H, Basta-Pljakic J, Cardoso L, Kennedy OD, Jasper H, Domene H, Karabatas L, Guida C, Schaffler MB, et al. 2013 Serum IGF-1 is insufficient to restore skeletal size in the total absence of the growth hormone receptor. *Journal of Bone and Mineral Research* **28** 1575–1586. (<https://doi.org/10.1002/jbmr.1920>)
- Wu S, Yang W & De Luca F 2015 Insulin-like growth factor-independent effects of growth hormone on growth plate chondrogenesis and longitudinal bone growth. *Endocrinology* **156** 2541–2551. (<https://doi.org/10.1210/en.2014-1983>)
- Wuster C, Abs R, Bengtsson BA, Bennmarker H, Feldt-Rasmussen U, Hernberg-Stahl E, Monson JP, Westberg B & Wilton P 2001 The influence of growth hormone deficiency, growth hormone replacement therapy, and other aspects of hypopituitarism on fracture rate and bone mineral density. *Journal of Bone and Mineral Research* **16** 398–405. (<https://doi.org/10.1359/jbmr.2001.16.2.398>)
- Xian L, Wu X, Pang L, Lou M, Rosen CJ, Qiu T, Crane J, Frassica F, Zhang L, Rodriguez JP, et al. 2012 Matrix IGF-1 maintains bone mass by activation of mTOR in mesenchymal stem cells. *Nature Medicine* **18** 1095–1101. (<https://doi.org/10.1038/nm.2793>)
- Yakar S & Isaksson O 2016 Regulation of skeletal growth and mineral acquisition by the GH/IGF-I axis: lessons from mouse models. *Growth Hormone and IGF Research* **28** 26–42. (<https://doi.org/10.1016/j.ghir.2015.09.004>)

- Yakar S, Liu JL, Stannard B, Butler A, Accili D, Sauer B & LeRoith D 1999 Normal growth and development in the absence of hepatic insulin-like growth factor I. *PNAS* **96** 7324–7329. (<https://doi.org/10.1073/pnas.96.13.7324>)
- Yakar S, Rosen CJ, Beamer WG, Ackert-Bicknell CL, Wu Y, Liu JL, Ooi GT, Setser J, Frystyk J, Boisclair YR, et al. 2002 Circulating levels of IGF-1 directly regulate bone growth and density. *Journal of Clinical Investigation* **110** 771–781. (<https://doi.org/10.1172/JCI0215463>)
- Yakar S, Buxsein ML, Canalis E, Sun H, Glatt V, Gundberg C, Cohen P, Hwang D, Boisclair Y, Leroith D, et al. 2006 The ternary IGF complex influences postnatal bone acquisition and the skeletal response to intermittent parathyroid hormone. *Journal of Endocrinology* **189** 289–299. (<https://doi.org/10.1677/joe.1.06657>)
- Yakar S, Canalis E, Sun H, Mejia W, Kawashima Y, Nasser P, Courtland HW, Williams V, Buxsein M, Rosen C, et al. 2009a Serum IGF-1 determines skeletal strength by regulating subperiosteal expansion and trait interactions. *Journal of Bone and Mineral Research* **24** 1481–1492. (<https://doi.org/10.1359/jbmr.090226>)
- Yakar S, Rosen CJ, Buxsein ML, Sun H, Mejia W, Kawashima Y, Wu Y, Emerton K, Williams V, Jepsen K, et al. 2009b Serum complexes of insulin-like growth factor-1 modulate skeletal integrity and carbohydrate metabolism. *FASEB Journal* **23** 709–719. (<https://doi.org/10.1096/fj.08-118976>)
- Yamaguchi M, Ogata N, Shinoda Y, Akune T, Kamekura S, Terauchi Y, Kadowaki T, Hoshi K, Chung UI, Nakamura K, et al. 2005 Insulin receptor substrate-1 is required for bone anabolic function of parathyroid hormone in mice. *Endocrinology* **146** 2620–2628. (<https://doi.org/10.1210/en.2004-1511>)
- Yeh JK, Chen MM & Aloia JF 1997 Effects of estrogen and growth hormone on skeleton in the ovariectomized rat with hypophysectomy. *American Journal of Physiology* **273** E734–E742. (<https://doi.org/10.1152/ajpcell.1997.273.2.C734>)
- Yeh LC, Wilkerson M, Lee JC & Adamo ML 2015 IGF-1 receptor insufficiency leads to age-dependent attenuation of osteoblast differentiation. *Endocrinology* **156** 2872–2879. (<https://doi.org/10.1210/en.2014-1945>)
- Yuen JS, Cockman ME, Sullivan M, Protheroe A, Turner GD, Roberts IS, Pugh CW, Werner H & Macaulay VM 2007 The VHL tumor suppressor inhibits expression of the IGF1R and its loss induces IGF1R upregulation in human clear cell renal carcinoma. *Oncogene* **26** 6499–6508. (<https://doi.org/10.1038/sj.onc.1210474>)
- Zhang M, Xuan S, Buxsein ML, von Stechow D, Akeno N, Faugere MC, Malluche H, Zhao G, Rosen CJ, Efstratiadis A, et al. 2002 Osteoblast-specific knockout of the insulin-like growth factor (IGF) receptor gene reveals an essential role of IGF signaling in bone matrix mineralization. *Journal of Biological Chemistry* **277** 44005–44012. (<https://doi.org/10.1074/jbc.M208265200>)
- Zhang M, Faugere MC, Malluche H, Rosen CJ, Chernauek SD & Clemens TL 2003 Paracrine overexpression of IGFBP-4 in osteoblasts of transgenic mice decreases bone turnover and causes global growth retardation. *Journal of Bone and Mineral Research* **18** 836–843. (<https://doi.org/10.1359/jbmr.2003.18.5.836>)
- Zhao G, Monier-Faugere MC, Langub MC, Geng Z, Nakayama T, Pike JW, Chernauek SD, Rosen CJ, Donahue LR, Malluche HH, et al. 2000 Targeted overexpression of insulin-like growth factor I to osteoblasts of transgenic mice: increased trabecular bone volume without increased osteoblast proliferation. *Endocrinology* **141** 2674–2682. (<https://doi.org/10.1210/endo.141.7.7585>)

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