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SPECIAL FEATURE

Integrins, insulin like growth factors, and the skeletal response to load

D. D. Bikle

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Abstract Bone loss during skeletal unloading, whether due to neurotrauma resulting in paralysis or prolonged immobilization due to a variety of medical illnesses, accelerates bone loss. In this review the evidence that skeletal unloading leads to bone loss, at least in part, due to disrupted insulin like growth factor (IGF) signaling, resulting in reduced osteoblast proliferation and differentiation, will be examined. The mechanism underlying this disruption in IGF signaling appears to involve integrins, the expression of which is reduced during skeletal unloading. Integrins play an important, albeit not well defined, role in facilitating signaling not only by IGF but also by other growth factors. However, the interaction between selected integrins such as $\alpha v\beta 3$ and $\beta 1$ integrins and the IGF receptor are of especial importance with respect to the ability of bone to respond to mechanical load. Disruption of this interaction blocks IGF signaling and results in bone loss.

Keywords Bone · IGF · Integrin · Mechanical load · Osteoblast · Osteoclast

The skeletal response to mechanical load is critical for maintenance of skeletal integrity. This review will assess the interacting roles that insulin like growth factor I (IGF-I) signaling and selected integrins play in this response. Skeletal unloading results in decreased integrin expression, resistance to the anabolic actions of IGF-I, and bone loss.

D. D. Bikle (✉)
Medicine and Dermatology,
University of California San Francisco,
San Francisco, CA, USA
e-mail: Daniel.bikle@ucsf.edu

D. D. Bikle
Special Diagnostic and Treatment Center,
Veterans Affairs Medical Center,
San Francisco, CA, USA

Abbreviations

BMSC	Bone marrow stromal cells
FAK	Focal adhesion kinase
grb2	Growth receptor binding protein-2
GEF	Guanine nucleotide exchange factor
IGF-R	Insulin like growth factor (IGF) and its receptor
IRS	Insulin receptor substrate
MAPK	Mitogen activated protein kinase
NO	Nitric oxide
PI3K	Phosphatidyl inositol 3 kinase
PIP_2 and PIP_3	Phosphatidyl inositol bis- and tris-phosphate
PDK	Phosphoinositide dependent kinase
PTB	Phosphotyrosine binding protein
Pyk	Phosphotyrosine kinase
PGE_2	Prostaglandin E_2
PKB/Akt	Protein kinase B
SOS	Son of sevenless
SH2	src homology 2
SHPS	SH2 domain containing protein tyrosine phosphatase (SHP) and its substrate

Impact of mechanical load on bone Mechanical loading has a profound influence on bone modeling and remodeling [1]. Bone formation occurs in those regions undergoing increased stress. For example when the ulna undergoes cyclic compression along its axis, because of its natural curvature, the medial surface is put under the greatest stress. This is the site of greatest bone formation following this procedure [2]. Similar results are found in other models of mechanical load. Both frequency and strain rate of the loading stimulus are important [3, 4]. The osteocyte is generally thought to be the major cell type in bone

responding to mechanical load. These cells form a syncytium in bone, with cellular processes connecting each other and to the lining cells and osteoblasts on the surface of bone via gap junctions. The osteocyte lacunae may serve as stress risers to amplify the overall strain stimulus in the vicinity of the osteocyte enhancing the fluid shear stress thought to be key for mechanotransduction [5]. This initial signal involves calcium influx through stretch activated and L type channels [6, 7], ATP release which acts through its purinergic receptors to further increase intracellular calcium concentrations, prostaglandin E_2 (PGE_2) production [8, 9], and nitric oxide (NO) release [10]. These cellular responses are found in vitro in studies of both osteocytes and osteoblasts, so osteoblasts as well as osteocytes may respond directly to stress in vivo. Of particular relevance for this review is that IGF-I and integrin expression are also increased by mechanical load in osteocytes and osteoblasts in vivo and in vitro [11, 12], and the IGF-I receptor can be activated by fluid sheer stress in osteoblasts in vitro [13]. As will be emphasized in this review, the combined signaling from selected integrins and IGF-I are critical for the skeletal response to mechanical load.

Skeletal unloading, on the other hand, as occurs during prolonged bed rest and immobilization resulting from spinal injuries, amputations, fractures, and arthritic conditions, leads to bone loss. The most extreme example is the microgravity environment of space flight, which results in the near cessation of bone formation, as evidenced by the appearance of an extensive arrest line in the periosteum of cortical bone in both the tibia and humerus [14, 15]. Skeletal unloading leads to a decrease in osteoblast number and activity [16–23], likely due to a decrease in proliferation of osteoprogenitor cells [24]. The hindlimb elevation (tail suspension) model was developed to simulate skeletal unloading without the trauma associated with other manipulations such as nerve resection. This procedure is well tolerated by the animals with minimal evidence of stress as indicated by continued weight gain [25] and normal levels and circadian rhythms of corticosterone [26]. Skeletal unloading by hindlimb elevation results in decreased bone formation, mineralization, and maturation [25, 27–31], decreased osteoblast numbers [32], reduced serum and skeletal osteocalcin levels [33], lowered ash content of bone [25, 27, 28], and decreased bone strength [28, 34]. When bone marrow stromal cells (BMSC) from the bones of the unloaded limbs are cultured in vitro, there are fewer osteoprogenitors, and they proliferate more slowly [35], indicating that skeletal unloading causes a persistent change in cell function that can be assessed in vitro. In contrast to the unloaded bones of the hindlimbs, no significant change in bone mass or bone formation is observed in the humeri, mandible, and cervical vertebrae during hindlimb elevation [25]. The lack of effect of hindlimb elevation on these normally loaded bones indicates that local factors rather than systemic factors dominate the response of bone to skeletal unloading. To seek a local factor that could explain these changes, we first examined the role of insulin like growth factors (IGF).

IGF signaling pathways IGF-I and its homologous family member IGF-II are both made by bone cells. IGF-I is the dominant IGF in postnatal murine bone, whereas IGF-II dominates in human bone. Although IGF-II has its own receptor (IGF-IIR or mannose-6 phosphate receptor), the IGF-I receptor (IGF-IR) is the major means by which these growth factors regulate cell growth and differentiation in that the IGF-IIR has no known signaling function. Since much of the data I will discuss have been generated in rats and mice, I will discuss primarily the results with IGF-I and its receptor. The IGF-I receptor is comprised of two alpha and two beta subunits (Fig. 1) (review in [36]). IGF-I binding to the receptor results in activation of its intrinsic tyrosine kinase. The kinase domain resides within amino acids 956–1256; activation entails the sequential tyrosine phosphorylation of residues Y1135, Y1131, and Y1136 which alters the structure of the β chain enabling its kinase activity to be expressed [37]. Mutation of these tyrosines to phenylalanine impairs the ability of IGF-IR to complex with other signaling molecules, including specific integrins [38]. These and subsequent phosphorylations create multiple docking sites for a variety of endogenous substrates including members of the insulin receptor substrate (IRS) family which associate with IGF-IR via phosphotyrosine binding (PTB) and src homology 2 (SH2) domains, growth receptor binding protein-2 (Grb2), which binds to specific motifs in the IGF-I receptor as well as in IRS, and the p85 subunit of phosphatidyl inositol 3 kinase (PI3K), which binds to other specific motifs within IRS. Src homology collagen (Shc), when tyrosine phosphorylated in response to IGF-I, binds to the SH2 domain of Grb2, which in turn forms a complex with son of sevenless (Sos), a guanine nucleotide exchange factor (GEF) that mediates GDP/GTP exchange in ras and thus activates it. Ras then activates Raf (MAPKKK), which phosphorylates and activates MEK (MAPKK), which in turn phosphorylates and activates extracellular signal-regulated kinase (ERK1/2 or MAPK). These are serine/threonine phosphorylations. Activated ERK enters the nucleus to phosphorylate and so activate transcription factors (e.g., elk-1 and c-jun) leading to increased cyclin D_1 and reduced $p21^{cip}$ and $p27^{kip}$ expression. The increased levels of cyclin D_1 and reduced levels of the cell cycle inhibitors $p21^{cip}$ and $p27^{kip}$ stimulate cell cycle progression from G1 to S, thus completing the pathway by which IGF-I and other growth factors promote proliferation. Activation of PI3K sets up a different pathway. PI3K phosphorylates phosphatidyl inositol

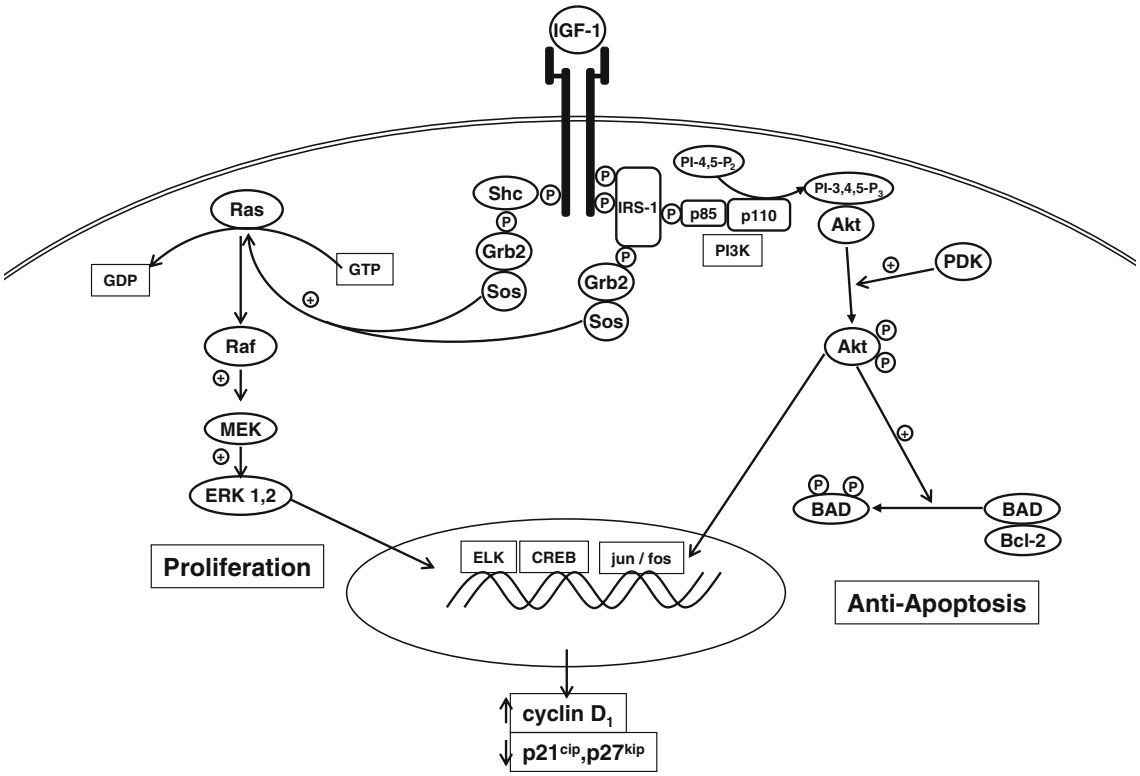

Fig. 1 IGF signaling pathway. IGF I or II binds to the IGF-I receptor, a heterotetramer comprised of two alpha and two beta subunits. Upon binding of IGF, the receptor undergoes autophosphorylation of critical tyrosine residues, and two major pathways are activated. The first pathway leads to activation of Ras and the MAPK pathway eventuating in the activation of ERK1/2, which can enter the nucleus to induce genes important for proliferation. The second pathway leads to the activation of PI3K resulting in activation of Akt, which exerts anti-apoptotic actions by phosphorylating and so inactivating Bad, a proapoptotic regulator of Bcl-2

bisphosphate (PIP_2) to phosphatidyl inositol trisphosphate (PIP_3) in the membrane, recruiting protein kinase B (PKB or Akt) to the membrane where it is phosphorylated and activated by phosphoinositide dependent kinase 1/2 (PDK1/2). The activated Akt then phosphorylates and inactivates Bad, a proapoptotic member of the bcl-2 family. This pathway blocks apoptosis. However, PI3K and Akt can enter the nucleus and by phosphorylating critical transcription factors also lead to increased cyclin D1 levels.

IGF-I signaling in bone We have focused on IGF-I because it is abundantly produced by murine bone [39], is a well studied regulator of osteoblast proliferation and differentiation [40], and when deleted by genetic engineering results in animals with retarded bone growth [41, 42] and bone formation [43]. These abnormalities are readily reversed with exogenous IGF-I [43]. Overexpression of IGF-I in bone under the osteocalcin [44] or collagen 1 [45] promoter increases bone formation. Deletion of the IGF-I receptor (IGF-IR) from mature osteoblasts results in mice with poorly mineralized bone [46]. Although the number of colony forming units in BMSC cultures from such mice is normal, the colonies fail to mineralize [47], indicating an important role for IGF-I signaling in osteoblast differentiation as well as proliferation. Similarly, IGF-1 is required for normal osteoclastogenesis in that the IGF-I null mouse has increased trabecular bone, fewer and smaller osteoclasts, and defective bone resorption [48]. On the other hand overexpression of IGF-I in osteoblasts increases osteoclast number in vivo [45], and addition of IGF-I to co-cultures of osteoblasts and osteoclast precursors promotes osteoclast formation in vitro [48]. These data indicate that IGF-I signaling in both osteoblasts and osteoclasts is required for all aspects of bone remodeling.

IGF signaling in mechanotransduction As previously mentioned, IGF-I production is increased by mechanical loading, and fluid flow is synergistic with IGF-I in the activation of the IGF-IR [13]. On the other hand, skeletal unloading results in resistance to IGF-I with respect to its anabolic actions [39, 49–52]. When IGF-I is infused into unloaded growing rats, their unloaded bones (tibiae) do not increase in size as assessed by changes in fat-free weight as do the bones of normally loaded animals [39]. Furthermore, IGF-I fails to stimulate bone formation (BFR) in the unloaded bones (tibiae), although stimulation of BFR in the humerus is equivalent to that seen in the normally loaded rats [51, 52]. Proliferation of osteoblasts in vivo is

depressed whereas apoptosis is increased in the unloaded bones, and neither respond to IGF-I infusion, unlike the situation in normally loaded bones [52]. BMSC from unloaded bones have normal levels of the IGF-I receptor and normal binding of IGF-I to this receptor, but fail to respond to IGF-I with receptor activation as assessed by phosphorylation [52, 53]. The downstream pathways are likewise impacted in that ras is not activated, and ERK1/2 are not phosphorylated in response to IGF-I in BMSC from unloaded bones, in contrast to that from normally loaded animals, and IGF-I stimulated phosphorylation of Akt is reduced [52]. These results indicate that the resistance to IGF-I in unloaded bone is primarily due to a failure of IGF-I to activate its own receptor. This is not a problem with binding of IGF-I to the receptor or receptor levels, which are not altered by skeletal unloading [52]. When the animals are reloaded after a period of unloading, they show an accentuated response to IGF-I resulting in bone formation rates above the normally loaded controls [49]. The resistance to IGF-I caused by skeletal unloading persists when the bone cells (BMSC) are studied in vitro. BMSC from the tibiae of hindlimb-elevated rats form fewer colony forming units in vitro and fail to respond to IGF-I administration with an increase in proliferation [52, 53]. Thus, mechanical loading (or unloading) of bone profoundly alters IGF signaling. The question is why. Based on our own work and that of others, we hypothesize that the key to understanding the impact of mechanical load on the skeletal response to IGF-I lies in integrin activation.

Integrin signaling Integrins are comprised of an alpha and beta subunit [54–56]. There are at least 18 α and 8 β genes so far identified in the human genome, several of which produce multiple transcripts by alternative splicing. These combine to form 24 different functional integrins in mammals. Each subunit can have multiple partners (Fig. 2). Seven of the human α subunits contain an I (interactive or inserted) domain (α1,α2,α10,α11,αL,αM, αX,αD, and αE) between the β propeller repeats 2 and 3 in the extracellular portion of the molecule which is involved in ligand binding and intercellular adhesion. Non-I domain containing α subunits include α3, α4, α5, α6, α7, α8, α9, αυ, and αIIb. All human integrin β subunits contain an I-like domain along with 4 EGF-like repeats in their extracellular domains. The RGD-binding site is located at the interface between the β−propeller domain of the α subunit and the I-like domain of the β subunit (Fig. 3). Different integrins bind preferentially to different matrix proteins, although both the integrins and the matrix proteins are promiscuous. Thus vitronectin binds αυβ5 or 3, osteopontin binds αυβ3, collagen binds α1 or 2β1, and fibronectin binds α3,5, or 8β1 and αυβ3, although the strength of binding varies. Limited crystallographic data

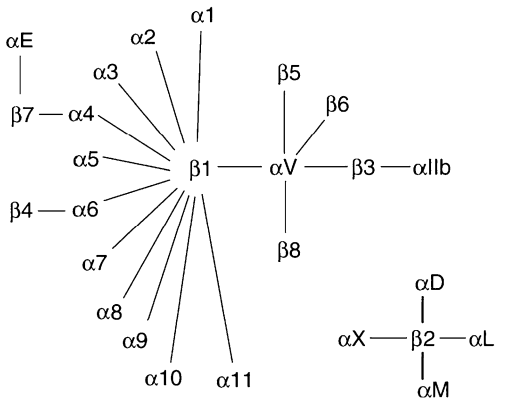

Fig. 2 The members of the human integrin superfamily and how they combine to form heterodimeric integrins. At least 18 α subunits and 8 β subunits have been identified in humans, which combine to generate 24 different integrins. Integrin subunits that can bind to each other to form a heterodimer are connected by solid lines

indicate that ligand binding induces major conformational changes in the integrin subunits. In general the cytoplasmic tails of the integrin subunits are short (less than 75 amino acids, β4 is an exception). The α subunits show little homology in their cytoplasmic tails except for a GFFKR motif near the transmembrane domain important for association with the β subunit. The tails of the β subunits are more homologous. Most β subunit tails have NPxY/F motifs as part of PTB (phosphotyrosine-binding) domains. The cytoplasmic tails of the integrin subunits recruit a number of proteins, the profile of which varies from integrin to integrin. β1and β3 bind talin, β1 binds α actinin, β1,2,7 bind filamin, α4 binds paxillin, and a number of integrins bind focal adhesion kinase (FAK). Such proteins link the integrins to the cytoskeleton, which is important for many of the functions of the integrins. In addition a select number of integrins such as αυβ3 bind to caveolin-1 in the membrane. Some integrins are found in only a limited number of cell types or tissues such as αIIbβ3 in platelets or α6β4 in keratinocytes, but other integrins such as αυβ3 are widely distributed. Thus it is not surprising that gene deletions of the different integrins produce many different phenotypes, a number of which are embryonic lethal [57].

Binding of integrins to their extracellular matrix proteins leads to clustering of the integrins with reorganization of the actin cytoskeleton and translocation of a number of proteins including signaling proteins to the focal adhesion plaque [58]. Focal adhesion kinase (FAK) or its homolog phosphotyrosine kinase 2 (Pyk2) plays an important role, acting as both a scaffolding protein to bring a number of proteins to the focal adhesion plaque including growth factor receptors, PI3K, Shc, Grb2 and src family kinases [59]. The src kinases phosphorylate tyrosines on FAK to provide docking sites for these proteins. Grb2 and Shc can

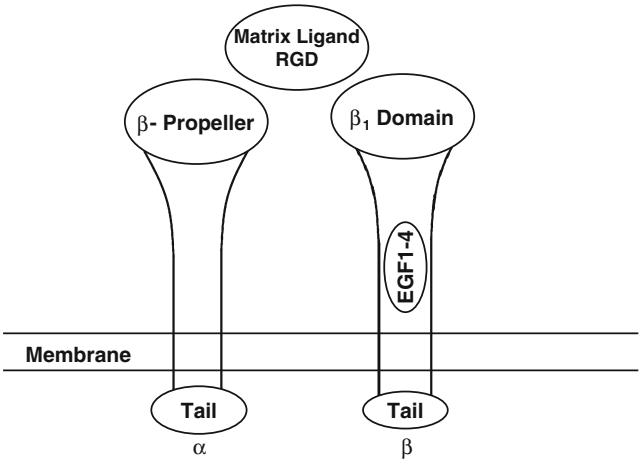

Fig. 3 A model of an integrin lacking the I domain in the α subunit (the typical integrin involved with IGF signaling in bone). The β-propeller domain of the α subunit and the β1 domain of the β subunit form the binding domain for the extracellular ligand, in this case a matrix RGD containing protein such as osteopontin, vitronectin, or fibronectin. Binding of the ligand causes a conformational change in the integrin subunits resulting in separation of the intracellular portions of the tails, enabling binding to a number of proteins to those tails such as FAK and talin that mediate integrin signaling

be activated by integrins through FAK, and by binding Sos can then activate the ras/MAPK pathway. Activation of this pathway can also take place by a FAK independent mechanism in which the activated integrin binds to caveolin-1, which serves as the scaffolding protein to bring the src family kinases and growth factor receptors to the complex. As for the src family kinases recruited to FAK, the src kinases recruited to the caveolin-1 complex may activate the ras/MAPK pathway by recruiting and activating Shc/Grb2/Sos. This pathway appears to be limited to certain integrins (e.g., α1β1, α5β1, αvβ3), and these integrins appear to be the ones most supportive of growth factor stimulation of proliferation [60, 61].

Integrins in bone Osteoblasts and BMSC express a number of integrins, including αv, 1, 2, 3, 5, 6, 8 and β1, 3, 5 [62–66]. BMP-2 increases a number of these integrins, increasing the adhesion of osteoblasts to vitronectin and osteopontin [67]. Blocking antibodies to αvβ prevent BMP-2 from stimulating osteoblasts differentiation [67]. Immunoprecipitation methods and clustering analysis following adhesion to different substrates indicate that favored αβ combinations include αv, 1, 2, 3, 5β1, αvβ1, 3, 5. Fibronectin, a ligand for several integrins including α4β1, α5β1, α8β1, αvβ1, and αvβ3 promotes bone cell adhesion and stimulation of osteoblast proliferation [68–70]. Addition of antibodies to fibronectin or soluble fibronectin fragments to the culture of differentiated osteoblasts results in apoptosis [70]. Within 60min of plating the osteoblasts on fibronectin or collagen, phosphorylation of FAK and ERK is observed as is a rise in the mRNA levels of fos and c-jun [71]. The Damsky laboratory [72] created a transgenic model overexpressing a dominant negative form of β1 under the osteocalcin promoter such that it was expressed only in mature osteoblasts. They found decreased bone mass and increased cortical porosity in the long bones and thinner flat bones of the skull, decreased bone formation rates in cortical bone, and failure of terminal differentiation of osteoblasts in vitro suggesting an important role for β1 in osteoblast function. The β2 null mouse also shows a reduction in trabecular bone with normal osteoclast numbers [73]. Bone marrow stromal cells from these mice show reduced osteogenic potential in vivo, and decreased mineralization in vitro. Overexpression of this integrin leads to increased bone formation [73].

The major integrin in the mature osteoclast is αvβ3, although osteoclast precursors express αvβ5 [74]. αvβ3 is critical for osteoclast function, but not for their formation. In particular, αvβ3 is required for the formation of the sealing ring necessary for the ability of osteoclasts to resorb bone. Mice null for β3 grow normally, but have increased bone mass and poorly functioning albeit abundant osteoclasts. In contrast, mice null for β5 show increased osteoclastogenesis especially after ovariectomy [75], suggesting a reciprocal interaction with β3. In the osteoclast when αvβ3 is activated, c-src binds to the β-subunit tail, recruits and activates another non-receptor tyrosine kinase syk, which mediates the activation of Vav3, a guanine nucleotide exchange factor for RhoA required for the formation of the sealing ring [76, 77]. The role of αvβ3 in regulating osteoclast function has been exploited clinically. An αvβ3 antagonist, L-000845704, was found to decrease bone turnover markers (resorption markers faster than formation markers) and increase lumbar spine BMD in post-menopausal osteoporotic women [78]. Whether such a drug would be useful to prevent or reverse bone loss accompanying skeletal unloading remains to be tested.

Integrins as sensors for mechanical load in bone Integrins form an important link between the extracellular matrix and the cytoskeleton, and thus are in a position to transduce mechanical signals imposed on bone to responses by the bone cells. During skeletal unloading by hindlimb elevation we found a reduction in integrin expression [52]. This result has recently been confirmed by others [79]. Reloading these animals restores integrin expression (Long et al. unpublished). Fluid shear stress or mechanical deformation of bone cells increases and/or activates selected integrins [80, 81], whereas culturing mesenchymal stem cells (presumed osteoblast precursors) in a rotary cell culture system decreases downstream integrin signaling (decreased FAK and pyk2 phosphorylation and RhoA activation) [82,

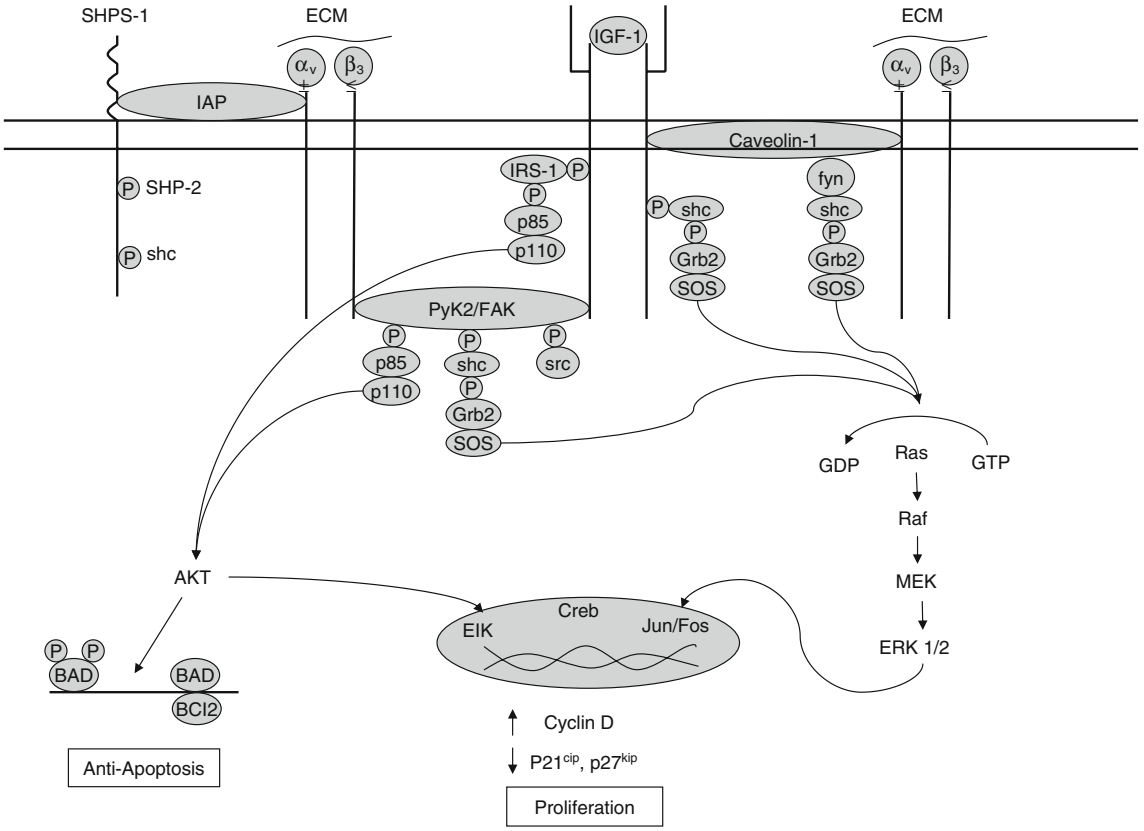

Fig. 4 Model for IGF/integrin signaling interactions in regulating the skeletal response to load. The IGF-IR forms a complex with αυβ3 integrin that is required for IGF-I activation of the IGF-IR. This complex may be formed via the scaffolding function of FAK/Pyk2 or that of caveolin-1. SHPS-1 may play a role by regulating access of the phosphatase SHP-2 to this complex. Mechanical load increases whereas unloading decreases formation of this complex and so regulates IGF-I responsiveness. Formation of the integrin/IGF-IR complex brings to the IGF-IR non-receptor kinases such as FAK and src family kinases which may activate the IGF-IR independently and/or synergistically with IGF-I. The source of IGF-I may originate from osteocytes and osteoblasts. Production of IGF-I as well as expression and activation of integrins in these cells are stimulated by mechanical load

83]. As mentioned previously, osteocytes are thought to be central to the ability of bone to sense mechanical load. These cells show attachments between their canalicular processes and the lining of the canalicular wall, attachments which contain αυβ3 [84], 3 is involved in both the osteoblastic and osteoclastic response to mechanical load. As mentioned above mice overexpressing a dominant negative β1 integrin subunit have been developed [72]. Although these mice showed a reduction in trabecular bone and loss of bone strength, their response to skeletal unloading (hindlimb elevation) did not differ significantly from wildtype controls [89]. However, they were not tested for their response to mechanical loading.

Role of integrins in regulating IGF-I responsiveness in bone As previously noted, activation of integrins by their extracellular ligands leads to clustering into focal adhesion plaques. Receptors for growth factors, including IGF-IR, may be included in these clusters [90]. Although this section is focused on IGF-IR/integrin interactions in osteoblasts, the c-fms (m-CSF receptor) and/or IGF-IR may have similar interactions with integrins in the osteoclast. The importance of IGF-IR/integrin interactions with respect to IGF signaling is supported by observations that the matrix on which bone cells are plated makes a difference with respect to the strength of IGF-I signaling. Vitronectin and fibronectin increase the binding of the β3 integrin subunit to IGF-IR and enhance the ability of IGF-I to activate its receptor.

The role of integrins in regulating IGF-I responsiveness has been demonstrated in a number of tissues. Perhaps the most extensive examination emanates from the Clemmons laboratory which has studied this phenomenon in aortic smooth muscle cells [91–93]. The laboratory found that echistatin (a disintegrin) or blocking antibodies to the integrin αυβ3 blocked IGF-I stimulated proliferation, IGF-I receptor autophosphorylation, IRS-1 phosphorylation, and binding of the p85 subunit of PI3K to IRS-1. Their proposed mechanism is that integrin activation recruits the src homology 2 domain containing protein

tyrosine phosphatase-2 (SHP-2) to the β3 integrin subunit. IGF-I in turn via the IGF-IR phosphorylates and so activates the transmembrane protein src homology domain containing protein tyrosine phosphatase substrate-1 (SHPS-1), which recruits SHP-2 to SHPS-1 for an as yet undefined role in further signaling. When αυβ3 activation is blocked, SHP-2 instead is recruited to the IGF-IR, where it dephosphorylates and so terminates the activation of the IGF-IR. Our results in bone cells indicate a different mechanism, however. We [52] have shown that although echistatin blocks IGF-I activation of the IGF-IR, neither skeletal unloading nor echistatin alters the recruitment of SHP-2 to the IGF-IR nor the timing of IGF-IR phosphorylation and dephosphorylation. Instead, the IGF-IR is just not phosphorylated in response to IGF-I in BMSC from unloaded bone or normal cells treated with echistatin. IGF-I responsiveness in bone cells may require direct binding of αυβ3 to the IGF-IR [94]. Indeed, IGF-I stimulates the phosphorylation of the β3 integrin subunit, and so activates it [94].

The matrix protein vitronectin is a potent stimulator of β3 integrin subunit phosphorylation, and potentiates the ability of IGF-I to do likewise [93, 95]. Mutation of the tyrosines involved (aa 773, 785) in the β3 integrin subunit blocks IGF-I signaling [93, 95]. Knocking down either the β1 or β3 integrin subunit in osteoblasts blocks IGF-I signaling, and the combined knockdown is especially inhibitory. Activation of the β3 integrin subunit may be the mechanism by which IGF-I and mechanical loading also activate (by phosphorylation) FAK and Pyk2 [96]. Skeletal unloading results in decreased expression of αυβ3 [52] as well as several other integrins including the β1 subunit, and decreased activation of FAK.

Our hypothesis is that mechanical load stimulates formation of an IGF-IR/integrin complex in osteoblasts. This process is maximized by activating the integrin with its matrix substrate (e.g., vitronectin) and the IGF-IR with IGF-I. We further hypothesize that the IGF-IR/integrin complex recruits non-receptor tyrosine kinases to the complex which are required for IGF-IR phosphorylation/activation in response to IGF-I. Skeletal unloading disrupts this mechanism by reducing integrin levels. Reloading restores integrin expression and enhances the formation of the integrin/IGF-IR complex accentuating the IGF-I responsiveness. Recent results from Kapur et al. [13] as well as our own laboratory support this hypothesis by demonstrating that fluid shear stress on bone cells stimulates IGF-IR phosphorylation independent of IGF-I, that this response is potentiated by fibronectin and vitronectin, and that the response is blocked by echistatin and knockdown of β1 and β3 integrin subunits. This model is shown in Fig. 4. This model need not exclude contributions from other mechanisms such as wnt signaling.

Clinical implications Disuse osteoporosis is a major health problem contributing to substantial morbidity through increased risk of fractures especially in the elderly. The bone lost during extended bed rest or immobilization is generally not regained in the elderly. The mechanisms underlying the imbalance between bone formation and resorption during skeletal unloading are not well understood. We are proposing that the skeletal response to mechanical load requires complex formation between selected integrins and IGF-IR. Disruption of this complex during skeletal unloading results in a relative reduction in bone formation leading to osteoporosis, but this can be restored by reloading. If this hypothesis turns out to be valid, we anticipate that drugs that selectively regulate the integrin signaling pathway will prove efficacious in modulating the skeletal response to IGF-I and in so doing prevent or reverse the loss of bone during disuse.

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Conflict of interest statement No conflict of interest.

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