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MUSCLE AND BONE (L BONEWALD AND M HAMRICK, SECTION EDITORS)

Biomechanical Aspects of the Muscle-Bone Interaction

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Abstract There is growing interest in the interaction between skeletal muscle and bone, particularly at the genetic and molecular levels. However, the genetic and molecular linkages between muscle and bone are achieved only within the context of the essential mechanical coupling of the tissues. This biomechanical and physiological linkage is readily evident as muscles attach to bone and induce exposure to varied mechanical stimuli via functional activity. The responsiveness of bone cells to mechanical stimuli, or their absence, is well established. However, questions remain regarding how muscle forces applied to bone serve to modulate bone homeostasis and adaptation. Similarly, the contributions of varied, but unique, stimuli generated by muscle to bone (such as low-magnitude, high-frequency stimuli) remains to be established. The current article focuses upon the mechanical relationship between muscle and bone. In doing so, we explore the stimuli that muscle imparts upon bone, models that enable investigation of this relationship, and recent data generated by these models.

Keywords Bone strain · Botox · Interstitial fluid flow · Intramedullary pressure · Muscle paralysis · Tail suspension · Vibration

Introduction

Interest in the interaction between skeletal muscle and bone continues to increase and broaden. The concomitant involution of both tissues during aging leads to declines in muscle and bone strength. This degeneration often manifests in reductions in mobility and function, an increased propensity for falls and fractures, and heightened morbidity and mortality in otherwise healthy, aging individuals [1, 2]. With the progressive aging of the population and increases in life expectancy, which allows for greater absolute declines in muscle and bone strength across the lifespan, the consequences of muscle and bone changes during aging are approaching epidemic status. To stem the tide and "kill two birds with one stone," there is a desire to develop interventions that simultaneously improve muscle and bone [3••]. The potential of such interventions would be enhanced if the two tissues interact by sharing common genetic and/or molecular pathways or interact in such a way that a positive change in one tissue directly modulates a similarly positive change in the other.

Muscle and bone are inextricably linked genetically, molecularly, and mechanically. The intertwining of the connections at the different organizational levels (subcellular, cellular, and supracellular) makes it difficult to tease out the relative contributions of each connection. For instance, a change in the molecular communication between the tissues on the cellular level will likely also change their mechanical linkage at the supracellular (i.e., tissue and organ) level and vice versa.

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Despite challenges in isolating the connections between muscle and bone, there is considerable interest in establishing and exploring genetic and molecular links between the tissues as knowledge in this area holds the key towards the development of novel therapies [3••, 4]. Muscle and bone share the same mesodermal origin and, thus, it is reasonable to hypothesize that the two tissues share genetic determinants. Accordingly, there is substantial effort aimed at establishing pleiotropic genes between the tissues [5]. Similarly, there is a burgeoning body of work exploring molecular "cross talk" between muscle and bone, with recent studies demonstrating that both tissues release endocrine, paracrine, and autocrine factors that may mediate intercellular communication between the tissues [6, 7].

The potential genetic and/or molecular links between muscle and bone are exciting and fertile areas of inquiry; however, we believe studies in these areas are most effectively pursued in the context of the essential functional mechanical interaction between muscle and bone. The mechanical relationship between the tissues is the most accepted and recognizable link as muscles attach to bone and generate motion via active contraction. By their direct physical attachment, muscles expose bone to a great variety of mechanical stimuli. The current article discusses the mechanical relationship between muscle and bone. In particular, we focus on the types of stimuli muscle imparts upon bone, models that hold potential to clarify this multifaceted relationship, and summarize current data in this area.

Muscle Forces on Bone During Locomotion

Skeletal muscle undeniably imparts force on bone, with the largest forces occurring during locomotion and lifting activities. Muscles attach directly to bone, but do so typically close to axes of motion resulting in small lever arms. As a result, large forces must be generated and transmitted to the skeleton in order to overcome the mechanical disadvantage and produce a required torque at the end of a lever (i.e., bone). For example, the biceps brachii muscle has a lever arm that is approximately one tenth that of the center of mass of the forearm and, thus, the muscle needs to generate a force over 10 times the weight of the forearm in order to produce elbow flexion. It has subsequently been proposed that muscle-derived forces are the primary source of mechanical loading that generates bone strain [8, 9]. In partial support of this hypothesis, Lu et al. [10] observed that axial loading of the femur during walking in a male participant fitted with an instrumented prosthesis was 3.5 times greater than suggested by externally measured ground reaction forces (GRFs), presumably due to the additional effect of internally (i.e., muscle) generated forces.

In addition to load transmission between muscle and bone, the tissues demonstrate codependent hypertrophic and hypotrophic adaptations. In exploring the mechanical interaction between muscle and bone underlying these adaptations, animal models typically disrupt muscle forces being applied to the skeleton and assess subsequent bone changes [11]. Some models utilize neurological approaches such as neurectomy or spinal cord injury to induce partial or complete muscle paralysis. These techniques demonstrate the musculoskeletal consequences of neurological injury (including rapid bone loss) and provide preclinical models of their respective human conditions. However, bone may be independently sensitive to the neurological changes associated with neurectomy or spinal cord injury [12, 13]. Also, neurectomy and spinal cord injury are inconsistently or slowly reversible, negating studies on muscle and bone recovery. Thus, studies utilizing neurectomy or spinal cord injury are complicated in isolating the mechanical link between muscle and bone. Alternative techniques of exploring the mechanical link between muscle and bone have involved introducing disuse via surgically induced tenotomy, splint or cast-induced immobilization, tail suspension, and intramuscular injection of botulinum toxin (Botox). The latter two models, given their differences, hold potential to begin to isolate the influence of mechanical stimuli in muscle/bone catabolic responses.

Tail suspension was developed as a model of space-induced weightlessness and involves maintaining an animal in 30° of head-down tilt in order to disallow weight-bearing by the hindlimbs [14]. Muscles are still able to contract while the animal is suspended; however, the removal of weight bearing reduces the resistance against which muscles need to contract in order to maintain posture and produce motion and, thereby, greatly diminishes the skeleton strain environment. Acutely (within 24 h), tail suspension results in rapid transcriptional repression of actin and myosin in affected skeletal muscle [15]. After 10–14 days, animals demonstrate bilateral reductions in hindlimb muscle and bone mass, with bone changes being most prevalent within trabecular regions and principally mediated by reduced bone formation [16] (though, there is some evidence that tail suspension is also associated with elevated bone resorption [17–19]). The caveat of tail suspension with regards to the investigation of muscle-bone interactions is that the technique also induces changes in a variety of other systems (including the cardiovascular, renal, and metabolic systems) that have potential musculoskeletal consequences [20].

In contrast, intramuscular injection of Botox directly impairs muscle function by inhibiting the release of acetylcholine to block neuromuscular transmission [21]. The paralysis causes a relatively minor reduction in gait-induced loading (e.g., 10–20 % reduction in peak GRFs) [22], but results in rapid muscle loss and substantial trabecular and cortical bone loss [23], acutely arising due to rapid osteoclastogenesis and resulting bone resorption within the marrow space [24•]. The catabolic effects are primarily unilateral, although higher doses of Botox have induced loss of body mass and have been associated with modest contralateral bone loss [23, 25]. Botox also impairs neuromuscular proprioceptive and nociceptive signaling [26, 27], complicating its use in isolating the mechanical link between muscle and bone. Both tail suspension and Botox models are potentially reversible, enabling muscle and bone interactions to be studied not only during disuse but also during subsequent reuse. However, trabecular bone resorption following transient muscle paralysis is so robust that individual trabecula become isolated and disconnected and restoration of trabecular BV/TV does not occur [28].

Studies using either tail suspension or Botox injection have attempted to explore the cause-and-effect relationship between changes in muscle and bone [28–33], with the hypothesis that if muscle loads bone, then muscle changes should proceed changes in bone during both disuse and subsequent reuse. Initial studies using Botox did not clearly support this hypothesis, with morphological changes in muscle and bone occurring somewhat concurrently during both disuse and reuse [28, 32]. However, Botox inhibits muscle activation (and, presumably, reduces muscle-induced skeletal loading) within hours of administration [34]. Thus, changes in muscle-induced skeletal loading, though modest in magnitude, appear to precede both muscle and bone morphological changes. Similarly, restoration of muscle activation and partial muscle function following Botox appears to precede gains in muscle morphology [35] and, thereby, muscle-induced skeletal loading is restored prior to subsequent gains in muscle and bone morphology.

While there appears to be a relationship between muscle-induced loading and bone cell function, study of the synchronization of muscle and bone morphological changes likely does not provide the most accurate picture of the interdependence between the tissues. Morphological changes in both muscle and bone result from cellular activities that are activated on a much quicker time scale than subsequent morphological changes, and it is possible that signaling cascades responsible for driving muscle and bone changes are induced almost in parallel as opposed to serially. For instance, we observed elevated osteoclast numbers and receptor activator for nuclear factor-κB ligand (RANKL) by 5 and 7 days following Botox-induced muscle paralysis, respectively [24•]. Similarly, using serial micro-computed tomography analyses, we detected initiation of trabecular resorption adjacent to the growth plate within 3 days following muscle paralysis [28, 36]. Given the 3 to 5-day time course for activation of in vivo osteoclastogenesis [37], it follows that the initial signaling events underlying bone catabolic responses following Botox-induced muscle paralysis occur within the marrow during the first 24 to 48 h post-paralysis, consistent with the loss of the ability of muscle to contract.

To further explore the relationship between muscle and bone, we investigated the combined effects of tail suspension and Botox-induced muscle paralysis [38••]. The premise was that by reducing the resistance against which muscles needed to contract (i.e., via tail suspension) as well as inhibiting the ability of muscles to be activated (i.e., via Botox injection), loading would be nearer to zero, resulting in a larger skeletal impact than with the introduction of either intervention alone. Indeed, combined introduction of Botox and tail suspension had greater detrimental effects on the skeleton than tail suspension or Botox injection alone. These data suggest a direct relationship between muscle and bone, which was supported by linear regression analyses showing that change in leg muscle cross-sectional area (a surrogate measure of muscle strength) explained more than half of the variance in change in midshaft cortical bone properties of the tibia and 41 % of the variance in proximal tibial bone volume fraction. The data were confirmed by a simultaneously conducted study by Ellman et al. [25] and furthered the findings of Manske et al. [39] who explored the combined effects of Achilles tenotomy and Botox-induced muscle inhibition.

An alternate line of evidence for a direct impact of muscle contractions on bone derives from experiments utilizing stimulated muscle contractions in the absence of weight bearing in anesthetized animals during periods of tail suspension. Moderate-intensity contractions (75 % of peak torque) of the lower leg musculature were capable of preventing disuse-induced bone loss in both the trabecular [40] and cortical [41•] bone compartments. In cortical bone, the effect was associated with a mitigation of the increased density of sclerostin-positive osteocytes typically observed during tail suspension [41•].

Further support for a skeletal effect of muscle-generated forces comes from embryonic studies exploring the influence of muscle on bone morphology development [42, 43]. It is desirable to develop bones that are structurally designed to resist deformation in the direction of physiological loading but are relatively lightweight to promote energy efficiency. Bones achieve these contrasting requirements by being hollow and shifting mass away from bending axes. As the rigidity of a unit area of bone is proportional to the fourth power of its distance from a bending axis, the same amount of bone mass positioned at a distance from a bending axis results in a disproportionate increase in rigidity. During embryonic development, muscle provides an epigenetic stimulus to facilitate the formation of a mechanically optimized bone shape. In particular, Zelzer and colleagues [44••] recently confirmed that muscle loads bone in utero and demonstrated that mice paralyzed due to muscular dysgenesis developed an abnormally circular-shaped long bone diaphysis that was less able to resist loading in physiological directions. Similar observations of aberrant long bone shape development have been reported in studies of amyogenic (muscle-less) mice [45–47]. In addition to the development of an optimal diaphyseal bone shape, muscle forces during embryonic development have also been shown to promote the formation of functional bone prominences for joint morphogenesis and tendon insertion and muscle action [45–48] and postnatally influence maturation of a functional tendon enthesis [49, 50].

The apparent direct effect of muscle on bone suggests that a change in the force-producing capacity of muscle should be coupled with a change in bone properties. However, there is evidence that muscle and bone can be uncoupled, with bone changes not necessarily following changes in muscle and vice versa. Muscle changes may be uncoupled from bone if increases in muscle strength are not as a result of or combined with an increase in physical activity and subsequent loads being applied to the skeleton [51]. Such a scenario may occur with pharmacological agents specifically targeting muscle. The most advanced of these agents are those targeting myostatin, a negative regulator of skeletal muscle growth [52]. Mice with null mutation of the myostatin gene have substantially greater muscle mass than wild-type mice, which is coupled with increased bone strength [53]. However, pharmacological treatment of mice with a myostatin-neutralizing antibody or propeptide increased muscle mass with no effect on bone [54•, 55]. The different skeletal influences of genetic and pharmacological inhibition of myostatin may have a number of explanations, including null mutant mice having greater muscle forces in utero and a longer duration of elevated muscle mass than wild-type pharmacologically treated mice. At this time, however, it appears that pharmacological inhibition of myostatin may need to be coupled with increased physical activity in order for the enhanced muscle properties to generate desirable skeletal changes.

It is also clear that bone cell function can be directly influenced outside of muscle. Pharmacological studies using both anti-catabolic and anabolic agents demonstrate that bone properties can be impacted independent of muscle changes [56, 57]. Similarly, studies introducing external loading to anesthetized animals demonstrate bone hypertrophy that is independent of muscle [58]. Given that the relation between muscle and bone is essential for homeostasis, it would be intuitive that any bone augmentation induced by an exogenous osteogenic/anti-resorptive stimulus outside of this relation would be lost when the stimulus ceases. Consistent with this thesis, discontinuation of external mechanical loading or pharmacological intervention is associated with a gradual loss of their bone mass benefits [59–65]. However, in certain conditions, it appears that bone size and strength benefits induced by an exogenous stimulus persist long-term and independent of muscle and bone mass. For example, mechanical loading during growth preferentially deposits new bone on the outer periosteal surface to increase bone size [66, 67], whereas the loss of bone mass during aging primarily occurs via intracortical bone loss adjacent to the endocortical surface [68]. The discordant bone surface effects of loading and its cessation enables the bone size benefits of loading when young to persist and have lasting benefits on bone strength, as the latter is most influenced by the distance of its material from the neutral axis (i.e., bone size) [64, 65].

The preceding evidence suggests muscle loads the skeleton to induce bone adaptation. However, there is also evidence that muscle can also be protective of bone loading. In particular, there is general consensus that muscle protects against, rather than causes, bone overuse injuries such as stress fractures [69]. During impact loading, muscle appears to act as an active shock attenuator helping to reduce loads as they are transmitted proximally along the kinetic chain. When muscles are dysfunctional (weakened, fatigued, or altered in their activation patterns), their ability to attenuate loads is compromised, potentially leading to increased or more rapid bone bending moments [70] and the distribution of loads to skeletal sites that may be less resistive to loading [71]. For instance, laboratory-based studies wherein strain gauges were attached to the tibia of human subjects illustrated that muscle fatigue caused an increase in both bone strain magnitude and rate during running [72, 73]. Similarly, in a kinematic and kinetic study, muscle fatigue was associated with increased peak rearfoot eversion, peak free moment, and vertical force loading rate—all factors associated with tibial stress fracture risk [74]. Further support for a protective role of muscle in reducing bone loading and subsequent overuse injuries comes from prospective clinical studies which have demonstrated that stress fracture susceptibility is inversely related to muscle size and strength [75–78].

Other Muscle-Generated Mechanical Stimuli

Although skeletal muscle imparts force on bone that engenders high strain magnitudes and induces strain-mediated adaptation during locomotion, there is growing appreciation that other components of the mechanical milieu created by muscle may also contribute to the biomechanical link between muscle and bone. In particular, there is interest in muscle-generated low-magnitude (<100 microstrain [$\mu\varepsilon$]), high-frequency (10–90 Hz) (LMHF) stimuli. The skeleton is exposed to a relatively constant barrage of LMHF stimuli, in contrast to the relatively infrequent high-magnitude strains (>2000 $\mu\varepsilon$) engendered at low frequencies (1–3 Hz) during locomotion [79]. Using vibromyography techniques to record muscle body accelerations generated during contraction, Huang et al. [80] demonstrated that LMHF stimuli originated from muscle, were essential to the maintenance of posture (even during activities such as quiet standing) and declined with age. Coupling these observations with others suggesting that the threshold for bone responses to mechanical stimuli is less when the stimuli are introduced at higher frequencies [81, 82], LMHF stimuli have been proposed to be important to skeletal homeostasis.

Rubin and colleagues [83] have championed LMHF stimuli as a modulator of bone properties. In a prominent initial study, they showed that adult sheep exposed to LMHF stimuli with a magnitude of $<0.3g$ (where g equals the Earth's gravitational field) and frequency of 30 Hz for 20 min per day over 1 year exhibited an impressive 34 % increase in proximal femur trabecular bone density compared to controls [84]. In subsequent clinical trials, Rubin and others [85–87] provided evidence suggestive of a beneficial skeletal effect of exogenously introduced LMHF stimuli as an inhibitor of bone loss in: (1) a subset of postmenopausal women, (2) young women with low bone density, and (3) children with neurologically derived disabling conditions. While each of these clinical studies possessed important limitations (such as a relatively small sample size, non-blinding of participants, and/or absence of group differences when using an intention-to-treat analysis), the data provide the impetus to further explore LMHF as a potential exogenous mechanical intervention for bone. Similarly, contrasting data provided by independent investigators introducing the same or alternative doses of LMHF stimuli to those introduced by Rubin and colleagues indicates some variability in site-specific bone responses to exogenous introduction of LMHF [88].

An alternative means of exploring the biomechanical link between muscle and bone is to electrically stimulate the muscle directly. While muscle stimulation is unlikely to engender high-magnitude bone strains consistent with those during locomotion, it may be able to recapitulate LMHF stimuli to modulate bone properties when such stimuli are diminished. Numerous animal and clinical studies have explored the virtues of LMHF stimuli generated via the electrical stimulation of muscle for the intervention of bone [12, 89]. The general consensus is that muscle stimulation can have beneficial skeletal effects, with recent work confirming the responsiveness of bone to high-frequency stimuli and furthering the field by exploring potential transduction pathways. For instance, Qin and Lam [90, 91] introduced oscillatory muscle stimulation to tail-suspended rats for 10 min per day for 4 weeks to show that stimulation at 20 or 50 Hz was able to maintain trabecular bone mass, whereas stimulation introduced at 1 Hz was ineffective. The stimulation at 20 Hz resulted in minimal matrix deformation (<100 microstrain), but resulted in a ninefold increase in oscillatory (peak-to-peak) intramedullary pressure (ImP) [91]. A change in ImP presents a potential means by which muscle-generated LMHF stimuli may be transduced into a bone cell response.

Although the process of mechanotransduction in bone remains an area of active inquiry, a growing body of evidence suggests it involves interstitial fluid flow (IFF) [92]. In addition to enhancing the transport of nutrients to individual cells embedded within the bone matrix, IFF may affect cellular function and trigger bone re/modeling. IFF can result from bone matrix deformation (i.e., strain) associated with muscle forces during locomotion which give rise to local pressure gradients within the matrix and drive interstitial fluid through the lacunocanalicular system. Alternatively, IFF can be generated through elevations in ImP [93, 94]. Pressurization of the intramedullary cavity causes an outward pressure gradient from the intramedullary cavity to the periosteal surface to also induce IFF within the lacunocanicular system [93, 94]. Numerous investigators have demonstrated that enhancement of ImP via differing means (including electrical stimulation of muscle) has osteogenic effects [94–98].

Conclusions

The mechanical link between muscle and bone is undeniable, with muscle providing forces acting directly on bone. Muscle not only generates active tension to engender high bone strains during locomotion but also produces LMHF stimuli and changes in ImP to which bone may be sensitive. Exogenous introduction of the latter, more subtle, muscle-generated stimuli may present novel avenues for enhancing bone morphology when high magnitude loads via impact loading are not possible, such as in the elderly and those experiencing disuse due to neurological or other conditions. While the preponderance of data reviewed in this paper suggests that muscle loading of bone is essential to maintain bone homeostasis and can induce hypertrophy, possible contributions of non-mechanical (i.e., genetic and molecular) links between muscle and bone were not accounted for in each of the reported studies. For instance, bone loss as a result of Botox-induced muscle paralysis may not only arise from reductions in muscle force but also due to alterations in molecular "cross talk" between muscle and bone. The intertwining of the different genetic, molecular, and mechanical links between muscle and bone makes it a challenge to tease out the relative contribution of each individual link. This is an issue that should be considered in future studies exploring biomechanical aspects of the muscle-bone interaction.

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Compliance with Ethics Guidelines

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Human and Animal Rights and Informed Consent All studies by Bloomfield, Gross, and Warden involving animal and/or human subjects were performed after approval by the appropriate institutional review boards. When required, written informed consent was obtained from all participants.

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• Of importance
•• Of major importance

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