Citation: BoneKEy Reports **2**, Article number: 437 (2013) | doi:10.1038/bonekey.2013.171
© 2013 International Bone & Mineral Society All rights reserved 2047-6396/13
www.nature.com/bonekey

REVIEW

IGF-1 regulation of key signaling pathways in bone

Anyonya R Guntur and Clifford J Rosen

Center for Clinical and Translational Research, Maine Medical Center Research Institute, Scarborough, ME, USA.

Insulin-like growth factor 1 (IGF-1) is an unique peptide that functions in an endocrine/paracrine and autocrine manner in most tissues. Although it was postulated initially that liver-derived IGF-1 was the major source of IGF-1 (that is, the somatomedin hypothesis), it is also produced in a wide variety of tissues and can function in numerous ways as both a proliferative and differentiative factor. One such tissue is bone and all cell lineages in the skeleton have been shown to not only require IGF-1 for normal development and function but also to respond to IGF-1 via the IGF-1 receptor. Ligand-receptor activation leads to several distinct downstream signaling cascades, which have significant implications for cell survival, protein synthesis and energy utilization. The novel role of IGF-1 in regulating metabolic demands of the bone remodeling unit is currently under investigation. More studies are likely to shed new light on various aspects of skeletal physiology and potentially may lead to new therapeutics.

BoneKEy Reports **2**, Article number: 437 (2013) | doi:10.1038/bonekey.2013.171

Introduction

Insulin-like growth factor 1 (IGF-1) is important for a number of different growth and differentiation processes across a wide variety of tissues.[1] Specifically, the growing skeleton is modulated by IGF-1 through endocrine/paracrine and autocrine mechanisms. Disruption of IGF-1 receptor (IGF1R) by genetic means in chondrocytes, osteoblasts and osteocytes has shown that IGF-1 signaling is necessary for controlling cell proliferation and differentiation.

IGF-1 binds to IGF1R, a type II tyrosine kinase, and leading to auto-phosphorylation of Tyr residues 1131,1135,1136 in the kinase domain, followed by phosphorylation of Tyr 950 in the juxtamembrane domain, which activates downstream substrates, insulin receptor substrate (IRS) proteins and Shc by tyrosine phosphorylations.[2] The IRS protein family consists of four isomers IRS1, 2, 3 and 4. Two of these proteins, IRS1 and IRS2, have been studied with respect to bone; IRS1 is expressed in chondrocytes and osteoblasts, IRS2 is expressed in osteoblasts and osteoclasts but not in chondrocytes. It is not known whether IRS3 and IRS4 are expressed in bone cells. Global deletion of IRS1 and IRS2 in mice leads to a decrease in bone mass, although there are no bone-specific conditional knockouts reported.[3,4] In case of IGF-1 induction, IRS1 activates phosphatidylinositol 3 kinase (PI3K),[5] and the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) network by binding to Shc and Grb2 (**Figure 1**).[6–8]

Activated PI3K phosphorylates phosphatidylinositol 4,5 bisphosphate converting it to phosphatidylinositol 3,4,5, trisphosphate. Phosphatidylinositol 3,4,5, trisphosphate recruits PDK1 (a Ser/Thr kinase), which can phosphorylate protein kinase B (AKT) at Thr 308 and then leads to partial activation of AKT (a AGC kinase). mTORC2 phosphorylates AKT on Ser 473;[9–11] these changes allow for complete activation of this signaling cascade. Activated AKT, of which there are three different isoforms AKT1, AKT2 and AKT3, in turn controls a number of cellular metabolic processes that affect development.[12]

Some of the substrates for phosphorylated AKT are essential for skeletal development. These include: (1) the Forkhead group (FoxO1,3,4) of transcriptional factors.[13,14] Deletion of FoxO1 specifically in osteoblasts leads to decreased bone mass and the deletion of FoxO1, 3 and 4 shows that the FoxO group of transcriptional factors are necessary for protecting osteoblasts from oxidative stress. (2) mTOR, which is a downstream target of AKT (also called mammalian target of rapamycin), regulates a number of processes critical for cell growth and regulation particularly protein synthesis. During IGF-1-mediated activation there is an increase in the activity of mTOR and a subsequent enhancement in osteoblast differentiation, which is inhibited in many cells by rapamycin. mTOR exists as two distinct complexes mTORC1 (mTOR, Raptor (regulatory-associated protein of mTOR), mLST8/GβL (G-protein β subunit-like protein) and DEPTOR (DEP domain containing mTOR interacting protein)), which is rapamycin sensitive and activated by growth factors like IGF-1/insulin and controls translation and cell proliferation through phosphorylation of its downstream substrates p70S6K and 4E-BP (eukaryotic initiation factor 4E-binding protein). p70S6K can feedback and inhibit IRS1 targeting it for degradation. mTORC2 (rapamycin insensitive

Correspondence: Dr CJ Rosen, Center for Clinical and Translational Research, Maine Medical Center Research Institute, 81 Research Drive, Scarborough, ME 04074, USA. E-mail: crofen@gmail.com

Received 2 May 2013; accepted 3 September 2013; published online 2 October 2013

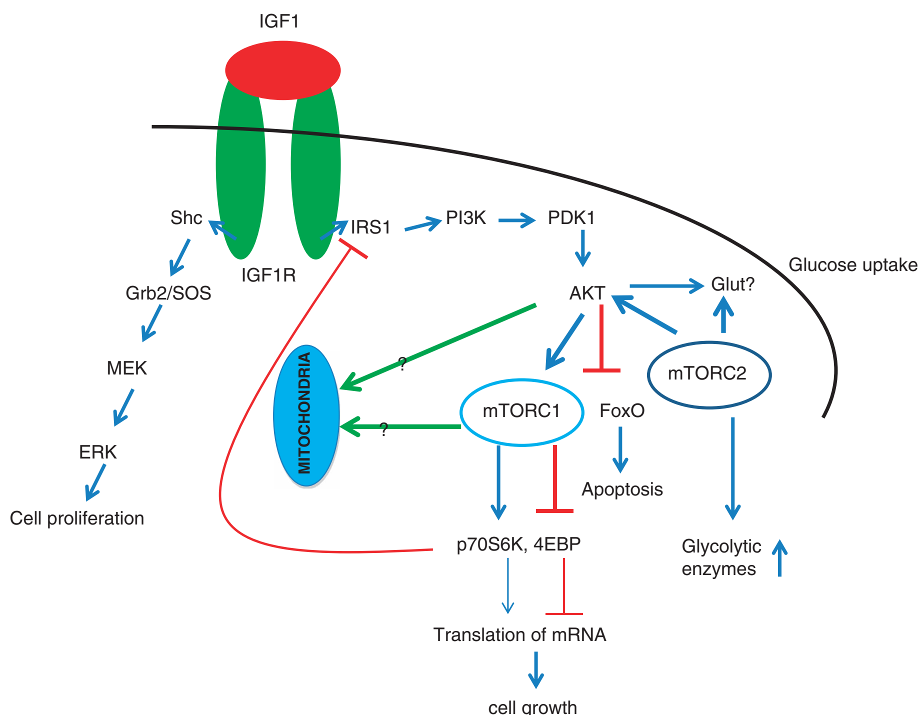

Figure 1 The figure represents a cartoon depicting the key signaling pathways that have been discussed in this review. IGF-1 can activate MAPK and PI3K signaling, as we have concentrated on AKT signaling in this review, the figure illustrates functions of AKT on its downstream targets in detail. The functions of IGF-1 on mitochondrial metabolism during chondrocyte and osteoblast differentiation and the role of IGF-1 on glucose metabolism have not been completely elucidated yet. There is some data showing that mTORC2 can activate the Warburg effect during Wnt-induced osteoblast differentiation. The functions of mTORC1 through activation of S6K and inhibition of 4EBP and the role of mTOR on oxidative phosphorylation and glycolysis should be studied further. PDK1, 3-phosphoinositide-dependent protein kinase-1.

during acute exposure), which consists of mTOR, Rictor (rapamycin insensitive companion of mTOR), mLST8/GβL, mSIN1 (mammalian stress-activated protein kinase-interacting protein 1) and PROTOR (protein observed with Rictor), can activate AKT by phosphorylating it at Ser 473 and can therefore inhibit FoxO1.[15,16] There are no conditional mouse models that have targeted the mTOR signaling pathway in osteoblasts.

The other important substrate that AKT can act on is glycogen synthase kinase3α,β (GSK3). GSK3 is a Ser/Thr kinase important for regulating the Wnt signaling pathway and glycogen synthesis. GSK3 is inactivated by phosphorylation of a key serine residue GSK3βSer9 and GSK3αSer21 by AKT and leads to glycogen synthesis.[17] In the absence of Wnt signaling, GSK3 can phosphorylate β-catenin at Thr 41, Ser 37 and Ser 33 sequentially in the N-terminus after the priming Ser 45 phosphorylation by casein kinase I and lead to its proteosomal degradation. On activation by Wnt ligands binding to frizzled and low-density lipoprotein receptor-related proteins 5 and 6 (LRP5/LRP6), GSK3 and casein kinase phosphorylate LRP6 and axin leading to a increase in active β-catenin resulting in its accumulation and nuclear translocation. The contribution of Wnt-mediated activation of β-catenin has been reported during skeletal development by a large number of studies (for extensive review see Williams and Insogna.[18] However, the role of IGF-1/AKT-mediated effect on β-catenin in skeletal development is not clear, though some studies have reported that IGF-1 can lead to active β-catenin.[19] Recent studies show that GSK3 inhibits chondrocyte proliferation, bone growth and hypertrophic chondrocyte turnover, and GSK3α can partially compensate for the loss of GSK3β. Additional deletion studies of compound knockout mice with a loss in both alleles of GSK3α and one allele of GSK3β have shown that GSK3α,β have an important role in early chondrocyte differentiation.[20,21]

IGF-1 in a number of different cell types can activate the Glut family of transporters for increasing the uptake of glucose. This is an important but often overlooked role for IGF-1 and is clearly important in systemic metabolism. Of the four class I Glut family members, Glut 1 and 3 have been identified in rat calvarial and osteosarcoma cell lines to be responsible for glucose uptake in osteoblasts.[22,23] Glut 4, which is mostly responsible for glucose uptake in insulin responsive tissue, has been identified in chondrocytes and there have been a number of recent reports that suggest that bone is responsive to insulin signaling. Thus, more studies need to be done to clearly identify which of the Glut receptors are expressed in skeletal cells and are utilized during energy utilization.[24,25] This review will specifically address the role of IGF-1 in bone development and the evidence that suggests a role for IGF-1 if any in skeletal energy metabolism. The first part of the review will focus on the loss of IGF-1 and IGF1R in bone and the effects on bone remodeling. This section will include the inter-relationship of IGF-1 with chondrocyte development, osteoblast differentiation, osteocytic activity and matrix IGF-1. The latter part of the review will discuss the role of IGF-1 in energy metabolism.

IGF-1 and Chondrocytes

Most of the long bones and small parts of the skull base develop through the process of endochondral ossification. During

endochondral ossification mesenchymal stem cells (MSCs) condense and then differentiate into chondrocytes to form a template of the skeletal element. The chondrocytes in the middle of the condensation then undergo proliferation to form the growth plate, whereas the cells at the periphery form a layer around the condensation, the perichondrium. The chondrocytes go through a series of steps where they proliferate and then hypertrophy. The hypertrophic chondrocytes secrete growth factors to signal blood vessel invagination and also entry of osteoblast precursors through a series of orchestrated and highly regulated processes to form the primary ossification center (for extensive review see refs 26–29). There are a number of secreted growth factor signaling families that have been shown to be involved in regulating these complex processes, including the fibroblast growth factor family, bone morphogenetic proteins and the hedgehog factors.

One of the most important regulators of chondrocyte function is IGF-1, which signals through PI3K and other secondary messengers (reviewed in Guntur and Rosen).[30] Chondrocytes in the long bones express IGF1R and also secrete IGF-1, global deletion of IGF-1 and its receptor IGF1R leads to both skeletal defects and cartilage changes including reduced bone length.[31] A chondrocyte-specific IGF-1 knockout has been described by using *Igf-1* flox/flox mice that were crossed to a collagen2α1 (*Col2α1*)-specific cre (chondrocyte-specific cre). The results revealed that loss of IGF-1 produced by these collagen2-expressing chondrocytes led to changes in the longitudinal growth and width of bone as well as a decrease in bone mineral density.[32] Although the width and the length of the femurs are smaller in the conditional knockouts, no effect was reported on the organization of the growth plate. The IGF1R has been deleted using the *Col2α1*, and its phenotype shows growth plate defects including disorganized chondrocytes, decreased proliferation and increased apoptosis.[33] The use of a tamoxifen inducible model to delete the IGF1R in chondrocytes 1 week postnatally revealed a significant reduction in growth plate chondrocyte proliferation and differentiation.[26] Recently, IGF-1 was deleted using the *Hoxb6* cre (this cre is expressed in both the hind and anterior part of the forelimb) and the phenotype revealed a critical role for IGF-1 in controlling the size of the limbs through the volume of hypertrophic chondrocytes. The bones of the mice were smaller by 30% but there were similar number of hypertrophic chondrocytes. The investigators in this work described growth of the hypertrophic chondrocytes as progressing through three distinct phases and the IGF-1 conditional knockouts had a defect in the last or third phase, which led to the hypertrophic chondrocytes not increasing in size.[34] The difference in size of the chondrocytes that have a loss in IGF-1 has also been observed with the global IGF-1 deletion. This fact is particularly interesting taking into account the fact that mTOR signaling can be activated by IGF-1 signaling and is active during growth plate development also has been shown to regulate cell size.[35] IGF-2 is the other ligand that can bind to the IGF1R. This paternal imprinted gene has been shown to have a role in skeletal development. The contribution of IGF-2 to skeletal development has been suggested to be mostly during embryonic development and that it is necessary to maintain progenitor cells.[36] But the comparison of the IGF-1 null and IGF1R chondrocyte-specific knockouts suggest that chondrocytes proliferate and hypertrophy mainly in response to IGF-1.

Finally, circulating IGF-1 is generated in response to growth hormone and can also affect bone. A number of different strategies have been used to study the role of circulating IGF-1 on bone development. As liver is the major source for secreted IGF-1, liver-specific deletion of IGF-1 resulting in reduced serum IGF-1 levels has shown that the decrease in serum IGF-1 levels leads to a minimal impact on trabecular bone but there is decreased cortical bone and bone strength.[24] The use of mouse strains with different genetic backgrounds, namely the C57Bl/6J and the C3H/HeJ mice that have low and high IGF-1 levels, respectively, has shown that the skeletal phenotype is dependent on the levels of circulating IGF-1. The B6 mice with low IGF-1 had lower bone mineral density compared with the C3H mice. Additional studies that have used inducible liver-specific IGF-1-deficient mice to tease out the effects of serum IGF-1 at different time points, found that depleting serum IGF-1 at 4 weeks in male mice led to a decrease in both trabecular and cortical bone by 16 weeks, deletion of serum IGF-1 at 8 weeks of age affected only the cortical bone at 32 weeks, whereas depletion after peak bone accretion did not have detrimental effects on bone.[31,37] These studies show that serum IGF-1 has an important role in skeletal development and maintenance.

IGF-1 and Osteoblasts

IGF-1 knockout studies. IGF-1 has been conditionally deleted in osteoblasts at various stages of development. The *Col1α2* cre deletes IGF-1 in osteoblasts that express collagen1, a major matrix protein secreted by osteoblasts, although *Col1α2* has been also identified to be expressed in a variety of non-skeletal tissue. The conditional knockout mice showed a decrease in bone formation[38] that led to an overall reduction in bone mass. On the other hand, overexpression of IGF-1 using the 3.6-kb *Col1α1* promoter led to increases in the length of the long bones and cortical width with minimal effect on trabecular bone. Calvarial bone width was also increased indicating that overexpression of IGF-1 in osteoblast lineage cells led to increased bone.[39] The use of *Osterix*-GFP cre to delete the IGF1R in early osteoprogenitor cells shows that osteoblasts are not able to differentiate normally and end up having lower bone mass compared with wild-type controls.[40] At a later time point, the use of the osteocalcin promoter to delete the IGF1R resulted in skeletal defects and impaired mineralization. The osteocalcin promoter is active at around e17dpc in mice and has been shown to be specifically expressed in mature osteoblasts.[41] The mice with the loss of IGF1R have decreased bone formation at 6 weeks of age and although they have a sufficient number of osteoblasts these mice have very low bone formation rates, suggesting the work of osteoblasts is impaired. One of the interesting observations from the knockout of IGF-1 is the requirement of IGF-1 for parathyroid hormone (PTH) action on bone.[31] In the absence of IGF-1 (global- or osteoblast-specific deletion), PTH did not have a positive effect on the rate of bone formation.[42] In addition, it appears that PTH also needs IRS1 and not IRS2 for it to have the full anabolic effect on bone. Because PTH is a therapeutic option for osteoporosis, understanding the principal mechanisms that are operative via the IGF signaling pathways remains a major priority.[43] All the data from both the chondrocyte and osteoblast deletion studies of IGF-1 and IGF1R show that activating IGF-1 signaling is important for maintaining the proliferation of both chondrocytes

and osteoblasts so that endochondral ossification occurs properly. Recent studies have shown that the cells adjacent to the zone of Ranvier in the perichondrium harbor progenitor cells.[44] Targeting osteoprogenitor-specific IGF-1 and IGF1R deletion utilizing *Prrx1*cre, *Dermo1*cre or *cathepsin K* cre (shown to be expressed in the perichondrial groove of Ranvier)[45] to study the role of IGF-1 in early mesenchymal cells would be important. This will enable us to understand the role of IGF-1 in regulating pathways important for early bone formation and also to identify if this signaling pathway has a role in regulating chondro/osteo progenitor cells.

IGF-1 Osteocytes and Osteoclasts

Osteocytes are important mechanosensors that are deeply embedded in bone and sense physical loading. These are terminally differentiated osteoblasts within bone matrix. Knockout of IGF-1 specifically in the osteocytes has been reported recently[46] using a dentin matrix protein1 (DMP1) cre to delete IGF-1 in osteocytes. The DMP1 cre has been generated by using the 10-kb promoter region of DMP1 and is active in odontoblasts and osteocytes and induced in response to mechanical loading. The Dmp1 cre used in this study is 10 kb cre, which has been used extensively to target cells of the osteocyte lineage. In this study, knockout of exon 4 of IGF-1 in osteocytes affected the bone longitudinal and cortical growth along with a decrease in the calvarial bone growth rate. The mice with an osteocyte-specific knockout also have a shorter growth plate compared with the wild-type control along with smaller hypertrophic chondrocytes.[34] There is expression of the IGF1R on osteoclasts and during bone remodeling. Matrix IGF-1 had been shown to be necessary for MSC differentiation, although whether osteoclasts secrete IGF-1 is not clear. The IGF-1 global knockout[47] showed an increase in bone volume/total volume primarily because of a decrease in osteoclast number, suggesting that IGF-1 is required for normal osteoclast differentiation.

IGF-1 and Bone Matrix

It has recently been shown that matrix IGF-1 is necessary for the activation and proliferation of MSCs during resorption and this is mediated via the mTOR signaling pathway.[30] IGFs are bound in the skeletal matrix and are one of the most prevalent non-collagen proteins. In the above-mentioned paper, MSCs that were treated with IGF-1 activated the IRS1/P13K/AKT pathway along with mTOR and it was observed that inhibiting this signaling pathway with Rapamycin affected only mTOR. In contrast, the LY294002, PI3K inhibitor, did not inhibit mTOR.[30] This (mTOR) activation led to an increase in osteoblast differentiation markers like osteocalcin and osteoblast transcriptional factors like Runx2. This study also showed that MSC isolated from *Igf1R* flox/flox mice and deleting IGF1R *in vitro* using adenocre fail to form new bone efficiently when embedded in Matrigel and placed under the renal capsule in *Rag2*$^{-/-}$ mice, compared with the adenoGFP-treated controls, the bone formation by the *Igf1*flox/flox adenoGFP cells could be inhibited by treating with rapamycin. Recent studies have also shown that osteoblasts that are differentiating in response to Wnt3a utilize the Warburg effect for energy consumption via the mTORC2 pathway by upregulating glycolytic enzymes and increasing lactate production.[48] Interestingly, the deletion of peroxisome proliferator-activated receptor (PPAR)γ specifically in osteoblasts leads to an increase in p70S6k, which is a downstream of mTORC1, suggesting that PPARγ has a negative control over mTOR. This signaling pathway should be extensively studied to define the relative contribution of the different complexes that are involved in osteoblast differentiation.[49]

IGF-1 and energy metabolism. All the studies to date with the skeletal IGF regulatory components have produced *in vivo* phenotypes through deletion or overexpression of the IGF-1 system in mice. However, the effect of these genetic manipulations on glucose homeostasis is less clear. The recent finding by the Ferron *et al.*[50] and Fulzele *et al.*[51] showed that there is a role for insulin signaling in the osteoblast and this can regulate the levels of osteocalcin and under carboxylated osteocalcin by binding to its receptor (the IGF1R knockout mice did not have a metabolic phenotype), suggesting that the metabolic effects observed are mainly mediated by insulin signaling under these experimental conditions. Subsequent activation can signal β-cells in the pancreas leading to an increase in proliferation and greater secretion of insulin. Mechanistically, further characterization has shown the following: (1) FoxO1 through its negative regulation of Runx2[52] downregulates *Bglap2*, (2) FoxO1 and ATF4 synergistically activating *Esp*, a negative regulator of insulin signaling,[53] affects glucose homeostasis. Another recent study examined an osteoblast-specific knockout of PDK1, a kinase that phosphorylates and partially activates AKT. Using the *Osterix* cre it was shown that the effects of IGF-1 and insulin on osteoblast differentiation through Runx2 expression are mediated by PDK1. The authors showed that the skeletal abnormalities manifested in the Rubinstein-Tyabi syndrome by mutations in CREB-binding protein lead to downregulation in the activity of CREB-binding protein, which can upregulate BMP2 through the master osteoblast transcriptional factor Runx2. In mice, Rubinstein-Tyabi syndrome-like skeletal abnormalities are seen when PDK1 is specifically deleted in osteoblasts. The mice with a osteoblast-specific deletion in PDK1 also have higher levels of non-fasting glucose and lower insulin levels once again suggesting that elements downstream of the IGF-1 ligand signaling pathway could regulate glucose homeostasis.[54] It is not yet clear if IGF-1 has similar effects as insulin in osteoblasts and if this would affect global glucose homeostasis, based on the current evidence.

Future directions for investigation: skeletal energy metabolism. IGF-1 is important for protein synthesis and subsequent cell growth and differentiation. Much of its effects on cell size seem to be mediated through the mTOR system. But, IGF-1 is also a nutrient sensor, which is modulated by available energy. This is exemplified in scenarios such as starvation or anorexia nervosa where circulating (that is, primarily the liver) and skeletal concentrations of IGF-1 are reduced and bone formation slows dramatically. The mechanisms responsible for this non-transcriptional effect is not well understood, although Nocturnin, another nutrient sensor, and 3′ deadenylase have been shown to rise in response to nutrient deprivation and decrease the half life of *Igf1* mRNA.[55] Of course, this compensation is evolutionarily important as cellular work must be reduced during nutrient deficiencies.

However, we have little understanding of the preferential substrates for optimal osteoblast differentiation, nor do we know precisely how IGF-I upregulates collagen synthesis relative to fuel sources as this process requires significant energy utilization. Future studies are certain to focus on local energy balance within the remodeling unit and its relationship to systemic substrate availability.

Conclusion

Although the last two decades have provided insight into the role of IGF-1 in regulating bone development, there is still a need to further study this pathway. For example, IGF-1 is critical in chondrocyte development. Moreover, a number of bones in the skull develop through endochondral formation and could impact the development of the skeletal elements in the skull base. In addition, gain-of-function mutations in IGF1R have been identified in some non-syndromic craniosynastosis where the mechanism seems to be through activation of IRS1.[56]

IGF-1 is likely to regulate both local and systemic energy metabolism. For example, it has been shown that during osteoblast differentiation the cells that are in a predifferentiation stage have lower number of active mitochondria and are more glycolytic in their energy consumption. Whereas during osteoblast differentiation when the cells have more need for energy consumption and need to synthesize more protein, there is an increase in mitochondrial transmembrane potential and the cells switch over to oxidative phosphorylation in tune with their differentiation status.[57] The effects of IGF-1 on mitochondrial function during both chondrogenic differentiation and osteogenic differentiation may be particularly important. These observations lead to a number of questions that require answers: (1) what are the energy requirements of MSC, which have the potential to differentiate into a number of different lineages? MSCs differentiating toward the adipogenic lineage are more glycolytic and need reactive oxygen species for adipogenesis, whereas MSCs differentiating toward the osteogenic lineages are more dependent on oxidative phosphorylation with increased reactive oxygen species being detrimental for *de novo* bone formation.[58] (2) What are the pathways that are utilized by differentiated osteoblasts to synthesize bone? TOR signaling has been shown to be important for regulating Wnt-mediated increases in glycolysis but are there other pathways? Thus, it is clear that although IGF-1 is ubiquitous, its role in the skeleton is multi-faceted and centers on both osteoblast proliferation and differentiation. Recent work suggesting that osteoclasts may respond to secreted IGFs from osteoblasts implies that this molecule is also a coupling factor for the remodeling unit. Further investigations are needed to define the role of IGF-1 in regulating energy metabolism.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

Research in CJR's Laboratory is supported by AR045433 from NIH. We thank Katherine J Motyl and Victoria E DeMambro for critical reading of the manuscript.

References

1. Efstratiadis A. Genetics of mouse growth. *Int J Dev Biol* 1998;**42**:955–976.
2. Hernández-Sánchez C, Blakesley V, Kalebic T, Helman L, LeRoith D. The role of the tyrosine kinase domain of the insulin-like growth factor-I receptor in intracellular signaling, cellular proliferation, and tumorigenesis. *J Biol Chem* 1995;**270**:29176–29181.
3. Akune T, Ogata N, Hoshi K, Kubota N, Terauchi Y, Tobe K *et al.* Insulin receptor substrate-2 maintains predominance of anabolic function over catabolic function of osteoblasts. *J Cell Biol* 2002;**159**:147–156.
4. Ogata N, Chikazu D, Kubota N, Terauchi Y, Tobe K, Azuma Y *et al.* Insulin receptor substrate-1 in osteoblast is indispensable for maintaining bone turnover. *J Clin Inves* 2000;**105**:935–943.
5. Baker J, Liu JP, Robertson EJ, Efstratiadis A. Role of insulin-like growth factors in embryonic and postnatal growth. *Cell* 1993;**75**:73–82.
6. Ling Y, Maile LA, Lieskovska J, Badley-Clarke J, Clemmons DR. Role of SHPS-1 in the regulation of insulin-like growth factor I–stimulated Shc and mitogen-activated protein kinase activation in vascular smooth muscle cells. *Mol Biol Cell* 2005;**16**:3353–3364.
7. Lieskovska J, Ling Y, Badley-Clarke J, Clemmons DR. The role of src kinase in insulin-like growth factor-dependent mitogenic signaling in vascular smooth muscle cells. *J Biol Chem* 2006;**281**:25041–25053.
8. Ornitz DM, Marie PJ. FGF signaling pathways in endochondral and intramembranous bone development and human genetic disease. *Genes Dev* 2002;**16**:1446–1465.
9. Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PRJ, Reese CB *et al.* Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Bα. *Curr Biol* 1997;**7**:261–269.
10. Burgering BMT, Coffer PJ. Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. *Nature* 1995;**376**:599–602.
11. Hresko RC, Mueckler M. mTOR · RICTOR is the Ser473 kinase for Akt/protein kinase B in 3T3-L1 adipocytes. *J Biol Chem* 2005;**280**:40406–40416.
12. Peng X-d XuP-Z, Chen M-L, Hahn-Windgassen A, Skeen J, Jacobs J, Crawford SE *et al.* Dwarfism, impaired skin development, skeletal muscle atrophy, delayed bone development, and impeded adipogenesis in mice lacking Akt1 and Akt2. *Genes Dev* 2003;**17**:1352–1365.
13. Ambrogini E, Almeida M, Martin-Millan M, Paik JH, Depinho RA, Han L *et al.* FoxO-mediated defense against oxidative stress in osteoblasts is indispensable for skeletal homeostasis in mice. *Cell Metab* 2010;**11**:136–146.
14. Kousteni S. FoxO1, the transcriptional chief of staff of energy metabolism. *Bone* 2012;**50**: 437–443.
15. Laplante M, Sabatini DM. mTOR signaling at a glance. *J Cell Sci* 2009;**122**:3589–3594.
16. Laplante M, Sabatini DM. Regulation of mTORC1 and its impact on gene expression at a glance. *J Cell Sci* 2013;**126**(Pt 8):1713–1719.
17. Doble BW, Woodgett JR. GSK-3: tricks of the trade for a multi-tasking kinase. *J Cell Sci* 2003;**116**:1175–1186.
18. Williams BO, Insogna KL. Where Wnts went: the exploding field of Lrp5 and Lrp6 signaling in bone. *J Bone Mineral Res* 2009;**24**:171–178.
19. Wang J, Zhou J, Bondy CA. Igf1 promotes longitudinal bone growth by insulin-like actions augmenting chondrocyte hypertrophy. *FASEB J* 1999;**13**:1985–1990.
20. Gillespie JR, Ulici V, Dupuis H, Higgs A, Dimattia A, Patel S *et al.* Deletion of glycogen synthase kinase-3beta in cartilage results in up-regulation of glycogen synthase kinase-3alpha protein expression. *Endocrinology* 2011;**152**:1755–1766.
21. Itoh S, Saito T, Hirata M, Ushita M, Ikeda T, Woodgett JR *et al.* GSK-3α and GSK-3β proteins are involved in early stages of chondrocyte differentiation with functional redundancy through RelA protein phosphorylation. *J Biol Chem* 2012;**287**:29227–29236.
22. Thorens B, Mueckler M. Glucose transporters in the 21st Century. *Am J Physiol Endocrinol Metab* 2010;**298**:E141–E145.
23. Thomas DM, Maher F, Rogers SD, Best JD. Expression and regulation by insulin of GLUT 3 in UMR 106-01, a clonal rat osteosarcoma cell line. *Biochem Biophys Res Commun* 1996;**218**:789–793.
24. Zoidis E, Ghirlanda-Keller C, Schmid C. Stimulation of glucose transport in osteoblastic cells by parathyroid hormone and insulin-like growth factor I. *Mol Cell Biochem* 2011;**348**:33–42.
25. Maor G, Karnieli E. The insulin-sensitive glucose transporter (GLUT4) is involved in early bone growth in control and diabetic mice, but is regulated through the insulin-like growth factor I receptor. *Endocrinology* 1999;**140**:1841–1851.
26. Karsenty G, Kronenberg HM, Settembre C. Genetic control of bone formation. *Annu Rev Cell Dev Biol* 2009;**25**:629–648.
27. Kronenberg HM. The role of the perichondrium in fetal bone development. *Ann New York Acad Sci* 2007;**1116**:59–64.
28. Schipani E, Maes C, Carmeliet G, Semenza GL. Regulation of osteogenesis-angiogenesis coupling by HIFs and VEGF. *J Bone Mineral Res* 2009;**24**:1347–1353.
29. Maes C, Kobayashi T, Selig MK, Torrekens S, Roth SI, Mackem S *et al.* Osteoblast precursors, but not mature osteoblasts, move into developing and fractured bones along with invading blood vessels. *Dev Cell* 2010;**19**:329–344.
30. Guntur AR, Rosen CJ. The skeleton: a multi-functional complex organ: new insights into osteoblasts and their role in bone formation: the central role of PI3Kinase. *J Endocrinol* 2011;**211**:123–130.
31. Yakar S, Courtland H-W, Clemmons D. IGF-1 and bone: new discoveries from mouse models. *J Bone Mineral Res* 2010;**25**:2543–2552.
32. Govoni KE, Lee SK, Chung Y-S, Behringer RR, Wergedal JE, Baylink DJ *et al.* Disruption of insulin-like growth factor-I expression in type IIαI collagen-expressing cells reduces bone length and width in mice. *Physiol Genomics* 2007;**30**:354–362.

33. Wang Y, Cheng Z, ElAlieh HZ, Nakamura E, Nguyen M-T, Mackem S *et al.* IGF-1R signaling in chondrocytes modulates growth plate development by interacting with the PTHrP/Ihh pathway. *J Bone Mineral Res* 2011;**26**:1437–1446.
34. Cooper KL, Oh S, Sung Y, Dasari RR, Kirschner MW, Tabin CJ. Multiple phases of chondrocyte enlargement underlie differences in skeletal proportions. *Nature* 2013;**495**: 375–378.
35. Bradley EW, Carpio LR, Westendorf JJ. Histone deacetylase 3 suppression increases PH domain and leucine-rich repeat phosphatase (Phlpp)1 expression in chondrocytes to suppress Akt signaling and matrix secretion. *J Biol Chem* 2013;**288**:9572–9582.
36. Hardouin SN, Guo R, Romeo P-H, Nagy A, Aubin JE. Impaired mesenchymal stem cell differentiation and osteoclastogenesis in mice deficient for Igf2-P2 transcripts. *Development* 2011;**138**:203–213.
37. Courtland H-W, Elis S, Wu Y, Sun H, Rosen CJ, Jepsen KJ *et al.* Serum IGF-1 affects skeletal acquisition in a temporal and compartment-specific manner. *PLoS One* 2011;**6**:e14762.
38. Govoni KE, Wergedal JE, Florin L, Angel P, Baylink DJ, Mohan S. Conditional deletion of insulin-like growth factor-I in collagen type 1alpha2-expressing cells results in postnatal lethality and a dramatic reduction in bone accretion. *Endocrinology* 2007;**148**:5706–5715.
39. Jiang J, Lichtler AC, Gronowicz GA, Adams DJ, Clark SH, Rosen CJ *et al.* Transgenic mice with osteoblast-targeted insulin-like growth factor-I show increased bone remodeling. *Bone* 2006;**39**:494–504.
40. Xian L, Wu X, Pang L, Lou M, Rosen CJ, Qiu T *et al.* Matrix IGF-1 maintains bone mass by activation of mTOR in mesenchymal stem cells. *Nat Med* 2012;**18**:1095–1101.
41. Fulzele K, DiGirolamo DJ, Liu Z, Xu J, Messina JL, Clemens TL. Disruption of the insulin-like growth factor type 1 receptor in osteoblasts enhances insulin signaling and action. *J Biol Chem* 2007;**282**:25649–25658.
42. Bikle DD, Sakata T, Leary C, Elalieh H, Ginzinger D, Rosen CJ *et al.* Insulin-like growth factor I is required for the anabolic actions of parathyroid hormone on mouse bone. *J Bone Mineral Res* 2002;**17**:1570–1578.
43. Yamaguchi M, Ogata N, Shinoda Y, Akune T, Kamekura S, Terauchi Y *et al.* Insulin receptor substrate-1 is required for bone anabolic function of parathyroid hormone in mice. *Endocrinology* 2005;**146**:2620–2628.
44. Karlsson C, Thornemo M, Henriksson HB, Lindahl A. Identification of a stem cell niche in the zone of Ranvier within the knee joint. *J Anat* 2009;**215**:355–363.
45. Yang W, Wang J, Moore DC, Liang H, Dooner M, Wu Q *et al.* Ptpn11 deletion in a novel progenitor causes metachondromatosis by inducing hedgehog signalling. *Nature* 2013;**499**:491–495.
46. Sheng MHC, Zhou X-D, Bonewald LF, Baylink DJ Lau KHW. Disruption of the insulin-like growth factor-1 gene in osteocytes impairs developmental bone growth in mice. *Bone* 2013;**52**:133–144.
47. Wang Y, Nishida S, Elalieh HZ, Long RK, Halloran BP, Bikle DD. Role of IGF-I signaling in regulating osteoclastogenesis. *J Bone Mineral Res* 2006;**21**:1350–1358.
48. Esen E, Chen J, Karner CM, Okunade AL, Patterson BW, Long F. WNT-LRP5 signaling induces Warburg effect through mTORC2 activation during osteoblast differentiation. *Cell metabolism* 2013;**17**:745–755.
49. Sun H, Kim JK, Mortensen RM, Mutyaba PL, Hankenson KD, Krebsbach PH. Osteoblast-targeted suppression of PPARγ increases osteogenesis through activation of Mtor signaling. *Stem Cells* 2013, N/A-N/A doi:10.1002/stem.1455.
50. Ferron M, Wei J, Yoshizawa T, Del Fattore A, DePinho RA, Teti A *et al.* Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. *Cell* 2010;**142**:296–308.
51. Fulzele K, Riddle RC, DiGirolamo DJ, Cao X, Wan C, Chen D *et al.* Insulin receptor signaling in osteoblasts regulates postnatal bone acquisition and body composition. *Cell* 2010;**142**: 309–319.
52. Yang S, Xu H, Yu S, Cao H, Fan J, Ge C *et al.* Foxo1 mediates insulin-like growth factor 1 (IGF1)/insulin regulation of osteocalcin expression by antagonizing Runx2 in osteoblasts. *J Biol Chem* 2011;**286**:19149–19158.
53. Kode A, Mosialou I, Silva BC, Joshi S, Ferron M, Rached MT *et al.* FoxO1 protein cooperates with ATF4 protein in osteoblasts to control glucose homeostasis. *J Biol Chem* 2012;**287**: 8757–8768.
54. Shim J-H, Greenblatt MB, Singh A, Brady N, Hu D, Drapp R *et al.* Administration of BMP2/7 *in utero* partially reverses Rubinstein-Taybi syndrome–like skeletal defects induced by Pdk1 or Cbp mutations in mice. *J Clin Inves* 2012;**122**:91–106.
55. Kawai M, Delany AM, Green CB, Adamo ML, Rosen CJ. Nocturnin Suppresses Igf1 expression in bone by targeting the 3′ untranslated region of Igf1 mRNA. *Endocrinology* 2010;**151**: 4861–4870.
56. Stamper BD, Mecham B, Park SS, Wilkerson H, Farin FM, Beyer RP *et al.* Transcriptome correlation analysis identifies two unique craniosynostosis subtypes associated with IRS1 activation. *Physiol Genomics* 2012;**44**:1154–1163.
57. Komarova SV, Ataullakhanov FI, Globus RK. Bioenergetics and mitochondrial transmembrane potential during differentiation of cultured osteoblasts. *Am J Physiol Cell Physiol* 2000;**279**:C1220–C1229.
58. Shyh-Chang N, Daley GQ, Cantley LC. Stem cell metabolism in tissue development and aging. *Development* 2013;**140**:2535–2547.