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Bone and Skeletal Muscle: Key Players in Mechanotransduction and Potential Overlapping Mechanisms

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Abstract

The development and maintenance of skeletal muscle and bone mass is critical for movement, health and issues associated with the quality of life. Skeletal muscle and bone mass are regulated by a variety of factors that include changes in mechanical loading. Moreover, bone mass is, in large part, regulated by muscle-derived mechanical forces and thus by changes in muscle mass/strength. A thorough understanding of the cellular mechanism(s) responsible for mechanotransduction in bone and skeletal muscle is essential for the development of effective exercise and pharmaceutical strategies aimed at increasing, and/or preventing the loss of, mass in these tissues. Thus, in this review we will attempt to summarize the current evidence for the major molecular mechanisms involved in mechanotransduction in skeletal muscle and bone. By examining the differences and similarities in mechanotransduction between these two tissues, it is hoped that this review will stimulate new insights and ideas for future research and promote collaboration between bone and muscle biologists.

Keywords

hypertrophy; mTORC1; growth; bone mineral density; mechanical loading

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INTRODUCTION

Skeletal muscle and bone play fundamental roles in human physiology, enabling locomotion and movement, enhancing blood flow to organs, and providing protection to vital organs, among others. Beyond the mechanical roles of these two organ systems, both are also major regulators of whole body metabolism. For instance, skeletal muscle serves as a storage site/consumer of amino acids and glucose, and secretes various myokines that affect metabolism in other tissues [1–3]. Bone serves as an ion bank for maintaining serum levels of physiologically crucial elements such as Ca^{2+} and Mg^{2+}, and also secretes active endocrine products [4–6]. In light of the far-reaching roles of these tissues in general health, it is imperative that the field comes to a better understanding of the conditions that concomitantly affect muscle and bone health, most notably reduced bone and/or skeletal muscle mass (either during pre/post-natal development or in adults). These conditions have the potential to increase the risk of injury and metabolic disease, reduce physical mobility, and ultimately affect the quality and duration of life.

Skeletal muscle mass and bone mass are regulated by a range of factors that include genetics, nutrition, hormones and growth factors and, in particular, mechanical stimuli [7, 8]. It is well known that an increase in mechanical loading of skeletal muscle results in an increase in skeletal muscle mass (i.e., muscle hypertrophy), while a decrease in mechanical loading leads to a reduction of skeletal muscle mass (i.e., muscle atrophy) (for reviews see [9–11]). The mechanical loading experienced by skeletal muscle typically comprises the internal longitudinal and lateral forces, of varying magnitudes and velocities, which are generated by active muscle contractions (e.g., shortening, lengthening, or isometric contractions) or by passive stretch. Changes in mechanical loading are also known to play a major role in the regulation of bone mass and strength; increased mechanical loading at critical stages of growth and development result in increased bone mineral accrual, bone mass and strength, while reduced mechanical loading results in the loss of bone mass and strength (for reviews see [12–14]). Importantly, evidence suggests that the development and maintenance of bone mass is, in large part, dependent on skeletal muscle-derived mechanical loading [15, 16].

Skeletal muscles contribute to the mechanical loading of bone in various ways that include the tensile forces developed by contracting muscles at their site of insertion, the compressive forces between bones developed by muscles contracting across joints, and bending forces experienced by long bones as muscles generate force for lifting distally held objects [15]. In support of a critical role for skeletal muscle-induced mechanical stimuli in the regulation of bone mass, studies have shown that a lack of muscle function in utero results in impaired fetal bone and joint development [17–21]. Furthermore, during post-natal skeletal growth (2–20 yr), there is a very strong positive correlation between muscle mass and bone mass, with gains in muscle mass preceding those in bone mass [22]. In fact, the associations between muscle/strength and bone mass in children are strong enough that clinical techniques for disease diagnosis can be founded upon them [23, 24]. For instance, deficiencies in the amount of bone per unit muscle strength versus deficiencies in both factors allow for classification of diagnoses into primary (true or intrinsic) and secondary

(physiologic) bone disorders. Combined, these data strongly suggest that greater forces, produced by larger muscles, may play a direct role in stimulating bone growth. Conversely, states of reduced muscle mass and function, such as various neuromuscular diseases (e.g. cerebral palsy and Duchenne muscular dystrophy [25, 26]), spinal cord injury [27] and space flight [28], are all associated with a loss of bone mass. It is also interesting to note, and perhaps telling with respect to the influence of muscle forces on bone mass, that in many disease states, bone mass is typically not over adapted for muscle mass [29].

Despite the fundamental dependence on mechanical stimuli for the development and/or maintenance of bone and skeletal muscle mass, the exact mechanism(s) by which changes in mechanical loading are transduced into anabolic or catabolic signaling events (i.e, mechanotransduction) in these tissues remains to be fully determined. A thorough understanding of the cellular mechanism(s) responsible for mechanotransduction in bone and skeletal muscle is essential for the development of effective exercise and pharmaceutical strategies aimed at increasing, and/or preventing the loss of, mass in these critical tissues. Furthermore, it is clear that a sizeable and powerful complement of skeletal muscle and a robust and rigid skeleton are desirable outcomes for optimal connective tissue health. Therefore, it is worthwhile to understand the molecular underpinnings of mechanotransduction in both tissues and to determine whether both can be optimized in tandem [30]. Hence, the purpose of this review is to summarize our understanding of the key mechano-sensitive signaling events that are thought play a role in the regulation of bone and skeletal muscle mass. Importantly, we will also highlight important gaps in the body of knowledge in the hope that this will stimulate further research, and potentially collaboration between bone and muscle biologists.

MECHANOTRANSDUCTION AND THE REGULATION OF SKELETAL MUSCLE MASS

Skeletal muscle mass is ultimately determined by the net difference in the rates of protein degradation and protein synthesis [31]. For example, a net increase in protein synthesis and/or a net decrease in protein degradation leads to muscle hypertrophy, while a net decrease in protein synthesis and/or increase in protein degradation results in muscle atrophy. Importantly, mechanical load-induced changes in muscle mass are associated with changes in protein synthesis, with increased mechanical load-induced muscle hypertrophy being associated with an increase in protein synthesis [10]. Despite the major role that protein synthesis plays in the mechanical regulation of skeletal muscle mass, the molecular mechanism(s) through which changes in mechanical loading regulate protein synthesis, and thus muscle mass, remain to be fully determined. Nevertheless, progress is being made and a significant body of evidence now indicates that the protein kinase called the mechanistic/mammalian target of rapamycin (mTOR) plays a central role in the pathway through which mechanical stimuli regulate protein synthesis and muscle mass [9, 11]. As such, the remainder of this section will focus on the role of mTOR in mechanically-induced increases in protein synthesis and muscle mass, and on several putative mechanically-sensitive factors that are proposed to play a role in the activation of mTOR signaling. It is important to note, however, that other signaling mechanisms also play important roles in the regulation of

protein synthesis and skeletal muscle mass during different stages of growth and development and under various conditions that may include increased mechanical loading [for recent reviews on these topics, see [32–41]. Furthermore, although somewhat controversial, there is also evidence that proliferation, differentiation and fusion of satellite cells (SC) may play a role in the mechanical regulation of skeletal muscle mass; however, the exact mechanism(s) responsible for the mechanically-induced activation of SCs remains to be determined [10, 42–45].

The Central Role of mTOR/mTORC1 in Mechanically-Induced Skeletal Muscle Growth

mTOR is a conserved serine/threonine kinase that is found in at least two multi-protein complexes: mTOR complex 1 (mTORC1), whose signaling is partly inhibited by the drug rapamycin, and mTORC2, which is largely rapamycin-resistant [46]. mTORC1 is a major regulator of cell growth, in part, by regulating mRNA translation, and thus protein synthesis, with the potential to regulate both translational efficiency (i.e., the rate of mRNA translation) and translational capacity (i.e., the number of ribosomes) (for a review, see [47]). Importantly, in skeletal muscle, mTORC1 signaling has been shown to be activated by a wide-range of different types of mechanical stimuli, and this effect can be observed in cell culture, and in whole muscle *ex vivo* and *in vivo* model systems [9]. Moreover, it has been demonstrated that the *in vivo* activation of mTORC1 signaling is sufficient to stimulate an increase in protein synthesis and induce muscle fiber hypertrophy [48, 49], and that the kinase activity of mTOR is necessary for mechanical load-induced muscle hypertrophy [50, 51]. However, despite the robust evidence for mTORC1's central role in mechanically-induced skeletal muscle growth, the identification of the upstream mechanism(s) that are responsible for the mechanical activation of mTORC1 remains a significant challenge. Below we will briefly describe the evidence for, and against, several putative mechanically-induced activators of mTORC1 signaling in skeletal muscle

Potential Candidates for the Mechanically-Induced Activation of mTORC1

Insulin-Like Growth Factor 1—One of the earliest molecules that was proposed to be involved in the mechanical activation of mTORC1 signaling is the insulin-like growth factor 1 (IGF-1). For example, early studies showed that IGF-1 expression was increased by mechanical loading, including a splice variant known as the mechano growth factor (MGF) which was proposed to act in an autocrine manner [52–55]. IGF-1 overexpression was also shown to be sufficient to activate PI3K/Akt/mTORC1 signaling, increase protein synthesis and induce muscle hypertrophy [56–65]. While there is some limited evidence that IGF-1 may play a role in the increase in protein synthesis several hours after resistance exercise in diabetic muscle [66], more recently, a significant body of evidence has accumulated which indicates that mechanical stimuli activate mTORC1 signaling, at least initially, via an IGF-1/PI3K/Akt-independent mechanism, and that IGF-1/PI3K/Akt signaling is not necessary for mechanically-induced skeletal muscle growth (for a more detailed review of this topic see [9]). Thus, while IGF-1 plays an important role in the development and/or maintenance of skeletal muscle mass [67], current evidence suggests that IGF-1 does not play a significant role in the acute mechanical activation of mTORC1 and that IGF-1 is not necessary for mechanically-induced skeletal muscle hypertrophy. Further work is required to definitively

determine the role, if any, of IGF-1 in the acute and more prolonged activation of mTORC1 and protein synthesis, and in muscle hypertrophy, in response to mechanical stimuli.

Extracellular Signal-Regulated Kinase 1 and 2—Another potential mechanically-activated upstream regulator of mTORC1 and skeletal muscle mass is the extracellular signal–regulated kinases 1 and 2 (ERK1/2). These members of the mitogen-activated protein kinase (MAPK) family form part of the Ras/Raf/MEK/ERK1/2 signaling pathway and are activated by various types of mechanical stimuli in cultured muscle cells and in rodent and human skeletal muscle *in vivo* [68–74]. ERK1/2-mediated signaling has the potential to positively regulate protein synthesis, in part, by the activation of mTORC1 signaling via p90RSK-mediated phosphorylation of TSC2 and Raptor [75–80]. While these studies suggest that ERK1/2 signaling could play a role in the mechanical activation of mTORC1 signaling, protein synthesis, and muscle hypertrophy, it was recently shown that ERK1/2 inhibition does not prevent stretch-induced hypertrophy in cultured myotubes [71]. Furthermore, while inhibition of ERK1/2 reduced basal mTORC1 signaling, it did not inhibit the stretch-induced increase in mTORC1 signaling or protein synthesis in isolated mouse muscles [74]. Thus, based on a limited number of studies, it appears that ERK1/2 signaling is not necessary for the mechanically-induced activation of mTORC1 signaling, protein synthesis, or muscle hypertrophy. Further research in rodents and humans, using different experimental models, is needed to further clarify these findings.

Phosphatidic Acid—In the absence of convincing evidence for either IGF-1 or ERK1/2 in the mechanical activation of mTORC1, protein synthesis or skeletal muscle growth, recent studies have begun to focus on the glycerophospholipid second messenger, phosphatidic acid (PA). PA is a direct activator mTORC1 signaling, most likely via its ability to bind to the FKBP12-rapamycin binding (FRB) domain of mTOR [74, 81, 82]. Studies have shown that the stimulation of cells with exogenous PA, or the overexpression of PA-generating enzymes, such as phospholipase D1 (PLD1), lysophosphatidic acid acyltransferase θ (LPAATθ) and diacylglycerol kinase ζ (DGKζ), activates mTORC1 signaling [74, 83–86]. Furthermore, the overexpression of PLD1 and DGKζ has been shown to be sufficient to induce skeletal muscle fiber hypertrophy in mice [87, 88]. Importantly, mechanical stimulation of skeletal muscle (i.e. passive stretch and eccentric contractions) induces an increase in muscle [PA] [74, 85, 88], suggesting that PA could indeed play a role in the mechanical activation of mTORC1. Initial studies investigating this hypothesis focused on the potential role of PLD as the source for the mechanically-induced increase in PA [85, 89]; however, more recent studies using a specific small-molecule PLD inhibitor found that PLD activity was not necessary for passive stretch-induced increases in PA [88]. Instead, it was found that passive stretch induced an increased in membrane associated DGK activity. Furthermore, in DGKζ knockout mice, it was shown that the stretch-induced increase in PA is almost completely abolished and that the activation of mTORC1 signaling is markedly impaired [74]. Thus, current evidence strongly suggests that DGKζ is predominantly responsible for the stretch-induced increase in PA and is a major contributor to the mechanical activation of mTORC1 signaling. Further research is still required, however, to confirm the role of DGKζ in other models of mechanical stimulation (e.g.

eccentric contractions and synergist ablation-induced mechanical overload) and to determine whether DGKζ-derived PA is sufficient to increase protein synthesis.

TSC2 Translocation and Rheb GTP-Loading Status—TSC2 and Rheb (Ras homologue enriched in the brain) form part of the canonical insulin/IGF-1 signaling pathway [90]. Rheb, located immediately upstream of mTOR, is a GTP-binding protein that possesses GTPase activity and its GTP/GDP-binding status is regulated by the GTPase activating protein (GAP) activity of TSC2 (a.k.a. Tuberin) [91]. Rheb interacts with mTOR's catalytic domain, and when in its GTP-bound state, Rheb can directly activate mTOR kinase activity [92, 93]. It is currently thought that, under basal conditions, the GAP domain of TSC2 stimulates Rheb's intrinsic GTPase activity which converts active GTP-Rheb into inactive GDP-Rheb, and thus leads to a repression of mTORC1 signaling [94–96]. Conversely, when stimulated by factors such as insulin, TSC2 GAP activity is proposed to be inhibited in a phosphorylation-dependent manner, leading to an increase in GTP-Rheb and the activation of mTORC1 signaling [90]. In skeletal muscle, the overexpression of Rheb is sufficient to activate mTORC1 signaling, induce an increase in protein synthesis, and stimulate muscle fiber hypertrophy [48, 49]. Despite this, there is currently very little known about the potential role of TSC2 and Rheb in the mechanical activation of mTORC1 signaling in skeletal muscle. Recently, however, it was shown that an acute bout of eccentric contractions almost completely abolished the association of TSC2 with late endosomal/lysosomal (LEL) structures [97]. As LEL structures are also the location of a population of mTOR and Rheb (for review see [98]), this finding suggests that the mechanically-induced translocation of TSC2 away from the LEL could lead to an increase in the amount of GTP-loaded Rheb at the LEL and subsequently promote the activation of mTORC1 signaling. While these findings appear promising, it remains to be determined whether: 1) the translocation of TSC2 away from the LEL plays an important role in the mechanical activation of mTORC1 signaling; 2) whether TSC2 translocation is mediated through a phosphorylation-mediated event and, if so, what kinase(s) are responsible for the changes in TSC2 phosphorylation; and 3) whether Rheb is necessary for the mechanical activation of mTORC1 signaling, protein synthesis and hypertrophy.

Increased Intracellular Calcium—Skeletal muscle contractions are initiated by an action potential-induced release of calcium (Ca^{2+}) from the sarcoplasmic reticulum (SR) and the subsequent Ca^{2+}-induced activation of the contractile apparatus [99]. Furthermore, passive stretch of myoblasts and skeletal muscles can also result in an increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$), most likely via the activation of stretch sensitive ion channels [100, 101]. Importantly, studies in non-muscle cells have shown that mTORC1 signaling may be regulated by changes in $[Ca^{2+}]_i$, with an increase in $[Ca^{2+}]_i$ activating mTORC1 signaling and a decrease in $[Ca^{2+}]_i$ leading to an inhibition of mTORC1 signaling [102, 103]. Increased $[Ca^{2+}]_i$ has also been shown to increase *ex vivo* rates of protein synthesis in resting skeletal muscle [104]. Thus, contraction- or stretch-induced increases in $[Ca^{2+}]_i$ would appear to be an ideal candidate for the mechanical activation of mTORC1 and protein synthesis in skeletal muscle. However, despite the potential role for $[Ca^{2+}]_i$, few studies have attempted to directly examine this hypothesis. Firstly, it was reported that the Ca^{2+} chelator, BAPTA-AM, did not inhibit *ex vivo* stretch-induced mTORC1 signaling in isolated

skeletal muscles [89]. In contrast, the *in vivo* inhibition of Ca^{2+} (and Na^{+}) permeable stretch-activated ion channels with gadolinium (Gd^{3+}) was shown to inhibit the eccentric contraction-induced increase in $[Ca^{2+}]_i$ and Gd^{3+} attenuated eccentric contraction-induced mTORC1 signaling [105, 106]. More recently, it was shown that the *in vivo* mechanical overload-induced activation of mTORC1 signaling and muscle hypertrophy was stimulated by an increase in $[Ca^{2+}]_i$ via the activation of SR transient receptor potential cation channel subfamily V (TrpV1) channels [107, 108]. Mechanistically, a contraction/stretch-induced increase in $[Ca^{2+}]_i$ could potentially stimulate mTORC1 signaling and protein synthesis by the activation of the Ca^{2+}/calmodulin-dependent protein kinase α (CaMKKα) which has been shown to up regulated by chronic mechanical overload [109, 110]. Furthermore, constitutively active CaMKKα was found to be sufficient to activate mTORC1 signaling, increase protein synthesis in a rapamycin-sensitive manner and induce muscle hypertrophy; however, paradoxically CaMKKα was also found to not be necessary for overload-induced muscle growth [110]. Thus, overall, while increases in $[Ca^{2+}]_i$ are an attractive candidate for the mechanical activation of mTORC1, further work is required to reconcile some of the conflicting results obtained to date, and to identify the molecular mechanism(s) through which changes in $[Ca^{2+}]_i$ could regulate mTORC1 signaling.

Reactive Nitrogen Species—Studies in non-muscle cells have shown that mTORC1 signaling can be regulated positively and negatively by changes in the cellular redox state [111]. It is well known that the acute mechanical stimulation of skeletal muscle results in changes in the cellular redox state via the production of reactive oxygen species [ROS; e.g., superoxide (O_2^-) and hydrogen peroxide (H_2O_2)] and reactive nitrogen species [RNS; e.g., nitric oxide (NO) and peroxynitrite ($ONOO^-$)] [112]. Therefore, changes in the production of ROS and/or RNS are potential candidates for the mechanical activation of mTORC1 signaling and skeletal muscle hypertrophy. In support of this possibility, it has been shown that the nitric oxide synthase (NOS) inhibitor, L-NAME, can inhibit stretch-induced hypertrophy of cultured myotubes and synergist ablation-induced muscle hypertrophy in rats [113–115]. Furthermore, muscles from neuronal NOS (nNOS)-null mice fail to undergo synergist ablation-induced muscle hypertrophy [107]. The exact mechanism behind the apparent role of NO in mechanically-induced hypertrophy remains to be determined, but recent evidence suggests that it may, in part, be due to increases in $[Ca^{2+}]_i$ [107]. Specifically, it was concluded that the synergist ablation-induced increase in $[Ca^{2+}]_i$ and mTORC1 signaling is stimulated by the formation of $ONOO^-$ that is produced from nNOS-derived NO and NADPH oxidase 4 (Nox4)-derived O_2^- [107, 108]. Although very attractive, significantly more work using different experimental models will be required to fully define the role of RNS in the mechanical activation of mTORC1 and skeletal muscle growth. Interestingly, nNOS-derived NO may also play a role in muscle atrophy induced by a reduction in mechanical loading [116]. For example, it was recently shown that hindlimb suspension-induced muscle atrophy was associated with the dislocation of nNOS from the membrane associated dystrophin glycoprotein complex to the cytoplasm and with increased NO production. Furthermore, the muscle atrophy was inhibited in nNOS-null mice and in mice treated with the nNOS-specific inhibitor, 7-nitroindazole [116]. Thus, RNS appear play a role in the regulation of muscle mass in response to both increased and decreased

mechanical loading. Further work is required to determine whether there is any link between muscle atrophy, NO and mTORC1 signaling.

Amino Acids—Amino acids are well known regulators of mTORC1 signaling, with amino acid depletion reducing mTORC1 signaling and increased amino acid availability leading to increased mTORC1 signaling [117]. It was recently demonstrated in non-muscle cells that amino acids regulate the association of mTOR with LEL structures via a mechanism that is dependent on the Rag family of GTPases [118–120]. Specifically, amino acid stimulation increases the association of mTOR with LEL structures by regulating the activity/GTP-loading state of the Rag GTPases [121]. Based on these data, it has been suggested that amino acid–induced changes in mTORC1 signaling are primarily regulated by spatially controlling the ability of mTOR to interact with the LEL-associated activator, Rheb. In skeletal muscle, amino acids (especially the branched-chain amino acid, leucine) are sufficient to activate mTORC1 signaling and increase protein synthesis via a rapamycin-sensitive mechanism [122, 123]. Therefore, it is plausible that an increase in the uptake of amino acids could play a role in the mechanical activation of mTORC1 signaling. To date, however, there is currently no direct evidence to support this hypothesis. Nevertheless, recent studies have shown that acute resistance exercise increases both mTORC1 signaling and the content of leucine in rat muscle [124], and increases the post-exercise activity of the class III PI3K, Vps34, which has been implicated in the amino acid–induced activation mTORC1 signaling [125–128]. On the contrary, a recent study has reported a decrease in endogenous muscle leucine content after acute resistance exercise despite an increase in mTORC1 signaling in middle aged humans [129]. Thus, while some indirect evidence suggests that amino acids could play a role in the mechanical activation of mTORC1 signaling, significantly more research is required to gain further insights into this possibility.

In summary, a significant body of evidence has established that mTORC1 plays a central role in the mechanical regulation of protein synthesis and skeletal muscle mass. Currently, there are several potential candidate molecules that have been proposed to play a role in the pathway through which mechanical stimuli activate mTORC1 signaling. While most of these molecules are likely to play important roles in the regulation mTORC1 signaling, protein synthesis and muscle mass during different stages of growth and development, current evidence suggests that DGKζ-derived PA plays a major role in the acute mechanical activation of mTORC1. This role, however, is not necessarily exclusive. Indeed, future research may identify that a complex range of factors contribute to the full mechanical activation of mTORC1 signaling. For example, the mechanically-induced dissociation of TSC2 from the LEL and the subsequent activation of Rheb, an increase in intracellular amino acids, and/or RNS-induced SR Ca^{2+} release may all combine with DGKζ-derived PA to promote the full activation of mTORC1 signaling. Ultimately, the relative contribution of each activating factor is likely to be specific to the type, intensity and duration of the mechanical stimulus, and to the time point examined following the initiation of the stimulus. This potential complexity highlights how limited our current understanding is, and underscores the need for further research into the process of mechanotransduction on regulation of mTORC1, protein synthesis and skeletal muscle mass. Furthermore, significantly more research is required to identify the upstream mechano-sensitive

element(s)/receptor(s) that ultimately stimulate the array of putative mTORC1 activators reviewed above.

MECHANOTRANSDUCTION AND THE REGULATION OF BONE MASS

Mechanoreception and tissue adaptation in bone: a different mechanism than muscle, involving division of labor among cell types

While skeletal muscle mass is ultimately determined by the net difference in the rates of protein degradation and protein synthesis within the cell, the regulation of bone mass represents a more complicated phenomenon because bone tissue is incapable of interstitial growth [130]. That is, the cross sectional size of a given muscle can change via hypertrophy or atrophy of preexisting myofibers, whereas cellular hypertrophy/atrophy of bone cells is not a mechanism that directly augments bone mass. In bone, changes in the mineralized matrix must be achieved by specialized cells (osteoblasts and osteoclasts) that are at the beck and call of the embedded master-regulatory cell type - the osteocyte [131]. Because changes in bone mass are enacted by the elaboration or removal of a complicated matrix, changes in bone mass occur much more slowly than the cell-trophic changes that occur in muscle tissue. Moreover, the physical environment of the osteocyte is completely different than that of bone cells localized on the bone surface. Osteocytes are entombed in a small, form-fitting cavity within the bone matrix (the lacuna). As the osteocyte cell processes emerge and course away from the cell body and lacuna, they travel in canaliculi (nano-canals) within the bone matrix [132]. Decades ago, it was postulated that the osteocyte was the best candidate for a sensor cell type, for several reasons [133]. First, the osteocytes are regularly distributed throughout cortical and trabecular bone, even in areas of mineralized matrix devoid of vasculature. Consequently, the network of osteocytes provides a widespread load-monitoring “net” that infiltrates every cubic millimeter of bone tissue. Second, osteocytes are connected to one another, and to bone surface cells, through long cellular processes that course through the bone and project to the bone surfaces. Osteocytes have a large number of these cell processes (~50/cell) emanating from the cell body and coursing in all directions [134]. The cell processes join similar cell processes from neighboring osteocytes and transmit information inter-cellularly *via* gap junctions, which facilitate rapid cell-cell communication [135]. Third, it is clear that osteocytes are not effector cells, as they are entombed in a bony matrix and therefore are incapable of adding or removing very much matrix, and can only remove it in the small area surrounding their lacunae [136]. This very localized activity of osteocytes, while potentially meaningful for regulating serum calcium levels, has little to no effect on bone size, shape, and structural properties. Because their role as an effector cell is precluded, they have been thought of historically (somewhat by default) as a sensor cell [137]. Beyond teleological arguments, experiments have supported the role of the osteocyte as the primary mechanosensory cell type in bone. Interestingly, a recent gene expression profile conducted on purified, flow-sorted populations of osteocytes extracted from living mouse bone, revealed a surprising number of highly expressed genes that are traditionally thought of as “muscle genes” [138]. While that experiment was not conducted under mechanically altered conditions, it is also worthwhile to consider that osteocytes might be more “muscle-like” than previously

thought, and that some of the mechanisms of strain sensation might overlap between these two cell types.

Ion Channels

Many mechanosensitive tissues in the body are regulated by ion channels. Ion channels are pore-forming proteins that traverse the plasma membrane and allow ions to flow into and out of a cell based on electrochemical gradient. Their activity (opening and closing) can be controlled by different mechanisms; among them are changes in voltage across the membrane, biochemical ligands, or physical stimuli such as mechanical perturbation of the membrane. It is well documented experimentally that ion channels play a crucial role in the process of mechanotransduction in bone [139, 140]. For example, Gd^{3+} is a potent blocking agent for mechanosensitive ion channels, and pretreatment of bone cells with Gd^{3+} prior to mechanical loading stimulation results in a loss of downstream mechanotransduction marker expression [141, 142]. However, Gd^{3+} is a fairly nonspecific inhibitor (e.g., some L-type voltage sensitive calcium channel inhibition has been reported), so it is difficult to be certain that the inhibitory effect of Gd^{3+} on mechanotransduction involves a mechanosensitive ion channel. More recently, the transient receptor potential (Trp) family of channels has been investigated regarding their role in bone cell mechanotransduction [143, 144]. Specifically, TrpV4, a receptor known to be sensitive to mechanical perturbation (most notably, cell swelling) and osmolarity in other tissues [145], was recently shown to modulate the response to mechanical disuse in mice. TrpV4 knockout mice subjected to tail suspension for 2 wk failed to lose bone and did not exhibit reduced bone formation rates, as was observed in tail suspended wild type mice [146]. The Trpv4 channel has been shown to be sensitive to shear stress in other cell types, thus its role in bone as a mechanosensor is promising. Another Trp channel that has received significant attention in bone cell mechanotransduction is TrpP1, also known as polycystin-1 (encoded by the Pkd1 gene). Several years ago, it was convincingly demonstrated that that polycystin-1 and polycystin-2 regulate mechanotransduction in kidney epithelial cells [147]. Polycystin-1 and -2 reside largely on the primary cilium—a nonmotile ~250 nm thick cytoskeletal stalk that extends into the tubular lumen [148]. Deflection of the cilium from fluid movement in the renal tubules activates polycystin-1, which causes the polycystin-2 channel to open, kicking off a Ca^{2+} cascade that has multiple downstream effects [149]. For example, in osteocyte-specific polycystin-1 knockout mice, *in vivo* ulnar loading exhibited a ~70% reduction in load-induced apposition rates compared to control mice, indicating that osteocytic polycystin-1 is an important protein in the anabolic response to skeletal loading [150]. Similar effects have been found in vitro, where cilium disruption via chemical treatment or gene silencing inhibits the response to matrix strain [151]. In light of the importance of fluid movement in the canaliculo-lacunar network, the primary cilium, and its compliment of associated proteins, is an attractive candidate for mechanosensing in the osteocytes. But it is difficult to envisage how the cilium would physically fit in the extracellular space because the distance between the canalicular wall and the osteocyte cell process/body is typically 50–80 nm and could be much smaller if measured on sections processed with newly refined fixation techniques that reduce cell shrinkage [152]. In summary, there is evidence suggesting that the primary cilium itself, or perhaps proteins normally associated with the cilium, mediate

mechanotransduction in bone; but greater experimentation in this area will be required to more fully understand the precise role of cilia in the mechanical signaling process.

G-protein coupled receptors

G-protein coupled receptors (GPCRs) represent the largest family of cell surface receptors, and are activated by a variety of ligands including neurotransmitters, hormones, small peptides, local cytokines, amino acids, and fatty acids, among others (reviewed in [153]). More than a decade ago, it was demonstrated that fluid flow activates G-proteins in osteoblasts, and that pharmacologically preventing G-protein activation prevents the normal response to fluid shear [154]. It is interesting to note that fluid shear stress leads to a conformational change in two GPCRs - the parathyroid hormone 1 receptor (PTH1R) and the B2 bradykinin receptor, in MC3T3 osteoblastic cells and BAEC endothelial cells [155, 156]. This result was detected using GPCR conformation-sensitive fluorescence resonance energy transfer (FRET), which revealed conformational changes in these GPCRs occurring within milliseconds of shear exposure. The changes were independent of the presence of either receptor's ligand. It was also reported that the responsiveness of the energy transfer signal could be modulated by membrane fluidity (e.g. modulation of membrane stiffness), indicating that these GPCRs might be direct sensors of mechanical perturbation of the membrane. While mechanotransduction may or may not involve either of these two particular receptors, the data make the larger point that other GPCRs, more crucial to the mechanotransduction response, might undergo similar conformational changes when the cell is mechanically stimulated. The premise that a ligand is not necessary for shear to activate intracellular G-proteins has been taken one step further, and it might be true that even the receptor itself is not necessary for shear-induced G-protein activation. For instance, the same group showed that when purified G-proteins are reconstituted into otherwise empty phospholipid vesicles, they could be activated (GDP hydrolysis) almost immediately upon fluid shear [157]. This flow-induced activation was independent of a GPCR presence, but rather, was modulated by membrane stiffness. Although, bone cells possess the G-protein machinery necessary to activate this pathway, verification of this mechanoreception mechanism in bone cells *per se* will require similar experiments in bone-specific models, which have not been done to date.

The search for the "mechanoreceptor" in bone is ongoing, with progress being made on several fronts. The molecule or mechanism that is at the forefront of mechanoreception—the protein that converts a physical signal into a biochemical signal—remains elusive. It is also possible that no single mechanism is responsible for initiating the entire event; numerous signaling systems in biology exhibit redundancy. While the mechanisms of signal reception are still being worked out, much greater progress has been made in the field of mechanically stimulated second messengers that are required for mechanically-induced changes in bone mass. Those mechanisms are described next.

Mechanically stimulated second messengers in bone

Once the mechanical signal is received by the local bone cell population and translated into an initial biological signal, a series of secondary biochemical signaling events must occur to propagate the signal within the cell and to other sensor/effector cells. Efforts to understand

the signaling pathways involved in mechanical signal propagation have uncovered a multitude of changes in the mechanically stimulated osteocyte/osteoblast, including gene expression changes, protein and lipid modifications (e.g., phosphorylation events), protein degradation, intracellular translocation events, release of secreted factors, and alterations in cell shape and size, among others. The challenge presented by these observations is to determine which among them are critical for mechanotransduction to occur, and which are simply auxiliary events that have few functional consequences for the mechanotransduction process.

Prostaglandins—One of the earliest pathways identified to be involved in bone cell mechanotransduction is the cyclooxygenase (Cox)/prostaglandin (PG) pathway. PGs are potent and short lived (sec/min) arachidonic acid (AA)-derived signaling molecules that act in a autocrine/paracrine manner [158]. In a multistep process, intracellular cyclooxygenase (Cox) enzymes convert AA to PGG_2, and then to PGH_2, after which various PG synthases generate specific PGs including PGE_2 [158]. PGs are secreted from the cell in response to a number of stimuli where they can then bind to specific GPCRs. Vigorous jumping exercises in humans induce an immediate release of PGE_2 from lower limb (loaded) bone tissue [159]. In rodents, mechanical loading up-regulates the mRNA and protein levels of Cox2 (the inducible isoform of Cox), whereas the constitutive isoform (Cox1) remains unchanged [160, 161]. The importance of PGE_2 signaling has been demonstrated *in vivo* by depleting the intracellular PGE_2 pool prior to mechanical loading. Pharmacologic inhibition of both Cox1 and Cox2 via indomethacin treatment, or selective inhibition of Cox2 alone via NS-398 treatment, was found to reduce osteogenic response to loading conducted several hours after administration of the inhibitors [162]. This result has been confirmed *in vitro* using fluid shear and stretch, where PGE_2 levels can more easily be measured from cell culture media [163–165]. The mechanism of PGE_2 release from mechanically stimulated cells is controversial, and might involve the opening of large, pore-forming connexin-43 hemichannels [166] or the purinergic P_2X_7 protein complex [167, 168]. Once released, PGE_2 binds in an autocrine or paracrine fashion to the heptahelical Ep receptors (Ep1-4), which mediate its effects [169, 170]. In summary, both *in vivo* and *in vitro* experiments point to a prominent role for prostaglandins in bone cell mechanotransduction, but the mechanism by which PGE_2 is released, and which receptors are important for its paracrine/autocrine effects, are unclear.

Wnt signaling—More recently, the Wnt (Wingless-related integration site) signaling pathway has been identified as a major intermediate player in bone cell mechanotransduction [171]. The Wnt family of secreted glycoproteins is made up of 19 different Wnt genes (in humans) whose protein products can activate multiple signaling pathways (reviewed in [172]). Canonical Wnt signaling involves secreted Wnts activating a receptor complex comprising a single-pass low-density lipoprotein receptor-related protein (Lrp) and a heptahelical Frizzled (Fzd) receptor which subsequently leads to the activation of the Dishevelled (Dsh) phosphoprotein [173]. Activated Dsh inhibits GSK3β activity which, in turn, results in reduced GSK3β-mediated phosphorylation of the transcription factor β-catenin and thus reduced β-catenin degradation [173]. This allows the stabilized β-catenin to act as a transcriptional co-activator by forming complexes with members of the

TCF/LEF family of transcription factors and increasing the expression of various Wnt/β-catenin target genes [173]. Importantly, numerous target genes of β-catenin are associated with enhanced osteogenesis and reduced resorption. Using a reporter construct, mechanical loading was shown to activate β-catenin mediated transcription both *in vivo* and *in vitro* [174, 175]. Remarkably, osteocytes appear to be the first cells to exhibit activated β-catenin transcriptional activity after loading [176], suggesting that Wnt signaling in osteocytes might be a sensor cell response pathway. Moreover, deletion of one of the canonical Wnt co-receptors-Lrp5-in mice appears to prevent load-induced bone formation [177, 178], which suggests that Lrp5/β-catenin signaling is vitally important for mechanotransduction in bone. The canonical Wnt pathway is negatively regulated by a number of endogenous secreted inhibitors that bind Lrp5/6 or Wnts. Among these inhibitors is a potent Lrp5/6 antagonist-sclerostin-that is highly expressed by mature osteocytes but not by other bone cells (e.g., osteoblasts, osteoclasts, bone lining cells). Sclerostin, the protein product of the *SOST* gene, is significantly down-regulated by mechanical loading, and significantly up-regulated by mechanical disuse [179]. Forced overexpression of a *SOST* transgene prevents load-induced bone formation [180], whereas Sost deletion improves mechanotransduction [181]. Moreover, inhibition of Sost during mechanical disuse prevents bone loss [182] In summary, Wnt has been identified as a major mediator of bone cell mechanotransduction. The modulation of numerous secreted Wnt inhibitors, as well as the downstream signaling nodes activated by Wnt, are still being defined.

IGF-1 signaling—Unlike skeletal muscle, in which IGF-1 does not appear to be necessary for the mechanically-induced increase in mTORC1 signaling and skeletal muscle mass, bone cell mechanotransduction requires IGF-1 signaling for the anabolic effects of loading. For instance, IGF-1 is known to play an essential role in embryonic bone development [183, 184] and early studies also suggested a potential role for IGF-1 in the regulation of bone mass in response to changes in mechanical loading. Specifically, studies conducted in mechanically stimulated osteocytes *in vitro*, and in mechanically stimulated rat vertebrae and tibia *in vivo*, revealed increased expression of IGF-1 mRNA expression after a bout of increased loading [185–187]. Furthermore, it was shown IGF-1 administration increases osteoblast proliferation *in vivo* [188] and that transgenic overexpression of IGF-1 in osteoblasts resulted in enhanced responsiveness to *in vivo* mechanical loading in mice [189]. Moreover, conditional disruption of the IGF-1 gene in type 1α(2) collagen-expressing cells *in vivo* effectively eliminated the osteogenic responses to increased mechanical loading in mice [190]. More recently, conditional osteocyte knockout of IGF-1 also ablated the osteogenic response to mechanical loading and this occurred despite normal IGF-1 expression in osteoblasts [191]. In reloading models, recombinant IGF-1 infusion has been shown to be sufficient to enhance the differentiation of osteoblast precursor cells and increase trabecular bone formation in rats subjected to mechanical unloading [192], while osteocyte IGF-1 receptor expression was found to be necessary for reloading-induced periosteal bone formation in mice [193]. Combined, these data suggest that IGF-1 does indeed play a critical role in the mechanical regulation of bone mass. However, the downstream effectors of activated IGF-1 are less clear. For instance, *in vitro* IGF-I treatment stimulates the activation of the IGF-1 receptor, Ras, ERK1/2 and Akt in cultured BMOp cells isolated from normally loaded bones [188], but the downstream targets of these

signaling events is unknown. In another study, conditional knockout of osteocyte IGF-1 reduced the load-induced increase in Wnt signaling-related gene expression and β-catenin protein levels. It also increased the loading-induced expression of the SOST gene and inhibited the load-induced increase in Cox-2 expression [191]. In summary, the IGF-1 axis is clearly important for mechanical signaling in bone cells, with more limited evidence suggesting that IGF-1 may regulate Wnt and PG signaling in response to increased mechanical loading. More studies are needed, however, to further investigate other possible IGF-1 mediated signaling pathways (e.g. Ras/ERK and Akt signaling).

Nitric oxide signaling—Similar to skeletal muscle, another pathway activated by mechanical stimulation in bone is nitric oxide signaling [194]. Nitric oxide (NO) is a free radical and as such can diffuse through the plasma membrane freely. NO is generated from the amino acid L-arginine by one of three isoforms of Nitric Oxide Synthase (NOS). *In vitro*, NO is released from mechanically stimulated osteoblasts and osteocytes [195–197]. *In vivo*, depletion of the NO pool prior to mechanical loading in rats using one of several NOS inhibitors significantly impairs the osteogenic response to loading [198]. Moreover, in mice lacking the inducible form of NOS ($iNOS^{-/-}$), a return to normal cage floor locomotion following 7 days of tail suspension failed to generate an otherwise osteogenic response, as was seen in iNOS replete mice [199]. These experiments suggest that the osteoblastic response to mechanical stimulation requires NO signaling. Perhaps equally noteworthy is the effect that NO signaling has on bone resorption. In addition to enhancing bone formation, mechanical loading impairs bone resorption and NO signaling might be one of the main mechanisms by which it is accomplished. For instance, mechanically-induced increases in NO cause a decrease in RankL which is a major regulator of osteoclast development and survival [200]. In summary, while NO appears to be an important second messenger in bone cell mechanotransduction, its target enzymes and cell type of origin (i.e., stromal cells, osteocytes, lining cells) are still open to investigation.

In conclusion, numerous second messenger cascades are activated in bone tissue during and after mechanical stimulation, including IGF-1, prostaglandins, nitric oxide, and components of the Wnt pathway. How these various cascades interact with one another is unclear. Recent experimental work has begun to link some of these otherwise disparate pathways together. For example, Kitase et al [201] have shown that mechanically induced PGE_2 in osteocytes participates in an autocrine loop that ultimately inactivated GSK3β and stimulates the downstream canonical Wnt target β-catenin. It remains to be determined whether other major cascades in bone cell mechanotransduction are linked, or whether they act as accessory pathways in the event that one is impaired. The need for clear delineation of the processes involved is obvious: recapitulation of the mechanotransduction cascades using non-mechanical stimuli holds great promise in preventing fractures, particularly in those with frail skeletons that could not endure significant loading episodes.

SIMILARITIES AND DIFFERENCES BEWTEEN SKELETAL MUSCLE AND BONE

While the differences in mechanotransduction mechanisms between skeletal muscle and bone appear to be great, there is also evidence that some common mechanisms might exist. For example, recent studies suggest that mechanically sensitive ion channels, such as TrpP1 channels in bone and TrpV1channels in skeletal muscle, may play significant roles in the anabolic response to increased mechanical loading. Moreover, a role for these mechanically sensitive ion channels suggests that ions, such as Ca^{2+}, may be an important second messenger in both of these tissues. In addition, NO, albeit from potentially different sources (iNOS in bone and nNOS in skeletal muscle), also appears to be involved in load-induced increases in bone and skeletal muscle mass. However, there are also mechanically sensitive mechanisms that are established in one tissue but remain to be convincingly demonstrated in the other tissue (see Table 1). For example, as described above, it has been convincingly shown that mTORC1 signaling plays a fundamental role in mechanically-induced increases in skeletal muscle mass; however, its role in bone mechanotransduction remains to be determined. Furthermore, there is compelling evidence that PG and Wnt signaling are mechanically sensitive in bone and that they play important roles in load-induced increases in bone mass; but whether these molecules play a role in the mechanical regulation of skeletal muscle mass remains unclear. Therefore, in the hope of stimulating new research into these questions we briefly review the evidence for a potential role for PG and/or Wnt signaling in the mechanical regulation of skeletal muscle mass, and for a potential role of mTORC1 signaling in the mechanical regulation of bone mass.

A Role for Prostaglandins in the Mechanical Regulation of Skeletal Muscle Mass?

PGs are known to be produced in skeletal muscle and incubation with AA or $PGF_{2\alpha}$ has been shown to stimulate an increase in protein synthesis in isolated skeletal muscles and to induce hypertrophy of cultured myotubes [202–207]. Moreover, the $PGF_{2\alpha}$-induced myotube hypertrophy was recently shown to mediated through a PI3K/ERK/mTORC1-dependent mechanism [204]. Importantly, numerous studies have shown that mechanical stimulation of skeletal muscle induces an increase in PGs, including $PGF_{2\alpha}$ (for reviews see [208, 209]). Thus, an increase in PG synthesis and secretion could play a role in the mechanically activation of mTORC1, protein synthesis and muscle hypertrophy. Indeed, high doses of the general COX inhibitors, acetaminophen and ibuprofen, have been shown to be sufficient to inhibit the increase in protein synthesis that occurs following eccentric exercise in humans [210]. In contrast, ‘over-the-counter’ doses of general COX inhibitors do not inhibit resistance training-induced increases in muscle mass [208]. Furthermore, a COX-2 isoform specific inhibitor (NS398) has been shown to blunt synergist ablation-induced muscle hypertrophy in mice without inhibiting the activation of mTORC1 signaling [211]. Finally, not all PGs stimulate protein synthesis. For example, PGE_2 (and AA) stimulates protein degradation in skeletal muscle [203]. These disparate findings highlight the need for more studies, using a range of experimental models, to clarify the role of different PGs in the mechanical regulation of protein turnover and skeletal muscle mass. Moreover, given the current evidence that PI3K and ERK signaling are not required for the mechanical activation of mTORC1 signaling, this would suggest that the role of PGs in the

mechanical regulation of skeletal muscle mass may be exerted through an mTORC1-independent mechanism.

A Role for Wnt Signaling in the Mechanical Regulation of Skeletal Muscle Mass?

Wnt molecules play critical roles in the embryonic development of skeletal muscle and in the regulation skeletal muscle regeneration [212, 213]. Importantly, acute maximal exercise has been shown to increase Dsh/GSK3β interaction, and decrease GSK3β activity and β-catenin phosphorylation in humans [214]. Furthermore, resistance training-induced muscle fiber hypertrophy is associated with an increased expression of various Wnt signaling pathway members (e.g. Wnt1, β-catenin, LEF1, Cyclin D1) [215]. In mice, chronic mechanical overload-induced muscle hypertrophy is associated with an increase in β-catenin, Fzd-1, DSH-1 and LEF-1 protein expression, increased nuclear abundance of β-catenin and LEF-1, and increased expression of Wnt/β-catenin target genes c-myc and cyclin D1 [216]. Moreover, β-catenin has been shown to be necessary for chronic overload-induced muscle fiber hypertrophy [217]. Thus, current evidence strongly suggests that canonical Wnt/β-catenin signaling plays a significant role in mechanically-induced skeletal muscle growth. More recently, evidence has also demonstrated a link between Wnt signaling, muscle hypertrophy and the activation of mTORC1 signaling [218, 219]. Specifically, the overexpression of Wnt7a (but not Wnt 3a or 5a) induced hypertrophy of muscle fibers *in vivo*, and in myotubes that was independent of myoblast cell proliferation, differentiation and fusion [218]. This Wnt7a-induced hypertrophy was found to be associated with a non-canonical Wnt activated signaling pathway that required Fzd7 and G protein subunit α_s, and involved the activation of PI3K/Akt/mTORC1 signaling, independent of the IGF-1 receptor or IRS1 activation [218]. Moreover, Wnt7a-induced myotube hypertrophy was inhibited by rapamycin [218]. Overall, there is significant data implicating canonical Wnt signaling in the mechanical regulation of skeletal muscle mass, while more recent data suggests a possible role for non-canonical Wnt signaling in the activation of mTORC1 signaling. However, while it is tempting to speculate that the non-canonical Wnt signaling-induced activation of mTORC1 could potentially play a role in the mechanical activation of protein synthesis and skeletal muscle hypertrophy, several important questions remain to be answered. For example, are Wnt7a expression and/or secretion increased during or after different types of acute and chronic mechanical stimulation? Given the current evidence that the acute mechanical activation of mTORC1 occurs in a PI3K/Akt-independent manner, perhaps a Wnt7a could contribute to the more delayed (hours) activation of mTORC1. If so, what is the cellular origin of the secreted Wnt7a: skeletal muscle cells, cells of the extra cellular matrix and/or immune cells? Thus, while promising, significantly more work is required to establish a role, if any, for non-canonical Wnt signaling in the mechanical activation of mTORC1, protein synthesis and skeletal muscle growth.

A Role for mTORC1 in the Mechanical Regulation of Bone Mass?

Given the predominant role that mTORC1 signaling plays in mechanically-induced skeletal muscle growth, it is of interest to examine whether there is any evidence for a similar role for mTORC1 in the mechanical regulation of bone mass. To date, there is some evidence that mTORC1 does indeed play a role in bone growth and, more specifically, in the regulation of long bone length. For example, several studies have shown that the mTORC1

inhibitor, rapamycin, inhibits long bone growth in young rodents, possibly by directly inhibiting chondrocyte differentiation and/or indirectly by inhibiting growth plate angiogenesis which leads to reduced chondrogenesis [220–224]. Thus, mTORC1 may play a vital role in long bone growth providing a mechanism for mitogens and nutrients to stimulate bone growth via increased growth plate activity. Importantly, recent evidence also implicates mTORC1 in mechanically-induced cartilage growth [225]. Specifically, the mechanical activation of mTORC1 was found to be necessary for cell proliferation, chondrogenesis, and cartilage growth during embryonic bone development [225]. Other evidence suggests that mTORC1 not only regulates chondrocytes, but also regulates osteoblasts. For instance, rapamycin has been shown to inhibit the proliferation and differentiation of preosteoblastic cells, in part, by inhibiting the expression of cyclins A and D1, and the transcription factor Runx2, respectively [226]. Furthermore, rapamycin has also been reported to inhibit erythropoietin-induced osteoblast differentiation in some preosteoblastic cell lines [227]. These data suggest that mTORC1 could play an important role on regulating bone mass, in part, by modulating the abundance of osteoblasts. This is further supported by a recent study that implicated mTORC1 in a Wnt signaling pathway that enhances post-natal bone mass via an increase in osteoblast numbers and activity [228]. Specifically, in this study, the induction of Wnt7b expression, either during embryonic development or at 4 wk post-natal, resulted in profound increases in bone mass that were related to increased osteoblast numbers [228]. Furthermore, it was found that Wnt7b (and Wnt3a) activated mTORC1 signaling, *in vitro* and *in vivo*, via a non-canonical PI3K/Akt pathway, and that the inducible deletion of the mTORC1 component, Raptor, markedly reduced the Wnt7b-induced increase in osteoblast activity and bone mass [228]. Interestingly, rapamycin analogs (e.g., evirolimus) appear to inhibit osteoclast survival and activity, suggesting that the activation of mTORC1 might also increase bone resorption [229]; however, the Wnt7b-induced increase in mTORC1 signaling and bone mass was not associated with changes in bone resorption [228]. These exciting data suggest that the activation of mTORC1 may indeed play a role in the regulation of bone mass by stimulating an increase in osteoblast numbers and activity.

In summary, an expanding body of evidence suggests that mTORC1 plays an important role in promoting long bone growth by increasing chondrocyte proliferation and growth plate activity. Importantly, mTORC1 has also been shown to promote increases in developmental and post-natal bone mass by stimulating an increase in osteoblast proliferation, differentiation and activity; however, there is currently no direct evidence linking mTORC1 signaling with mechanically-induced increases in osteoblast function and bone mass. Nevertheless, given the evidence that Wnt signaling plays a major role in mechanotransduction in bone, and the recent finding that Wnt7a is sufficient to increase bone mass via an mTORC1-depenent mechanism, future research may lead to the establishment of mTORC1 as a *bona fide* regulator of mechanically-induced increases in bone mass.

CONCLUSION

In conclusion, the molecular mechanisms that regulate mechanotransduction in skeletal muscle and bone are complex, with multiple mechanisms likely operating in synergy. In

skeletal muscle mTORC1 has been established as a central mediator of mechanically-induced changes in protein synthesis and muscle cell size, with several putative candidates proposed to play a role in mTORC1's activation (e.g. PA, amino acids, TSC2 translocation, Ca^{2+} and NO). However, despite these advances, still very little is known about the upstream mechanically-activated sensor(s) that ultimately trigger these second messengers to promote mTORC1 signaling. In bone, a much more complicated picture of mechanotransduction has emerged with the necessity to consider the role of multiple cell types in this process. Nonetheless, current evidence has shown that mechanically-sensitive ion channels and G-protein coupled receptors play significant roles in detecting changes in mechanical stress in bone mass. Furthermore, IGF-1, NO, PG and Wnt signaling have all been implicated as second messengers in bone mechanotransduction. Major questions that remain to be resolved include whether PG and non-canonical Wnt signaling plays a role in the mechanical activation of mTORC1 in skeletal muscle and whether mTORC1 signaling is involved in bone mechanotransduction. This review highlights that there is still much be discovered about the process of mechanotransduction in skeletal muscle and bone. By examining the differences and similarities in mechanotransduction between these two tissues it is hoped that this review will stimulate new insights and ideas for future research and collaboration between bone and muscle biologists. Ultimately, advances in our knowledge of the mechanisms of mechanotransduction will assist in the development of effective exercise and pharmaceutical strategies aimed at increasing, and/or preventing the loss of, bone and skeletal muscle mass.

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ABBREVIATIONS

AA	arachidonic acid
Akt	v-Akt Murine Thymoma Viral Oncogene
BAPTA-AM	1,2-Bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid tetrakis(acetoxymethyl ester)
CaMKKα	Ca^{2+}/calmodulin-dependent protein kinase alpha
Cox	cyclooxygenase
DGK	diacylglycerol kinase
DGKζ	diacylglycerol kinase zeta
Dsh	Dishevelled
ERK	extracellular signal-regulated kinase
FRB	FKBP12-rapamycin binding
FRET	fluorescence resonance energy transfer
Fzd	Frizzled

GAP	GTPase activating protein
Gd^{3+}	gadolinium
GDP	guanosine diphosphate
GPCR	G-protein coupled receptor
GSK3β	glycogen synthase kinase 3 beta
GTP	Guanosine-5'-triphosphate
H_2O_2	hydrogen peroxide
IGF-1	insulin-like growth factor 1
iNOS	inducible nitric oxide synthase
LEF	Lymphoid enhancer-binding factor
LEL	late endosomal/lysosomal
L-NAME	L-N^G-Nitroarginine methyl ester
LPAATθ	lysophosphatidic acid acyltransferase theta
Lrp	lipoprotein receptor-related protein
MAPK	mitogen-activated protein kinase
MEK	Mitogen/Extracellular signal-regulated Kinase
MGF	mechano growth factor
mTOR	mechanistic or mammalian target of rapamycin
mTORC1	mTOR complex1
mTORC2	mTOR complex2
NO	nitric oxide
NOS	nitric oxide synthase
nNOS	neuronal nitric oxide synthase
Nox4	NADPH oxidase 4
O_2^-	superoxide
$ONOO^-$	peroxynitrite
p90RSK	90 kDa ribosomal S6 kinase
PA, PDL1	phospholipase D1; phosphatidic acid
PG	prostaglandin
PI3K	phosphatidylinositide 3-kinase
Pkd	polycystic kidney disease
PTH1R	parathyroid hormone 1 receptor

RAF	rapidly accelerated fibrosarcoma
RANKL	Receptor activator of nuclear factor kappa-B ligand
RAS	rat sarcoma oncogene
Rheb	Ras homologue enriched in the brain
RNS	reactive nitrogen species
ROS	reactive oxygen species
RUNX2	Runt-related transcription factor 2
SR	sarcoplasmic reticulum
SC	satellite cells
TCF	T-cell factor
Trp	transient receptor potential
TrpV1	transient receptor potential cation channel subfamily V1
TrpV4	transient receptor potential cation channel subfamily V4
TSC2	Tuberous Sclerosis Complex 2
Vps34	vacuolar protein sorting 34
Wnt, Wnt	Wingless-related integration site.

REFERENCES

1. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nat Rev Endocrinol. 2012; 8:457–465. [PubMed: 22473333]
2. Yang, J. Enhanced Skeletal Muscle for Effective Glucose Homeostasis. In: Ya-Xiong, T., editor. Progress in Molecular Biology and Translational Science: Academic Press. 2014. p. 133-163.
3. Wolfe RR. The underappreciated role of muscle in health and disease. Am J Clin Nutr. 2006; 84:475–482. [PubMed: 16960159]
4. DiGirolamo DJ, Kiel DP, Esser KA. Bone and Skeletal Muscle: Neighbors With Close Ties. Journal of Bone and Mineral Research. 2013; 28:1509–1518. [PubMed: 23630111]
5. Karsenty G, Oury F. Biology Without Walls: The Novel Endocrinology of Bone. Annual Review of Physiology. 2012; 74:87–105.
6. DiGirolamo DJ, Clemens TL, Kousteni S. The skeleton as an endocrine organ. Nat Rev Rheumatol. 2012; 8:674–683. [PubMed: 23045255]
7. Schiaffino S, Dyar KA, Ciciliot S, Blaauw B, Sandri M. Mechanisms regulating skeletal muscle growth and atrophy. FEBS Journal. 2013; 280:4294–4314. [PubMed: 23517348]
8. Robling AG. The interaction of biological factors with mechanical signals in bone adaptation: recent developments. Curr Osteoporos Rep. 2012; 10:126–131. [PubMed: 22538521]
9. Goodman CA. The Role of mTORC1 in Regulating Protein Synthesis and Skeletal Muscle Mass in Response to Various Mechanical Stimuli. Rev Physiol Biochem Pharmacol. 2014; 166:1–53. [PubMed: 23784619]
10. Adams GR, Bamman MM. Characterization and Regulation of Mechanical Loading-Induced Compensatory Muscle Hypertrophy. Comprehensive Physiology. 2012; 2:2829–2870. [PubMed: 23720267]

11. Hornberger TA. Mechanotransduction and the regulation of mTORC1 signaling in skeletal muscle. Int J Biochem Cell Biol. 2011; 43:1267–1276. [PubMed: 21621634]
12. Arfat Y, Xiao W-Z, Iftikhar S, Zhao F, Li D-J, Sun Y-L, Zhang G, Shang P, Qian A-R. Physiological Effects of Microgravity on Bone Cells. Calcified Tissue International. 2014; 94:569–579. [PubMed: 24687524]
13. Klein-Nulend J, Bacabac RG, Bakker AD. Mechanical loading and how it affects bone cells: the role of the osteocyte cytoskeleton in maintaining our skeleton. Eur Cell Mater. 2012; 24:278–291. [PubMed: 23007912]
14. Robling AG, Turner CH. Mechanical Signaling for Bone Modeling and Remodeling. Cirt Rev Eukaryot Gene Expr. 2009; 19:319–338.
15. Lu TW, Taylor SJ, O'Connor JJ, Walker PS. Influence of muscle activity on the forces in the femur: an in vivo study. J Biomech. 1997; 30:1101–1106. [PubMed: 9456377]
16. Burr DB. Muscle Strength, Bone Mass, and Age-Related Bone Loss. Journal of Bone and Mineral Research. 1997; 12:1547–1551. [PubMed: 9333114]
17. Rodríguez JI, Garcia-Alix A, Palacios J, Paniagua R. Changes in the long bones due to fetal immobility caused by neuromuscular disease. A radiographic and histological study. J Bone Joint Surg Am. 1988; 70:1052–1060. [PubMed: 3403574]
18. Rodríguez J, Palacios J, García-Alix A, Pastor I, Paniagua R. Effects of immobilization on fetal bone development. A morphometric study in newborns with congenital neuromuscular diseases with intrauterine onset. Calcified Tissue International. 1988; 43:335–339. [PubMed: 3146421]
19. Sharir A, Stern T, Rot C, Shahar R, Zelzer E. Muscle force regulates bone shaping for optimal load-bearing capacity during embryogenesis. Development. 2011; 138:3247–3259. [PubMed: 21750035]
20. Hall BK, Herring SW. Paralysis and growth of the musculoskeletal system in the embryonic chick. Journal of Morphology. 1990; 206:45–56. [PubMed: 2246789]
21. Hall JG. Importance of muscle movement for normal craniofacial development. J Craniofac Surg. 2010; 21:1336–1338. [PubMed: 20818259]
22. Rauch F, Bailey DA, Baxter-Jones A, Mirwald R, Faulkner R. The ‘muscle-bone unit’ during the pubertal growth spurt. Bone. 2004; 34:771–775. [PubMed: 15121007]
23. Rauch F, Schoenau E. Peripheral quantitative computed tomography of the proximal radius in young subjects--new reference data and interpretation of results. J Musculoskelet Neuronal Interact. 2008; 8:217–226. [PubMed: 18799854]
24. Fricke O, Beccard R, Semler O, Schoenau E. Analyses of muscular mass and function: the impact on bone mineral density and peak muscle mass. Pediatr Nephrol. 2010; 25:2393–2400. [PubMed: 20458595]
25. Larson CM, Henderson RC. Bone mineral density and fractures in boys with Duchenne muscular dystrophy. J Pediatr Orthop. 2000; 20:71–74. [PubMed: 10641693]
26. Tasdemir HA, Buyukavci M, Akcay F, Polat P, Yildiran A, Karakelleoglu C. Bone mineral density in children with cerebral palsy. Pediatrics International. 2001; 43:157–160. [PubMed: 11285068]
27. Eser P, Frotzler A, Zehnder Y, Wick L, Knecht H, Denoth J, Schiessl H. Relationship between the duration of paralysis and bone structure: a pQCT study of spinal cord injured individuals. Bone. 2004; 34:869–880. [PubMed: 15121019]
28. Lang T, LeBlanc A, Evans H, Lu Y, Genant H, Yu A. Cortical and Trabecular Bone Mineral Loss From the Spine and Hip in Long-Duration Spaceflight. Journal of Bone and Mineral Research. 2004; 19:1006–1012. [PubMed: 15125798]
29. Schoenau E. From mechanostat theory to development of the "Functional Muscle-Bone-Unit". J Musculoskelet Neuronal Interact. 2005; 5:232–238. [PubMed: 16172514]
30. Binkley N, Krueger D, Buehring B. What's in a name revisited: should osteoporosis and sarcopenia be considered components of "dysmobility syndrome?". Osteoporos Int. 2013; 24:2955–2959. [PubMed: 23903951]
31. Goodman CA, Mayhew DL, Hornberger TA. Recent Progress towards Understanding the Molecular Mechanisms that Regulate Skeletal Muscle Mass. Cell Signal. 2011; 23:1896–1906. [PubMed: 21821120]

32. Lynch GS, Ryall JG. Role of {beta}-Adrenoceptor Signaling in Skeletal Muscle: Implications for Muscle Wasting and Disease. Physiol. Rev. 2008; 88:729–767. [PubMed: 18391178]
33. Glass DJ. PI3 Kinase Regulation of Skeletal Muscle Hypertrophy and Atrophy. Curr Top Microbiol Immunol. 2010; 346
34. Huang Z, Chen X, Chen D. Myostatin: A novel insight into its role in metabolism, signal pathways, and expression regulation. Cell Signal. 2011; 23:1441–1446. [PubMed: 21609762]
35. Lee NKL, MacLean HE. Polyamines, androgens and skeletal muscle hypertrophy. J Cell Physiol. 2011; 226:1453–1460. [PubMed: 21413019]
36. McCarthy JJ. The MyomiR network in skeletal muscle plasticity. Exerc Sport Sci Rev. 2011; 39:150–154. [PubMed: 21467943]
37. Phillips BE, Hill DS, Atherton PJ. Regulation of muscle protein synthesis in humans. Curr Opin Clin Nutr Metab Care. 2012; 15:58–63. [PubMed: 22037010]
38. Schiaffino S, Mammucari C. Regulation of skeletal muscle growth by the IGF1-Akt/PKB pathway: insights from genetic models. Skeletal Muscle. 2011; 1:4. [PubMed: 21798082]
39. Berdeaux R, Stewart R. cAMP signaling in skeletal muscle adaptation: hypertrophy, metabolism, and regeneration. American Journal of Physiology - Endocrinology And Metabolism. 2012; 303:E1–E17. [PubMed: 22354781]
40. Dubois V, Laurent M, Boonen S, Vanderschueren D, Claessens F. Androgens and skeletal muscle: cellular and molecular action mechanisms underlying the anabolic actions. Cellular and Molecular Life Sciences. 2012; 69:1651–1667. [PubMed: 22101547]
41. Piccirillo R, Demontis F, Perrimon N, Goldberg AL. Mechanisms of muscle growth and atrophy in mammals and Drosophila. Developmental Dynamics. 2013 *in press*.
42. Blaauw B, Reggiani C. The role of satellite cells in muscle hypertrophy. J Muscle Res Cell Motil. 2014; 35:3–10. [PubMed: 24505026]
43. McCarthy JJ, Mula J, Miyazaki M, Erfani R, Garrison K, Farooqui AB, Srikuea R, Lawson BA, Grimes B, Keller C, Van Zant G, Campbell KS, Esser KA, Dupont-Versteegden EE, Peterson CA. Effective fiber hypertrophy in satellite cell-depleted skeletal muscle. Development. 2011; 138:3657–3666. [PubMed: 21828094]
44. Fornaro M, Hinken AC, Needle S, Hu E, Trendelenburg AU, Mayer A, Rosenstiel A, Chang C, Meier V, Billin AN, Becherer JD, Brace AD, Evans WJ, Glass DJ, Russell AJ. Mechano Growth Factor peptide (MGF) has no apparent effect on muscle myoblasts or primary muscle stem cells. Am J Physiol Endocrinol Metab. 2013 *in press*.
45. O'Connor RS, Pavlath GK, McCarthy JJ, Esser KA. Last Word on Point: Counterpoint: Satellite cell addition is/is not obligatory for skeletal muscle hypertrophy. J Appl Physiol. 2007; 103:1107. [PubMed: 17724310]
46. Laplante M, Sabatini David M. mTOR Signaling in Growth Control and Disease. Cell. 2012; 149:274–293. [PubMed: 22500797]
47. Mahoney SJ, Dempsey JM, Blenis J. Cell Signaling in Protein Synthesis Ribosome Biogenesis and Translation Initiation and Elongation. Prog Mol Biol Transl Sci. 2009; 90C:53–107. [PubMed: 20374739]
48. Goodman CA, Mabrey DM, Frey JW, Miu MH, Schmidt EK, Pierre P, Hornberger TA. Novel insights into the regulation of skeletal muscle protein synthesis as revealed by a new nonradioactive in vivo technique. FASEB J. 2011; 25:1028–1039. [PubMed: 21148113]
49. Goodman CA, Miu MH, Frey JW, Mabrey DM, Lincoln HC, Ge Y, Chen J, Hornberger TA. A Phosphatidylinositol 3-Kinase/Protein Kinase B-independent Activation of Mammalian Target of Rapamycin Signaling Is Sufficient to Induce Skeletal Muscle Hypertrophy. Mol. Biol. Cell. 2010; 21:3258–3268. [PubMed: 20668162]
50. Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, Bauerlein R, Zlotchenko E, Scrimgeour A, Lawrence JC, Glass DJ, Yancopoulos GD. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. Nat Cell Biol. 2001; 3:1014–1019. [PubMed: 11715023]
51. Goodman CA, Frey JW, Mabrey DM, Jacobs BL, Lincoln HC, You J-S, Hornberger TA. The role of skeletal muscle mTOR in the regulation of mechanical load-induced growth. J Physiol. 2011; 589:5485–5501. [PubMed: 21946849]

52. Perrone CE, Fenwick-Smith D, Vandenburgh HH. Collagen and Stretch Modulate Autocrine Secretion of Insulin-like Growth Factor-1 and Insulin-like Growth Factor Binding Proteins from Differentiated Skeletal Muscle Cells. J Biol Chem. 1995; 270:2099–2106. [PubMed: 7530717]
53. Yang S, Alnaqeeb M, Simpson H, Goldspink G. Cloning and characterization of an IGF-1 isoform expressed in skeletal muscle subjected to stretch. J Muscle Res Cell Motil. 1996; 17:487–495. [PubMed: 8884603]
54. McKoy G, Ashley W, Mander J, Yang SY, Williams N, Russell B, Goldspink G. Expression of insulin growth factor-1 splice variants and structural genes in rabbit skeletal muscle induced by stretch and stimulation. J Physiol. 1999; 516:583–592. [PubMed: 10087355]
55. Hameed M, Orrell RW, Cobbold M, Goldspink G, Harridge SD. Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise. J Physiol. 2003; 547:247–254. [PubMed: 12562960]
56. Monier S, Le Cam A, Le Marchand-Brustel Y. Insulin and insulin-like growth factor I. Effects on protein synthesis in isolated muscles from lean and goldthioglucose-obese mice. Diabetes. 1983; 32:392–397. [PubMed: 6404679]
57. Gulve EA, Dice JF. Regulation of protein synthesis and degradation in L8 myotubes. Effects of serum, insulin and insulin-like growth factors. Biochem J. 1989; 260:377–387. [PubMed: 2669733]
58. Coleman ME, DeMayo F, Yin KC, Lee HM, Geske R, Montgomery C, Schwartz RJ. Myogenic Vector Expression of Insulin-like Growth Factor I Stimulates Muscle Cell Differentiation and Myofiber Hypertrophy in Transgenic Mice. J Biol Chem. 1995; 270:12109–12116. [PubMed: 7744859]
59. Barton-Davis ER, Shoturma DI, Musaro A, Rosenthal N, Sweeney HL. Viral mediated expression of insulin-like growth factor I blocks the aging-related loss of skeletal muscle function. Proc Natl Acad Sci U S A. 1998; 95:15603–15607. [PubMed: 9861016]
60. Musaro A, McCullagh K, Paul A, Houghton L, Dobrowolny G, Molinaro M, Barton ER, L Sweeney H, Rosenthal N. Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. Nat Genet. 2001; 27:195–200. [PubMed: 11175789]
61. Dardevet D, Sornet C, Vary T, Grizard J. Phosphatidylinositol 3-kinase and p70 s6 kinase participate in the regulation of protein turnover in skeletal muscle by insulin and insulin-like growth factor I. Endocrinology. 1996; 137:4087–4094. [PubMed: 8828461]
62. Frost RA, Lang CH. Differential Effects of Insulin-Like Growth Factor I (IGF-I) and IGF-Binding Protein-1 on Protein Metabolism in Human Skeletal Muscle Cells. Endocrinology. 1999; 140:3962–3970. [PubMed: 10465265]
63. Rommel C, Bodine SC, Clarke BA, Rossman R, Nunez L, Stitt TN, Yancopoulos GD, Glass DJ. Mediation of IGF-1-induced skeletal myotube hypertrophy by PI(3)K/Akt/mTOR and PI(3)K/Akt/GSK3 pathways. Nat Cell Biol. 2001; 3:1009–1013. [PubMed: 11715022]
64. Latres E, Amini AR, Amini AA, Griffiths J, Martin FJ, Wei Y, Lin HC, Yancopoulos GD, Glass DJ. Insulin-like growth factor-1 (IGF-1) inversely regulates atrophy-induced genes via the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway. J Biol Chem. 2005; 280:2737–2744. [PubMed: 15550386]
65. Park IH, Erbay E, Nuzzi P, Chen J. Skeletal myocyte hypertrophy requires mTOR kinase activity and S6K1. Exp Cell Res. 2005; 309:211–219. [PubMed: 15963500]
66. Fedele MJ, Lang CH, Farrell PA. Immunization against IGF-I prevents increases in protein synthesis in diabetic rats after resistance exercise. Am J Physiol Endocrinol Metab. 2001; 280:E877–E885. [PubMed: 11350769]
67. Liu J-P, Baker J, Perkins AS, Robertson EJ, Efstratiadis A. Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). Cell. 1993; 75:59–72. [PubMed: 8402901]
68. Martineau LC, Gardiner PF. Insight into skeletal muscle mechanotransduction: MAPK activation is quantitatively related to tension. J Appl Physiol. 2001; 91:693–702. [PubMed: 11457783]
69. Drummond MJ, Fry CS, Glynn EL, Dreyer HC, Dhanani S, Timmerman KL, Volpi E, Rasmussen BB. Rapamycin administration in humans blocks the contraction-induced increase in skeletal muscle protein synthesis. J Physiol. 2009; 587:1535–1546. [PubMed: 19188252]

70. Tannerstedt J, Apro W, Blomstrand E. Maximal lengthening contractions induce different signaling responses in the type I and type II fibers of human skeletal muscle. J Appl Physiol. 2009; 106:1412–1418. [PubMed: 19112158]
71. Sasai N, Agata N, Inoue-Miyazu M, Kawakami K, Kobayashi K, Sokabe M, Hayakawa K. Involvement of PI3K/Akt/TOR pathway in stretch-induced hypertrophy of myotubes. Muscle & Nerve. 2010; 41:100–106. [PubMed: 19768770]
72. Miyazaki M, McCarthy JJ, Fedele MJ, Esser KA. Early activation of mTORC1 signalling in response to mechanical overload is independent of phosphoinositide 3-kinase/Akt signalling. J Physiol. 2011; 589:1831–1846. [PubMed: 21300751]
73. Hulmi JJ, Walker S, Ahtiainen JP, Nyman K, Kraemer WJ, Hakkinen K. Molecular signaling in muscle is affected by the specificity of resistance exercise protocol. Scand J Med Sci Sports. 2012; 22:240–248. [PubMed: 21204993]
74. You JS, Frey JW, Hornberger TA. Mechanical Stimulation Induces mTOR Signaling via an ERK-Independent Mechanism: Implications for a Direct Activation of mTOR by Phosphatidic Acid. PLoS ONE. 2012; 7:e47258. [PubMed: 23077579]
75. Roux PP, Ballif BA, Anjum R, Gygi SP, Blenis J. Tumor-promoting phorbol esters and activated Ras inactivate the tuberous sclerosis tumor suppressor complex via p90 ribosomal S6 kinase. Proc Natl Acad Sci U S A. 2004; 101:13489–13494. [PubMed: 15342917]
76. Ma L, Chen Z, Erdjument-Bromage H, Tempst P, Pandolfi PP. Phosphorylation and Functional Inactivation of TSC2 by Erk: Implications for Tuberous Sclerosisand Cancer Pathogenesis. Cell. 2005; 121:179–193. [PubMed: 15851026]
77. Carrière A, Cargnello M, Julien L-A, Gao H, Bonneil É, Thibault P, Roux PP. Oncogenic MAPK Signaling Stimulates mTORC1 Activity by Promoting RSK-Mediated Raptor Phosphorylation. Curr Biol. 2008; 18:1269–1277. [PubMed: 18722121]
78. Fonseca BD, Alain T, Finestone LK, Huang BPH, Rolfe M, Jiang T, Yao Z, Hernandez G, Bennett CF, Proud CG. Pharmacological and Genetic Evaluation of Proposed Roles of Mitogen-activated Protein Kinase/Extracellular Signal-regulated Kinase Kinase (MEK), Extracellular Signal-regulated Kinase (ERK), and p90RSK in the Control of mTORC1 Protein Signaling by Phorbol Esters. J Biol Chem. 2011; 286:27111–27122. [PubMed: 21659537]
79. Anjum R, Blenis J. The RSK family of kinases: emerging roles in cellular signalling. Nat Rev Mol Cell Biol. 2008; 9:747–758. [PubMed: 18813292]
80. Salto R, Vílchez JD, Cabrera E, Guinovart JJ, Girón MD. Activation of ERK by sodium tungstate induces protein synthesis and prevents protein degradation in rat L6 myotubes. FEBS Lett. 2014; 588:2246–2254. [PubMed: 24846141]
81. Fang Y, Vilella-Bach M, Bachmann R, Flanigan A, Chen J. Phosphatidic Acid-Mediated Mitogenic Activation of mTOR Signaling. Science. 2001; 294:1942–1945. [PubMed: 11729323]
82. Yoon MS, Sun Y, Arauz E, Jiang Y, Chen J. Phosphatidic Acid Activates Mammalian Target of Rapamycin Complex 1 (mTORC1) Kinase by Displacing FK506 Binding Protein 38 (FKBP38) and Exerting an Allosteric Effect. J Biol Chem. 2011; 286:29568–29574. [PubMed: 21737445]
83. Avila-Flores A, Santos T, Rincon E, Merida I. Modulation of the Mammalian Target of Rapamycin Pathway by Diacylglycerol Kinase-produced Phosphatidic Acid. J. Biol. Chem. 2005; 280:10091–10099. [PubMed: 15632115]
84. Tang W, Yuan J, Chen X, Gu X, Luo K, Li J, Wan B, Wang Y, Yu L. Identification of a novel human lysophosphatidic acid acyltransferase, LPAAT-theta, which activates mTOR pathway. J Biochem Mol Biol. 2006; 39:626–635. [PubMed: 17002884]
85. O'Neil TK, Duffy LR, Frey JW, Hornberger TA. The role of phosphoinositide 3-kinase and phosphatidic acid in the regulation of mammalian target of rapamycin following eccentric contractions. J Physiol. 2009; 587:3691–3701. [PubMed: 19470781]
86. Foster DA. Regulation of mTOR by Phosphatidic Acid? Cancer Res. 2007; 67:1–4. [PubMed: 17210675]
87. Jaafar R, De Larichaudy J, Chanon S, Euthine V, Durand C, Naro F, Bertolino P, Vidal H, Lefai E, Nemoz G. Phospholipase D regulates the size of skeletal muscle cells through the activation of mTOR signaling. Cell Commun Signal. 2013; 11:55. [PubMed: 23915343]

88. You J-S, Lincoln HC, Kim C-R, Frey JW, Goodman CA, Zhong X-P, Hornberger TA. The Role of Diacylglycerol Kinase ζ and Phosphatidic Acid in the Mechanical Activation of Mammalian Target of Rapamycin (mTOR) Signaling and Skeletal Muscle Hypertrophy. J Biol Chem. 2014; 289:1551–1563. [PubMed: 24302719]

89. Hornberger TA, Chu WK, Mak YW, Hsiung JW, Huang SA, Chien S. The role of phospholipase D and phosphatidic acid in the mechanical activation of mTOR signaling in skeletal muscle. Proc Natl Acad Sci U S A. 2006; 103:4741–4746. [PubMed: 16537399]

90. Huang J, Manning BD. The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. Biochem J. 2008; 412:179–190. [PubMed: 18466115]

91. Aspuria P-J, Tamanoi F. The Rheb family of GTP-binding proteins. Cellular Signalling. 2004; 16:1105–1112. [PubMed: 15240005]

92. Long X, Lin Y, Ortiz-Vega S, Yonezawa K, Avruch J. Rheb Binds and Regulates the mTOR Kinase. Current Biology. 2005; 15:702–713. [PubMed: 15854902]

93. Sato T, Nakashima A, Guo L, Tamanoi F. Specific Activation of mTORC1 by Rheb G-protein in Vitro Involves Enhanced Recruitment of Its Substrate Protein. J. Biol. Chem. 2009; 284:12783–12791. [PubMed: 19299511]

94. Inoki K, Li Y, Xu T, Guan K-L. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. Genes Dev. 2003; 17:1829–1834. [PubMed: 12869586]

95. Tee AR, Manning BD, Roux PP, Cantley LC, Blenis J. Tuberous Sclerosis Complex Gene Products, Tuberin and Hamartin, Control mTOR Signaling by Acting as a GTPase-Activating Protein Complex toward Rheb. Current Biology. 2003; 13:1259–1268. [PubMed: 12906785]

96. Zhang Y, Gao X, Saucedo LJ, Ru B, Edgar BA, Pan D. Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. Nat Cell Biol. 2003; 5:578–581. [PubMed: 12771962]

97. Jacobs BL, You J-S, Frey JW, Goodman CA, Gundermann DM, Hornberger TA. Eccentric Contractions Increase TSC2 Phosphorylation and Alter the Targeting of TSC2 and mTOR to the Lysosome. J Physiol. 2013; 591:4611–4620. [PubMed: 23732640]

98. Jacobs BL, Goodman CA, Hornberger TA. The mechanical activation of mTOR signaling: an emerging role for late endosome/lysosomal targeting. J Muscle Res Cell Motil. 2013; 35:11–21. [PubMed: 24162376]

99. Stephenson DG, Lamb GD, Stephenson GM. Events of the excitation-contraction-relaxation (E-CR) cycle in fast- and slow-twitch mammalian muscle fibres relevant to muscle fatigue. Acta Physiol Scand. 1998; 162:229–245. [PubMed: 9578368]

100. Armstrong RB, Duan C, Delp MD, Hayes DA, Glenn GM, Allen GD. Elevations in rat soleus muscle [Ca2+] with passive stretch. J Appl Physiol. 1993; 74:2990–2997. [PubMed: 8396114]

101. Franco A, Lansman JB. Stretch-sensitive channels in developing muscle cells from a mouse cell line. The Journal of Physiology. 1990; 427:361–380. [PubMed: 2170636]

102. Graves LM, He Y, Lambert J, Hunter D, Li X, Earp HS. An Intracellular Calcium Signal Activates p70 but Not p90 Ribosomal S6 Kinase in Liver Epithelial Cells. Journal of Biological Chemistry. 1997; 272:1920–1928. [PubMed: 8999881]

103. Conus NM, Hemmings BA, Pearson RB. Differential Regulation by Calcium Reveals Distinct Signaling Requirements for the Activation of Akt and p70S6k. J. Biol. Chem. 1998; 273:4776–4782. [PubMed: 9468542]

104. Kameyama T, Etlinger JD. Calcium-dependent regulation of protein synthesis and degradation in muscle. Nature. 1979; 279:344–346. [PubMed: 377097]

105. Sonobe T, Inagaki T, Poole DC, Kano Y. Intracellular calcium accumulation following eccentric contractions in rat skeletal muscle in vivo: role of stretch-activated channels. Am J Physiol Regul Integr Comp Physiol. 2008; 294:R1329–R1337. [PubMed: 18199588]

106. Spangenburg EE, McBride TA. Inhibition of stretch-activated channels during eccentric muscle contraction attenuates p70S6K activation. J Appl Physiol. 2006; 100:129–135. [PubMed: 16179399]

107. Ito N, Ruegg UT, Kudo A, Miyagoe-Suzuki Y, Takeda Si. Activation of calcium signaling through Trpv1 by nNOS and peroxynitrite as a key trigger of skeletal muscle hypertrophy. Nat Med. 2013; 19:101–106. [PubMed: 23202294]

108. Ito N, Ruegg UT, Kudo A, Miyagoe-Suzuki Y, Takeda Si. Capsaicin mimics mechanical load-induced intracellular signaling events: Involvement of TRPV1-mediated calcium signaling in induction of skeletal muscle hypertrophy. Channels. 2013; 7:221–224. [PubMed: 23584166]
109. McGee SL, Mustard KJ, Hardie DG, Baar K. Normal hypertrophy accompanied by phosphoryation and activation of AMP-activated protein kinase α1 following overload in LKB1 knockout mice. J Physiol. 2008; 586:1731–1741. [PubMed: 18202101]
110. Ferey JLA, Brault JJ, Smith CAS, Witczak CA. Constitutive Activation of CaMKKα Signaling is Sufficient But Not Necessary for mTORC1 Activation and Growth in Mouse Skeletal Muscle. Am J Physiol Endocrinol and Metab. 2014 *in press:*
111. Corradetti MN, Guan KL. Upstream of the mammalian target of rapamycin: do all roads pass through mTOR? Oncogene. 2006; 25:6347–6360. [PubMed: 17041621]
112. Powers SK, Jackson MJ. Exercise-Induced Oxidative Stress: Cellular Mechanisms and Impact on Muscle Force Production. Physiol. Rev. 2008; 88:1243–1276. [PubMed: 18923182]
113. Smith LW, Smith JD, Criswell DS. Involvement of nitric oxide synthase in skeletal muscle adaptation to chronic overload. J Appl Physiol. 2002; 92:2005–2011. [PubMed: 11960951]
114. Soltow QA, Betters JL, Sellman JE, Lira VA, Long JH, Criswell DS. Ibuprofen inhibits skeletal muscle hypertrophy in rats. Med Sci Sports Exerc. 2006; 38:840–846. [PubMed: 16672835]
115. Soltow QA, Zeanah EH, Lira VA, Criswell DS. Cessation of cyclic stretch induces atrophy of C2C12 myotubes. Biochem Biophys Res Commun. 2013; 434:316–321. [PubMed: 23541574]
116. Suzuki N, Motohashi N, Uezumi A, Fukada S-i, Yoshimura T, Itoyama Y, Aoki M, Miyagoe-Suzuki Y, Takeda Si. NO production results in suspension-induced muscle atrophy through dislocation of neuronal NOS. J. Clin. Invest. 2007; 117:2468–2476. [PubMed: 17786240]
117. Jewell JL, Russell RC, Guan K-L. Amino acid signalling upstream of mTOR. Nat Rev Mol Cell Biol. 2013; 14:133–139. [PubMed: 23361334]
118. Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, Sabatini DM. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science. 2008; 320:1496–1501. [PubMed: 18497260]
119. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. Ragulator-Rag Complex Targets mTORC1 to the Lysosomal Surface and Is Necessary for Its Activation by Amino Acids. Cell. 2010; 141:290–303. [PubMed: 20381137]
120. Zoncu R, Bar-Peled L, Efeyan A, Wang S, Sancak Y, Sabatini DM. mTORC1 Senses Lysosomal Amino Acids Through an Inside-Out Mechanism That Requires the Vacuolar H+-ATPase. Science. 2011; 334:678–683. [PubMed: 22053050]
121. Efeyan A, Zoncu R, Sabatini DM. Amino acids and mTORC1: from lysosomes to disease. Trends in Molecular Medicine. 2012; 18:524–533. [PubMed: 22749019]
122. Anthony JC, Yoshizawa F, Anthony TG, Vary TC, Jefferson LS, Kimball SR. Leucine Stimulates Translation Initiation in Skeletal Muscle of Postabsorptive Rats via a Rapamycin-Sensitive Pathway. J Nutr. 2000; 130:2413–2419. [PubMed: 11015466]
123. Dickinson JM, Fry CS, Drummond MJ, Gundermann DM, Walker DK, Glynn EL, Timmerman KL, Dhanani S, Volpi E, Rasmussen BB. Mammalian Target of Rapamycin Complex 1 Activation Is Required for the Stimulation of Human Skeletal Muscle Protein Synthesis by Essential Amino Acids. J Nutr. 2011; 141:856–862. [PubMed: 21430254]
124. MacKenzie MG, Hamilton DL, Murray JT, Baar K. mVps34 is activated by an acute bout of resistance exercise. Biochem Soc Trans. 2007; 035:1314–1316. [PubMed: 17956340]
125. Gulati P, Gaspers LD, Dann SG, Joaquin M, Nobukuni T, Natt F, Kozma SC, Thomas AP, Thomas G. Amino Acids Activate mTOR Complex 1 via Ca2+/CaM Signaling to hVps34. Cell Metab. 2008; 7:456–465. [PubMed: 18460336]
126. Gran P, Cameron-Smith D. The actions of exogenous leucine on mTOR signalling and amino acid transporters in human myotubes. BMC Physiology. 2011; 11:10. [PubMed: 21702994]
127. Xu L, Salloum D, Medlin PS, Saqcena M, Yellen P, Perrella B, Foster DA. Phospholipase D Mediates Nutrient Input to Mammalian Target of Rapamycin Complex 1 (mTORC1). J Biol Chem. 2011; 286:25477–25486. [PubMed: 21622984]

128. Yoon M-S, Du G, Backer JM, Frohman MA, Chen J. Class III PI-3-kinase activates phospholipase D in an amino acid-sensing mTORC1 pathway. J Cell Biol. 2011; 195:435–447. [PubMed: 22024166]
129. D'Souza RF, Marworth JF, Figueiredo VC, Della Gatta PA, Petersen AC, Mitchell CJ, Cameron-Smith D. Dose-dependent increases in p70S6K phosphorylation and intramuscular branched-chain amino acids in older men following resistance exercise and protein intake. Physiol Rep. 2014; 2
130. Enlow, DH. Principles of Bone Remodeling. IL: Charles C. Thomas; 1963.
131. Bonewald LF. Osteocytes as dynamic multifunctional cells. Ann N Y Acad Sci. 2007; 1116:281–290. [PubMed: 17646259]
132. Bonewald LF. Generation and function of osteocyte dendritic processes. J Musculoskelet Neuronal Interact. 2005; 5:321–324. [PubMed: 16340122]
133. Lanyon LE. Osteocytes, strain detection, bone modeling and remodeling. Calcif Tissue Int. 1993; 53(Suppl 1):S102–S106. discussion S106-7. [PubMed: 8275362]
134. Klein-Nulend J, Bakker AD, Bacabac RG, Vatsa A, Weinbaum S. Mechanosensation and transduction in osteocytes. Bone. 2013; 54:182–190. [PubMed: 23085083]
135. Loiselle AE, Jiang JX, Donahue HJ. Gap junction and hemichannel functions in osteocytes. Bone. 2013; 54:205–212. [PubMed: 23069374]
136. Qing H, Bonewald LF. Osteocyte remodeling of the perilacunar and pericanalicular matrix. Int J Oral Sci. 2009; 1:59–65. [PubMed: 20687297]
137. Frost, HM. Bone Modeling and Skeletal Modeling Errors. IL: Charles C. Thomas; 1973.
138. Paic F, Igwe JC, Nori R, Kronenberg MS, Franceschetti T, Harrington P, Kuo L, Shin DG, Rowe DW, Harris SE, Kalajzic I. Identification of differentially expressed genes between osteoblasts and osteocytes. Bone. 2009; 45:682–692. [PubMed: 19539797]
139. Charras GT, Williams BA, Sims SM, Horton MA. Estimating the sensitivity of mechanosensitive ion channels to membrane strain and tension. Biophys J. 2004; 87:2870–2884. [PubMed: 15454477]
140. Gong X, Fan Y, Zhang Y, Luo C, Duan X, Yang L, Pan J. Inserted rest period resensitizes MC3T3-E1 cells to fluid shear stress in a time-dependent manner via F-actin-regulated mechanosensitive channel(s). Biosci Biotechnol Biochem. 2014; 78:565–573. [PubMed: 25036951]
141. Chen NX, Ryder KD, Pavalko FM, Turner CH, Burr DB, Qiu J, Duncan RL. Ca(2+) regulates fluid shear-induced cytoskeletal reorganization and gene expression in osteoblasts. Am J Physiol Cell Physiol. 2000; 278:C989–C997. [PubMed: 10794673]
142. Ryder KD, Duncan RL. Parathyroid hormone enhances fluid shear-induced [Ca2+]i signaling in osteoblastic cells through activation of mechanosensitive and voltage-sensitive Ca2+ channels. J Bone Miner Res. 2001; 16:240–248. [PubMed: 11204424]
143. Lieben L, Carmeliet G. The Involvement of TRP Channels in Bone Homeostasis. Front Endocrinol (Lausanne). 2012; 3:99. [PubMed: 22934090]
144. Guilak F, Leddy HA, Liedtke W. Transient receptor potential vanilloid 4: The sixth sense of the musculoskeletal system? Ann N Y Acad Sci. 2010; 1192:404–409. [PubMed: 20392266]
145. Hill-Eubanks DC, Gonzales AL, Sonkusare SK, Nelson MT. Vascular TRP Channels: Performing Under Pressure and Going with the Flow. Physiology (Bethesda). 2014; 29:343–360. [PubMed: 25180264]
146. Mizoguchi F, Mizuno A, Hayata T, Nakashima K, Heller S, Ushida T, Sokabe M, Miyasaka N, Suzuki M, Ezura Y, Noda M. Transient receptor potential vanilloid 4 deficiency suppresses unloading-induced bone loss. J Cell Physiol. 2008; 216:47–53. [PubMed: 18264976]
147. Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, Elia AE, Lu W, Brown EM, Quinn SJ, Ingber DE, Zhou J. Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. Nat Genet. 2003; 33:129–137. [PubMed: 12514735]
148. Malicki J, Avidor-Reiss T. From the cytoplasm into the cilium: bon voyage. Organogenesis. 2014; 10:138–157. [PubMed: 24786986]
149. Patel A, Honore E. Polycystins and renovascular mechanosensory transduction. Nat Rev Nephrol. 2010; 6:530–538. [PubMed: 20625375]

150. Xiao Z, Dallas M, Qiu N, Nicolella D, Cao L, Johnson M, Bonewald L, Quarles LD. Conditional deletion of Pkd1 in osteocytes disrupts skeletal mechanosensing in mice. FASEB J. 2011; 25:2418–2432. [PubMed: 21454365]

151. Wang H, Sun W, Ma J, Pan Y, Wang L, Zhang W. Polycystin-1 mediates mechanical strain-induced osteoblastic mechanoresponses via potentiation of intracellular calcium and Akt/beta-catenin pathway. PLoS One. 2014; 9:e91730. [PubMed: 24618832]

152. McNamara LM, Majeska RJ, Weinbaum S, Friedrich V, Schaffler MB. Attachment of osteocyte cell processes to the bone matrix. Anat Rec (Hoboken). 2009; 292:355–363. [PubMed: 19248169]

153. Zhou L, Bohn LM. Functional selectivity of GPCR signaling in animals. Curr Opin Cell Biol. 2014; 27:102–108. [PubMed: 24680435]

154. Reich KM, McAllister TN, Gudi S, Frangos JA. Activation of G proteins mediates flow-induced prostaglandin E2 production in osteoblasts. Endocrinology. 1997; 138:1014–1018. [PubMed: 9048603]

155. Chachisvilis M, Zhang YL, Frangos JA. G protein-coupled receptors sense fluid shear stress in endothelial cells. Proc Natl Acad Sci U S A. 2006; 103:15463–15468. [PubMed: 17030791]

156. Zhang YL, Frangos JA, Chachisvilis M. Mechanical stimulus alters conformation of type 1 parathyroid hormone receptor in bone cells. Am J Physiol Cell Physiol. 2009; 296:C1391–C1399. [PubMed: 19369447]

157. Gudi S, Nolan JP, Frangos JA. Modulation of GTPase activity of G proteins by fluid shear stress and phospholipid composition. Proc Natl Acad Sci U S A. 1998; 95:2515–2519. [PubMed: 9482917]

158. Yang Y, Tang LQ, Wei W. Prostanoids receptors signaling in different diseases/cancers progression. J Recept Signal Transduct Res. 2013; 33:14–27. [PubMed: 23327583]

159. Thorsen K, Kristoffersson AO, Lerner UH, Lorentzon RP. In situ microdialysis in bone tissue. Stimulation of prostaglandin E2 release by weight-bearing mechanical loading. J Clin Invest. 1996; 98:2446–2449. [PubMed: 8958205]

160. Forwood MR, Kelly WL, Worth NF. Localisation of prostaglandin endoperoxide H synthase (PGHS)-1 and PGHS-2 in bone following mechanical loading in vivo. Anat Rec. 1998; 252:580–586. [PubMed: 9845208]

161. Forwood MR. Inducible cyclo-oxygenase (COX-2) mediates the induction of bone formation by mechanical loading in vivo. J Bone Miner Res. 1996; 11:1688–1693. [PubMed: 8915776]

162. Li J, Burr DB, Turner CH. Suppression of prostaglandin synthesis with NS-398 has different effects on endocortical and periosteal bone formation induced by mechanical loading. Calcif Tissue Int. 2002; 70:320–329. [PubMed: 12004337]

163. Gong X, Yang W, Wang L, Duncan RL, Pan J. Prostaglandin E2 modulates F-actin stress fiber in FSS-stimulated MC3T3-E1 cells in a PKA-dependent manner. Acta Biochim Biophys Sin (Shanghai). 2014; 46:40–47. [PubMed: 24296051]

164. Castillo AB, Triplett JW, Pavalko FM, Turner CH. Estrogen receptor-beta regulates mechanical signaling in primary osteoblasts. Am J Physiol Endocrinol Metab. 2014; 306:E937–E944. [PubMed: 24619882]

165. Li Y, Luo Y, Huang K, Xing J, Xie Z, Lin M, Yang L, Wang Y. The responses of osteoblasts to fluid shear stress depend on substrate chemistries. Arch Biochem Biophys. 2013; 539:38–50. [PubMed: 24051006]

166. Batra N, Burra S, Siller-Jackson AJ, Gu S, Xia X, Weber GF, DeSimone D, Bonewald LF, Lafer EM, Sprague E, Schwartz MA, Jiang JX. Mechanical stress-activated integrin alpha5beta1 induces opening of connexin 43 hemichannels. Proc Natl Acad Sci U S A. 2012; 109:3359–3364. [PubMed: 22331870]

167. Liu D, Genetos DC, Shao Y, Geist DJ, Li J, Ke HZ, Turner CH, Duncan RL. Activation of extracellular-signal regulated kinase (ERK1/2) by fluid shear is Ca(2+)- and ATP-dependent in MC3T3-E1 osteoblasts. Bone. 2008; 42:644–652. [PubMed: 18291742]

168. Li J, Liu D, Ke HZ, Duncan RL, Turner CH. The P2X7 nucleotide receptor mediates skeletal mechanotransduction. J Biol Chem. 2005; 280:42952–42959. [PubMed: 16269410]

169. Galea GL, Sunters A, Meakin LB, Zaman G, Sugiyama T, Lanyon LE, Price JS. Sost down-regulation by mechanical strain in human osteoblastic cells involves PGE2 signaling via EP4. FEBS Lett. 2011; 585:2450–2454. [PubMed: 21723865]

170. Sanuki R, Mitsui N, Suzuki N, Koyama Y, Yamaguchi A, Isokawa K, Shimizu N, Maeno M. Effect of compressive force on the production of prostaglandin E(2) and its receptors in osteoblastic Saos-2 cells. Connect Tissue Res. 2007; 48:246–253. [PubMed: 17882700]

171. Bonewald LF, Johnson ML. Osteocytes, mechanosensing and Wnt signaling. Bone. 2008; 42:606–615. [PubMed: 18280232]

172. Clevers H, Nusse R. Wnt/beta-catenin signaling and disease. Cell. 2012; 149:1192–1205. [PubMed: 22682243]

173. Kikuchi A, Kishida S, Yamamoto H. Regulation of Wnt signaling by protein-protein interaction and post-translational modifications. Exp Mol Med. 2006; 38:1–10. [PubMed: 16520547]

174. Hens JR, Wilson KM, Dann P, Chen X, Horowitz MC, Wysolmerski JJ. TOPGAL mice show that the canonical Wnt signaling pathway is active during bone development and growth and is activated by mechanical loading in vitro. J Bone Miner Res. 2005; 20:1103–1113. [PubMed: 15940363]

175. Norvell SM, Alvarez M, Bidwell JP, Pavalko FM. Fluid shear stress induces beta-catenin signaling in osteoblasts. Calcif Tissue Int. 2004; 75:396–404. [PubMed: 15592796]

176. Kamel MA, Picconi JL, Lara-Castillo N, Johnson ML. Activation of beta-catenin signaling in MLOY4 osteocytic cells versus 2T3 osteoblastic cells by fluid flow shear stress and PGE2: Implications for the study of mechanosensation in bone. Bone. 2010; 47:872–881. [PubMed: 20713195]

177. Sawakami K, Robling AG, Ai M, Pitner ND, Liu D, Warden SJ, Li J, Maye P, Rowe DW, Duncan RL, Warman ML, Turner CH. The Wnt co-receptor LRP5 is essential for skeletal mechanotransduction but not for the anabolic bone response to parathyroid hormone treatment. J Biol Chem. 2006; 281:23698–23711. [PubMed: 16790443]

178. Saxon LK, Jackson BF, Sugiyama T, Lanyon LE, Price JS. Analysis of multiple bone responses to graded strains above functional levels, and to disuse, in mice in vivo show that the human Lrp5 G171V High Bone Mass mutation increases the osteogenic response to loading but that lack of Lrp5 activity reduces it. Bone. 2011; 49:184–193. [PubMed: 21419885]

179. Robling AG, Niziolek PJ, Baldridge LA, Condon KW, Allen MR, Alam I, Mantila SM, Gluhak-Heinrich J, Bellido TM, Harris SE, Turner CH. Mechanical stimulation of bone in vivo reduces osteocyte expression of Sost/sclerostin. J Biol Chem. 2008; 283:5866–5875. [PubMed: 18089564]

180. Tu X, Rhee Y, Condon KW, Bivi N, Allen MR, Dwyer D, Stolina M, Turner CH, Robling AG, Plotkin LI, Bellido T. Sost downregulation and local Wnt signaling are required for the osteogenic response to mechanical loading. Bone. 2012; 50:209–217. [PubMed: 22075208]

181. Morse A, McDonald MM, Kelly NH, Melville KM, Schindeler A, Kramer I, Kneissel M, van der Meulen MCH, Little DG. Mechanical Load Increases in Bone Formation via a Sclerostin-Independent Pathway. Journal of Bone and Mineral Research. 2014; 29:2456–2467. [PubMed: 24821585]

182. Lin C, Jiang X, Dai Z, Guo X, Weng T, Wang J, Li Y, Feng G, Gao X, He L. Sclerostin Mediates Bone Response to Mechanical Unloading Through Antagonizing Wnt/β-Catenin Signaling. Journal of Bone and Mineral Research. 2009; 24:1651–1661. [PubMed: 19419300]

183. Wang Y, Nishida S, Sakata T, Elalieh HZ, Chang W, Halloran BP, Doty SB, Bikle DD. Insulin-like growth factor-I is essential for embryonic bone development. Endocrinology. 2006; 147:4753–4761. [PubMed: 16857753]

184. 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. [PubMed: 23032105]

185. Raab-Cullen DM, Thiede MA, Petersen DN, Kimmel DB, Recker RR. Mechanical loading stimulates rapid changes in periosteal gene expression. Calcif Tissue Int. 1994; 55:473–478. [PubMed: 7895187]

186. Reijnders CMA, Bravenboer N, Tromp AM, Blankenstein MA, Lips P. Effect of mechanical loading on insulin-like growth factor-I gene expression in rat tibia. Journal of Endocrinology. 2007; 192:131–140. [PubMed: 17210750]

187. Lean JM, Jagger CJ, Chambers TJ, Chow JW. Increased insulin-like growth factor I mRNA expression in rat osteocytes in response to mechanical stimulation. Am J Physiol. 1995; 268:E318–E327. [PubMed: 7864109]

188. Sakata T, Wang Y, Halloran BP, Elalieh HZ, Cao J, Bikle DD. 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. 2004; 19:436–446. [PubMed: 15040832]

189. Gross TS, Srinivasan S, Liu CC, Clemens TL, Bain SD. Noninvasive loading of the murine tibia: an in vivo model for the study of mechanotransduction. J Bone Miner Res. 2002; 17:493–501. [PubMed: 11874240]

190. Kesavan C, Wergedal JE, Lau KH, Mohan S. Conditional disruption of IGF-I gene in type 1alpha collagen-expressing cells shows an essential role of IGF-I in skeletal anabolic response to loading. Am J Physiol Endocrinol Metab. 2011; 301:E1191–E1197. [PubMed: 21878662]

191. Lau KH, Baylink DJ, Zhou XD, Rodriguez D, Bonewald LF, Li Z, Ruffoni D, Muller R, Kesavan C, Sheng MH. Osteocyte-derived insulin-like growth factor I is essential for determining bone mechanosensitivity. Am J Physiol Endocrinol Metab. 2013; 305:E271–E281. [PubMed: 23715728]

192. Machwate M, Zerath E, Holy X, Pastoureau P, Marie PJ. Insulin-like growth factor-I increases trabecular bone formation and osteoblastic cell proliferation in unloaded rats. Endocrinology. 1994; 134:1031–1038. [PubMed: 8119139]

193. Kubota T, Elalieh HZ, Saless N, Fong C, Wang Y, Babey M, Cheng Z, Bikle DD. Insulin-like growth factor-1 receptor in mature osteoblasts is required for periosteal bone formation induced by reloading. Acta Astronaut. 2013; 92:73–78. [PubMed: 23976802]

194. Klein-Nulend J, van Oers RF, Bakker AD, Bacabac RG. Nitric oxide signaling in mechanical adaptation of bone. Osteoporos Int. 2014; 25:1427–1437. [PubMed: 24322479]

195. Gonzalez O, Fong KD, Trindade MC, Warren SM, Longaker MT, Smith RL. Fluid shear stress magnitude, duration, and total applied load regulate gene expression and nitric oxide production in primary calvarial osteoblast cultures. Plast Reconstr Surg. 2008; 122:419–428. [PubMed: 18626357]

196. McGarry JG, Klein-Nulend J, Prendergast PJ. The effect of cytoskeletal disruption on pulsatile fluid flow-induced nitric oxide and prostaglandin E2 release in osteocytes and osteoblasts. Biochem Biophys Res Commun. 2005; 330:341–348. [PubMed: 15781270]

197. McAllister TN, Du T, Frangos JA. Fluid shear stress stimulates prostaglandin and nitric oxide release in bone marrow-derived preosteoclast-like cells. Biochem Biophys Res Commun. 2000; 270:643–648. [PubMed: 10753677]

198. Turner CH, Takano Y, Owan I, Murrell GA. Nitric oxide inhibitor L-NAME suppresses mechanically induced bone formation in rats. Am J Physiol. 1996; 270:E634–E639. [PubMed: 8928770]

199. Watanuki M, Sakai A, Sakata T, Tsurukami H, Miwa M, Uchida Y, Watanabe K, Ikeda K, Nakamura T. Role of inducible nitric oxide synthase in skeletal adaptation to acute increases in mechanical loading. J Bone Miner Res. 2002; 17:1015–1025. [PubMed: 12054156]

200. Rahnert J, Fan X, Case N, Murphy TC, Grassi F, Sen B, Rubin J. The role of nitric oxide in the mechanical repression of RANKL in bone stromal cells. Bone. 2008; 43:48–54. [PubMed: 18440890]

201. Kitase Y, Barragan L, Qing H, Kondoh S, Jiang JX, Johnson ML, Bonewald LF. Mechanical induction of PGE2 in osteocytes blocks glucocorticoid-induced apoptosis through both the beta-catenin and PKA pathways. J Bone Miner Res. 2010; 25:2657–2668. [PubMed: 20578217]

202. Karim SM, Sandler M, Williams ED. Distribution of prostaglandins in human tissues. Br J Pharmacol Chemother. 1967; 31:340–344. [PubMed: 12262295]

203. Rodemann HP, Goldberg AL. Arachidonic acid, prostaglandin E2 and F2 alpha influence rates of protein turnover in skeletal and cardiac muscle. Journal of Biological Chemistry. 1982; 257:1632–1638. [PubMed: 6799511]

204. Markworth JF, Cameron-Smith D. Prostaglandin F2α stimulates PI3K/ERK/mTOR signaling and skeletal myotube hypertrophy. American Journal of Physiology - Cell Physiology. 2011; 300:C671–C682. [PubMed: 21191105]

205. Smith RH, Palmer RM, Reeds PJ. Protein synthesis in isolated rabbit forelimb muscles. The possible role of metabolites of arachidonic acid in the response to intermittent stretching. Biochem. J. 1983; 214:153–161. [PubMed: 6412693]

206. Markworth JF, Cameron-Smith D. Arachidonic acid supplementation enhances in vitro skeletal muscle cell growth via a COX-2-dependent pathway. American Journal of Physiology - Cell Physiology. 2013; 304:C56–C67. [PubMed: 23076795]

207. Korotkova M, Lundberg IE. The skeletal muscle arachidonic acid cascade in health and inflammatory disease. Nat Rev Rheumatol. 2014; 10:295–303. [PubMed: 24468934]

208. Trappe TA, Liu SZ. Effects of prostaglandins and COX-inhibiting drugs on skeletal muscle adaptations to exercise. Journal of Applied Physiology. 2013; 115:909–919. [PubMed: 23539318]

209. Palmer RM. Prostaglandins and the control of muscle protein synthesis and degradation. Prostaglandins Leukot Essent Fatty Acids. 1990; 39:95–104. [PubMed: 2188265]

210. Trappe TA, White F, Lambert CP, Cesar D, Hellerstein M, Evans WJ. Effect of ibuprofen and acetaminophen on postexercise muscle protein synthesis. Am J Physiol Endocrinol Metab. 2002; 282:E551–E556. [PubMed: 11832356]

211. Novak ML, Billich W, Smith SM, Sukhija KB, McLoughlin TJ, Hornberger TA, Koh TJ. COX-2 inhibitor reduces skeletal muscle hypertrophy in mice. Am J Physiol Regul Integr Comp Physiol. 2009; 296:R1132–R1139. [PubMed: 19176887]

212. von Maltzahn J, Chang NC, Bentzinger CF, Rudnicki MA. Wnt signaling in myogenesis. Trends in Cell Biology. 2012; 22:602–609. [PubMed: 22944199]

213. Tsivitse S. Notch and wnt signaling, physiological stimuli and postnatal myogenesis. Int J Biol Sci. 2010; 6:268–281. [PubMed: 20567496]

214. Aschenbach WG, Ho RC, Sakamoto K, Fujii N, Li Y, Kim Y-B, Hirshman MF, Goodyear LJ. Regulation of Dishevelled and beta-catenin in rat skeletal muscle: an alternative exercise-induced GSK- 3beta signaling pathway. Am J Physiol Endocrinol Metab. 2006; 291:E152–E158. [PubMed: 16478782]

215. Leal ML, Lamas L, Aoki MS, Ugrinowitsch C, Ramos MS, Tricoli V, Moriscot AS. Effect of different resistance-training regimens on the WNT-signaling pathway. Eur J Appl Physiol. 2011; 111:2535–2545. [PubMed: 21365345]

216. Armstrong DD, Esser KA. Wnt/{beta}-catenin signaling activates growth-control genes during overload-induced skeletal muscle hypertrophy. Am J Physiol Cell Physiol. 2005; 289:C853–C859. [PubMed: 15888552]

217. Armstrong DD, Wong VL, Esser KA. Expression of beta-catenin is necessary for physiological growth of adult skeletal muscle. Am J Physiol Cell Physiol. 2006; 291:C185–C188. [PubMed: 16436469]

218. von Maltzahn J, Bentzinger CF, Rudnicki MA. Wnt7a-Fzd7 signalling directly activates the Akt/mTOR anabolic growth pathway in skeletal muscle. Nat Cell Biol. 2012; 14:186–191. [PubMed: 22179044]

219. von Maltzahn J, Zinoviev R, Chang NC, Bentzinger CF, Rudnicki MA. A truncated Wnt7a retains full biological activity in skeletal muscle. Nat Commun. 2013; 4

220. Phornphutkul C, Lee M, Voigt C, Wu K-Y, Ehrlich MG, Gruppuso PA, Chen Q. The effect of rapamycin on bone growth in rabbits. Journal of Orthopaedic Research. 2009; 27:1157–1161. [PubMed: 19382193]

221. Sanchez C, He Y-Z. Bone growth during rapamycin therapy in young rats. BMC Pediatrics. 2009; 9:3. [PubMed: 19144108]

222. Alvarez-Garcia O, Garcia-Lopez E, Loredo V, Gil-Pena H, Rodriguez-Suarez J, Ordonez FA, Carbajo-Perez E, Santos F. Rapamycin induces growth retardation by disrupting angiogenesis in the growth plate. Kidney Int. 2010; 78:561–568. [PubMed: 20555322]

223. Álvarez-García Ó, García-López E, Loredo V, Gil-Peña H, Mejía-Gaviria N, Rodríguez-Suárez J, Ordóñez FÁ, Santos F. Growth Hormone Improves Growth Retardation Induced by Rapamycin without Blocking Its Antiproliferative and Antiangiogenic Effects on Rat Growth Plate. PLoS ONE. 2012; 7:e34788. [PubMed: 22493717]

224. Phornphutkul C, Wu K-Y, Auyeung V, Chen Q, Gruppuso PA. mTOR signaling contributes to chondrocyte differentiation. Developmental Dynamics. 2008; 237:702–712. [PubMed: 18265001]

225. Guan Y, Yang X, Yang W, Charbonneau C, Chen Q. Mechanical activation of mammalian target of rapamycin pathway is required for cartilage development. The FASEB Journal. 2014

226. Singha UK, Jiang Y, Yu S, Luo M, Lu Y, Zhang J, Xiao G. Rapamycin inhibits osteoblast proliferation and differentiation in MC3T3-E1 cells and primary mouse bone marrow stromal cells. Journal of Cellular Biochemistry. 2008; 103:434–446. [PubMed: 17516572]

227. Kim J, Jung Y, Sun H, Joseph J, Mishra A, Shiozawa Y, Wang J, Krebsbach PH, Taichman RS. Erythropoietin mediated bone formation is regulated by mTOR signaling. Journal of Cellular Biochemistry. 2012; 113:220–228. [PubMed: 21898543]

228. Chen J, Tu X, Esen E, Joeng KS, Lin C, Arbeit JM, Rüegg MA, Hall MN, Ma L, Long F. WNT7B Promotes Bone Formation in part through mTORC1. PLoS Genet. 2014; 10:e1004145. [PubMed: 24497849]

229. Hadji P, Coleman R, Gnant M. Bone effects of mammalian target of rapamycin (mTOR) inhibition with everolimus. Critical Reviews in Oncology/Hematology. 2013; 87:101–111. [PubMed: 23838481]

Highlights

- We review the primary molecular mechanisms that have been implicated in skeletal muscle and bone mechanotransduction.
- mTORC1 signaling plays a fundamental role in the mechanical regulation of skeletal muscle mass and has several putative activators.
- GPCRs, ion channels, prostaglandins, IGF-1, Wnts and NO play roles in bone mechanostransduction.
- It remains to be determined whether mTORC1 signaling plays a role in the mechanical regulation of bone mass.
- It remains unknown whether prostaglandins and/or Wnts play a role in the mechanical activation of mTORC1 in skeletal muscle.

Table 1

Summary of the pathways/molecules involved in muscle and/or bone mechanotransduction[1,2]

	Muscle	Bone
mTORC1	✓✓✓	
IGF-1	✓	✓✓
Erk1/2	✓	✓✓
PA	✓✓	
TSC2/Rheb	✓	
iCa^{2+}	✓	✓✓✓
ROS/RNS	✓	✓✓
AA	✓	
GPCRs		✓
PGs	✓	✓✓
Wnt	✓	✓✓✓

[1] the number of check marks indicate the strength of the role in mechanotransduction (based on the literature).

[2] see text for full names of abbreviated molecules