J Musculoskelet Neuronal Interact 2010; 10(1):25-34

Review Article

Hylonome

The effect of muscle dysfunction on bone mass and morphology

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Abstract

There is little doubt that skeletal development and subsequent maintenance of bone mass and morphology during adulthood is greatly influenced by viable muscle function. In this review, we will summarize human observations that support this concept, then focus on models that have enabled (or may enable in the future) insight into the co-dependency of muscle and bone. Specifically, we will summarize data generated with three types of models: 1) spinal cord injury models, 2) transgenic mice with altered muscle function, and 3) experimental models affecting one hindlimb or a single muscle group. In sum, these data clearly support the concept that muscle function is critical for the successful development of the skeleton and is likely to play an important role in mediating bone health through life. The specific signaling pathways by which this interdependency is achieved, however, remain to be clarified.

Keywords: Bone Mass, Muscle Atrophy, Disuse, Botox

Of muscle and bone

Giovanni Borelli, a 17th century mathematician and scientist, was one of the first to apply the concept of levers to understand how muscles load the skeleton. One seminal insight in his monograph, "*De Motu Animalium (On the Movement of Animals)*" was the observation that muscle forces required to enable lifting of loads greatly exceed the actual weight being lifted due to mechanical disadvantage. While this observation would be obvious to any current first year bioengineering student, at the time, the concept was provocative and controversial given the contemporary view of the divine "perfection" of the human body[1,2]. Of relevance to this discussion, Borelli's observation emphasizes that muscle function during activity gives rise to the very loading events that are thought to be required for successful development and maintenance of bone mass and morphology[3]. In a broader perspective, this concept formed the basis for Roux's later development of the paradigm of functional adaptation[4,5]. In the last century, as noted elsewhere[6,7], D'Arcy Thompson, in his seminal book, "On Growth and Form", observed that, "... between muscle and bone there can be no change in the one but it is correlated with changes in the other...". These historical references highlight the long acknowledged role of muscle function in skeletal development and health, and emphasize the role that skeletal loading plays in achieving this end. As D'Arcy Thompson also remarked (pg. 237-238), "... bone is not only a living, but a highly plastic structure; the little trabeculae are constantly being formed and deformed ... under the direct action and control of the forces to which the system is exposed"[8].

Given that muscle and bone share a common mesodermal origin, it is not surprising that the dependent association of muscle and bone arises at the earliest stages of development[9,10]. Throughout skeletal growth (i.e., 2 to 20 years of age), there is a strong correlation between muscle mass and bone mass[11]. During pubertal growth, whole body and regional accretion of lean body mass slightly precedes whole body and regional accretion of bone mineral content (BMC). That muscle gains precede bone gains, dependent upon site and gender (by 0.22 to 0.71 years), implies the potential for a causative influence[12]. Muscle and bone development are simultaneously influenced by a variety of circulating hormones and growth factors that include (but are certainly not limited to) the somatotropin/IGF-1 axis, androgens, estrogens, and vitamin D[13]. Within this milieu, genetic factors strongly underlie the coupling of an individual's muscle strength

The authors have no conflict of interest.

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Edited by: M. Hamrick
Accepted 23 January 2010

and bone structural properties[14,15]. In fact, it is difficult to identify cross-sectional human data in which a population of greater than average muscle mass does not also demonstrate greater than average bone mass or enhanced skeletal structural properties. Over a lifetime, the general pattern of muscle accretion and bone accretion, age of maximal lean muscle mass and achievement of peak bone mass, and the onset of sarcopenia and age-induced bone degradation are all temporally consistent[16]. Acutely, however, alterations in bone mass and morphology have been observed prior to or in the absence of muscle hypertrophy[17,18].

Another similarity between adaptation of muscle mass and bone mass through life is that mechanical stimuli can serve to enhance peak bone mass during growth and diminish the loss of mass later in life[19]. The role of muscle function in achieving this end, either via direct mechanical stimuli[6] or other physiologic pathways, is a clear example of Darwinian developmental plasticity, whereby environmental influences modulate tissue morphology[20]. A number of studies have generally associated bone density and lean body mass[21,22]. However, given that mechanical deformation serves as an epigenetic guiding stimulus for the skeletal morphology[23], it is not surprising that strong correlations can be identified between muscle mass and specific geometric aspects of the skeleton such as cross-sectional area[24,25]. Certainly, the series of studies that have examined the bone mass of the dominant arm in racquet sports participants demonstrate the potential for profoundly enhanced bone mass and morphology, particularly when exercise is initiated during skeletal development[26-29]. One only has to watch Roger Federer raising another grand slam trophy to realize that unilateral muscle hypertrophy also results from such endeavors.

On the other hand, degraded muscle function, arising by disease or age, is clearly accompanied by diminished bone mass and morphology. Thus, increased muscle function not only has the potential to generate anabolic mechanical signals for bone, but normal muscle function is *required* for maintaining a healthy skeleton. In humans, a number of muscle related diseases illustrate this observation. For example, myasthenia gravis (MG) is an autoimmune disease that destroys acetylcholine signaling in neuromuscular junctions. MG patients experience a loss of muscle strength with secondary osteoporosis[30]. The relative contributions of neuromuscular degradation and treatment side effects (in this case, systemic corticosteroids) to the observed bone loss remains to be determined. A second example can be found in Duchenne muscular dystrophy, a disease in which the lack of dystrophin results in profound muscular degeneration[31]. Duchenne's patients suffer from diminished bone properties and elevated fracture risk[32,33]. Cerebral palsy is a third example of a disease that phenotypically manifests via muscle dysfunction, reduced skeletal loading and bone degradation[34,35].

Muscle and bone loss following Spinal Cord Injury (SCI)

Rapid and permanent connective tissue degradation ensues following spinal cord injury (SCI). Sensory and motor innervation of the musculoskeletal system is disrupted due to damage of the neural tissue within the spinal canal, leading to debilitating atrophy of sublesional muscle and bone. In the absence of supraspinal input, rapid, profound and permanent loss of muscle mass has been long recognized as a hallmark of complete SCI[36]. Within 6 weeks post-SCI, the mean cross sectional areas (CSAs) of muscle biopsies from the vastus lateralis were only 62% of the values of age, weight and height-matched control patients[37]. Prospective follow up of SCI patients demonstrates continued deterioration of muscle mass as data from cross-sectional studies indicate that muscle atrophy (as measured by computer tomography) appears to stabilize in long-term SCI patients (17±2.64 yr post-injury) with no significant differences in either CSA or force generation when compared to mid-term patients (2.2±0.5 yr post-injury)[38].

Not surprisingly, decrements in muscle size are accompanied by severe degeneration of muscle function. Electrically elicited torque in muscle paralyzed less than 6 weeks is quite low, reflecting a rapid and near complete loss of muscle activity[39]. In the soleus muscle, endurance also declines rapidly over the first year[40]. For example, a chronically paralyzed soleus muscle (>2 years) is able to generate only 20 to 30 % of its initial peak torque after a bout of repetitive activation[41]. Similarly, both peak knee extension torque and patella tendon force during electrical stimulation in SCI patients reaches only 25% of values measured in controls[42].

Impaired muscle function diminishes large scale deformations of bone as gait activity and ground reaction forces during activity are reduced compared to normal. Thus, minimal skeletal loading associated with SCI has been presumed to underlie bone loss following such injuries. Immediately upon injury, bone mineral density (BMD) declines rapidly, particularly at sites rich in trabecular bone[43]. In the first few months after SCI, BMD declines 2 to 4 percent a month[44] a rate that has been described as 5 to 20 times greater than losses from purely metabolic etiologies[45]. The magnitude of bone lost in the lower limbs following SCI has been quantified in a number of cross-sectional studies using both DXA and pQCT. Early research suggested that the rate of bone loss after SCI is rapid and linear in the acute stages, establishing a lower steady-state bone mass level 1 to 2 years after the event[46,47]. The time course of bone loss (and attainment of a new homeostasis) may depend on the bone compartment. For example, at sites with a high proportion of trabecular bone, the time course followed a log curve leveling off from 1 to 3 years post injury, whereas at the tibial diaphysis, a cortical bone site, bone mass appeared to decrease progressively beyond 10 years post injury[48]. Consistent with observations in cross-sectional studies, initial bone mass losses are greater in trabecular than in cortical compartments[43,44,49].

The profound loss of BMD in SCI patients is also reflected in severe deterioration in bone architecture. For example, MRI studies have demonstrated that men and women with long-standing complete SCI had reduced bone volume and trabecular number resulting in increased trabecular spacing compared with controls[50,51]. Alterations in bone area and bone geometry following SCI have also been noted in studies that have utilized computed tomography[52-54]. Again, the decline in BMD and concomitant deterioration in bone structure appears

to continue for 2 to 8 years following SCI, depending on the anatomical site and the method of measurement, eventually reaching steady state values 50 to 60 percent lower than non-SCI values[55,56]. However, whether bone loss plateaus following SCI has been challenged by two recent studies of monozygotic twins, both of which suggested that bone loss may continue over the life span of SCI patients[57].

Mouse models of muscle and bone disuse

Mouse models provide the means to experimentally perturb specific aspects of muscle/bone interactions at levels that range from whole genome manipulations to experimental approaches that alter function in either both limbs or one limb. Muscle-bone interactions have been explored in both knockout and transgenic mice. In these studies the obvious advantage of isolating the perturbing effects of a single gene is balanced by the complexity arising when genes integral to skeletal development are congenitally altered. As a result, separating the role of the examined pathway in development of bone mass and morphology versus maintaining bone homeostasis during adulthood is experimentally challenging. For example, transgenic models of human spinal muscle atrophy (SMA), a severe disease of motor neurons that is characterized by progressive muscle weakness, exemplify the powerful linkage between motor neuron function and skeletal development. Transgenic mice with mutations in the survival motor neuron (SMN) gene exhibit proximal muscle atrophy adjacent to the spine and pathological changes in spinal cord and skeletal muscle that are similar to those observed in human patients with SMA[58,59]. These mice develop a severe osteoporotic phenotype with cortical bone porosities, a 33% lower bone volume and a 50% decline in trabecular number[60]. The severe bone deterioration is accompanied by significant elevations in serum and urine markers of bone resorption (64% higher levels of serum TRAP5b and 11 fold elevation of urinary NTx, respectively) and 57% greater osteoclast surface vs. WT (i.e. amount of bone surface occupied by osteoclasts). Together, these observations are indicative of the strong link between muscle dysfunction and pathological bone loss driven by elevated osteoclast recruitment and increased resorptive activity.

In addition to the skeletal consequences observed in loss of function models such as the SMA mutant, genetic gain of function models are also available to confirm the role of muscle on achievement of bone homeostasis. In particular, mice lacking myostatin (GDF8), a negative regulator of skeletal muscle growth, demonstrate increased BMD at both appendicular and axial skeletal sites[61,62]. In cortical bone, the skeletal effects in GDF8 mutants are most pronounced at muscle insertion sites with only modest effects on cortical diaphyses. Interestingly, these mutants have large increases in trabecular bone volume (30%) and trabecular thickness (50%) in vertebral bodies[62].

Understanding the pathogenesis of bone loss in SCI has been recently augmented by rodent models of SCI (primarily rat) where injury of the thoracic spine is induced via blunt trauma. Bone loss in this model is extremely rapid with BMD at trabecular rich sites declining 34% within just 10 days and trabecular bone volume loss reaching nearly 60%. As expected, such a rapid decline in bone volume is accompanied by decreases in trabecular number and thickness and increased trabecular spacing. Moreover, the cortical bone shell is also dramatically altered by endocortical resorption which increases the marrow cavity volume by 24% and thins the cortical width by 30.5%[63]. The magnitude and severity of bone deterioration observed in SCI models is placed in context when the skeletal effects of SCI are contrasted with other models of muscle atrophy and disuse such as hind limb cast immobilization (HCI). In a 4 week study comparing SCI with HCI, SCI resulted in more rapid bone loss with greater deterioration of trabecular microarchitecture and cortical bone geometry[64]. Taken together, findings in animal models underscore the severe nature of SCI-induced bone loss and point to a critical role of supraspinal input in the achievement and regulation of bone mass.

A variety of immobilization models have been implemented in rodents, for the most part initially in rats[65]. The most predominantly utilized model involves unweighting of both hindlimbs (i.e., usually via tail suspension) which has also been implemented in mice[66,67]. With regard to single hindlimb interventions that may provide more insight into focal mediation of muscle/bone interactions, rat models have included nerve resection[68,69], tendon resection[70,71], and casting or bandaging of one hindlimb[64,72]. Of these, a smaller subset have been implemented in mice (primarily sciatic neurectomy), in part due to challenges with smaller animals and in behavioral differences between animals. Given our primary focus on murine models, this section will consider the contrast between hindlimb suspension and sciatic neurectomy in mice from both a mechanical disuse and an acute cellular and tissue adaptation perspective.

A number of experimental issues arise when attempting to delineate whether muscle dysfunction is directly or indirectly effecting the bone cell populations responsible for altering bone morphology following either hindlimb suspension or neurectomy. Both models reliably induce muscle and bone loss compared to age-matched and zero-time control mice. Hindlimb suspension was originally developed as a model of space-induced bone degradation and eliminates hindlimb locomotion ground contact (i.e., no weight bearing) although hindlimb muscles are still able to contract[73]. Hindlimb/tail suspension also induces a variety of alterations with potential overlap upon muscle/bone interactions such as cardiovascular fluid shifts, elevated stress, and altered testosterone levels in male mice[74-77]. These systemic alterations mimic those observed due to microgravity exposure and, as such, may be transient (e.g., alterations in corticosteroid levels[78]), but may also confound explorations of the acute effects of diminished skeletal loading in 1 g environments. Sciatic neurectomy disables muscle contraction in a single hindlimb. While general locomotion is effected by this intervention, decreases in experimental limb weight bearing is surprisingly modest only (approximately 25%;[69]). Sciatic neurectomy also results in partial limb denervation as other sensory and sympathetic nerves still function (in contrast to complete paralysis as would be achieved via spinal cord

transection;[79]). Even though soleus and grastrocnemius muscle activity is only transiently reduced (and tibialis anterior activity is actually increased) during hindlimb suspension[80], one might surmise that the decrease in locomotion-induced bone deformations (i.e., peak normal strains) following hindlimb suspension greatly exceeds the decrement due to unilateral sciatic neurectomy. As we were not able to identify a confirmatory reference, we used inverse dynamics to estimate the inertial bending moment at the mouse knee joint during tail suspension. Assuming similar kinematics (i.e., angular acceleration) as during rapid walking (0.3 m/s;[81]), the maximal bending moment during hindlimb suspension would induce peak normal strains in the tibia that are less than 5% of those that are likely to be induced during locomotion following sciatic neurectomy. While clearly requiring experimental validation, this initial estimate does support our premise above.

Studies using both models have focused primarily upon either muscle or bone adaptation, with a few exceptions[82-84]. Both hindlimb/tail suspension and sciatic neurectomy result in rapid and significant muscle loss. Following 14 d of tail suspension, lower limb muscle loss ranges up to 50% depending upon muscle[85-87]. Following sciatic neurectomy, alterations are confined to the neurectomized limb and muscle wet weights are significantly diminished within 3 d (>10%) and diminished over 50% within 14 d[69]. Muscle fiber cross-sectional area is even more sensitive to this intervention and is significantly decreased within 24 hr (>18%;[88]). As would be expected with such a rapid morphologic response, mRNA expression of genes associated with muscle atrophy, such as myostatin, are also upregulated within 24 hr[89,90].

Hindlimb unweighting effects on mouse bone morphology are inbred strain specific. For example, C57Bl/6J mice demonstrate a catabolic response to hindlimb suspension that is not evident in C3H/HeJ mice[91]. The lack of response of C3H mice to this stimulus may suggest a diminished sensitivity of bone to mechanical stimuli[92], altered levels of initial osteoblast activity[93,94], or, perhaps, an altered role for muscle in modulating bone homeostasis. In general, hindlimb/tail suspension of adult C57 mice (>16 wk) results in an approximate decrease of trabecular BV/TV of 20% to 30% within two weeks with a 10% to 15% decrease in trabecular thickness and no change in trabecular number[91,95,96]. At a cellular level, this acute alteration is achieved by significantly decreasing osteoblast function and significantly increasing osteoclast resorption[97]. Alterations of cortical bone within 2 wk are minimal[95]. The degradation of trabecular bone following sciatic neurectomy is more profound, reaching 40% within 2 wk[79]. Further, cortical bone effects are also rapidly observed with cortical thickness decreased 10 to 20% in mice or rats[79,98]. An important difference between the models that is likely to underlie bone morphology responses is that the primary acute cellular response provoked by sciatic neurectomy is one of profound osteoclastic bone resorption which appears to greatly exceed acute osteoclastic resorption induced by hindlimb suspension. Given the temporal variability in cellular adaptation induced by these interventions, it is not surprising that systemic interventions have differential protective effects upon the skeleton in these models. For example, blockage of beta-adrenergic signaling inhibits loss of bone following hindlimb suspension, but not due to sciatic neurectomy[79,99].

In part to begin to more specifically explore the role of muscle function in maintaining bone homeostasis, our group has recently developed a mouse model of transient muscle paralysis-induced bone loss[100]. In our initial studies, we explored whether transient paralysis of the quadriceps (vastus lateralis, medialis, and intermedius and rectus femoris) and calf (gastrocnemius, soleus, plantaris) muscle groups (each muscle group receiving a single 2 Units/100 g injection of Botulinum Toxin A; Botox) would influence trabecular and cortical bone morphology in the distal femur and tibia. Voluntary gait activity (assessed via a qualitative inventory) was significantly impaired within 1 d, maximally impaired in 3 d, but had significantly improved from this low level within 14 d and demonstrated continued recovery through 21 d. Muscle mass in Botox injected mice was still profoundly diminished at 21 d compared to saline injection mice (-47% in the quadriceps, -60% in the calf). As assessed by high resolution micro-CT imaging, profound trabecular bone loss was observed at 21 d, both in the distal femur and proximal tibia (-43% and -54%, respectively), with cortical bone volume reduced nearly 15% in the tibial diaphysis. Morphologically, this degradation was achieved primarily via decreased trabecular thickness and endocortical expansion, suggestive of an osteoclast-driven response. Subsequently, studies by our group have confirmed that profound bone loss in the model is extremely rapid with a >75% BV/TV loss in the proximal tibia trabecular just 12 d after calf paralysis was induced (Figure 1;[101]). Further, the acute response to transient muscle paralysis is due to a RANKL mediated osteoclastic activation that is confined to the effected limb[102-104]. The acute cellular and tissue response in this model is therefore consistent with that observed in SCI and neuronal injury models where the magnitude of acute osteoclastic resorption exceeds that precipitated by hindlimb suspension, although a direct comparison between the models has not yet been reported. Likewise, while we have yet to rigorously quantify decreased locomotion strains following transient muscle paralysis, our qualitative observations suggest a relatively moderate diminishment of peak normal strains in the tibia following calf paralysis.

A number of other groups have used Botox-induced muscle paralysis to study bone alterations in both rats and mice. In a study that preceded ours, growing rats were exposed to quadriceps paralysis with BMC and radiographic texture analysis used as outcome measures[105]. Of these measures, only BMC differences were significantly altered. Based on our data and subsequent studies by that group and others[106-108], the minimal response compared to our initial study likely arose due to the limited resolution of implemented assays and the confounding influence of a growing skeleton. While the magnitude of the skeletal response varies substantially with sampling site, which muscle(s) are paralyzed, age of animals, and resolution of assay, each of these experiments has demonstrated muscle dysfunction has a profound catabolic influence of upon bone morphology.

In a more recent study, we have begun to explore whether

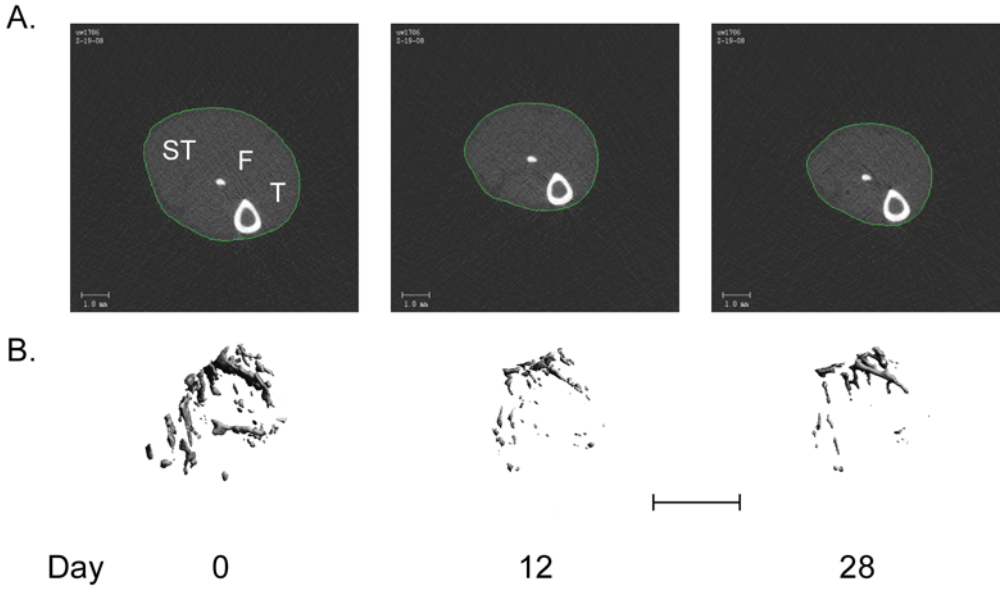

Figure 1. Micro-CT imaging of the right tibia of a mouse exposed to transient muscle paralysis of the calf muscles. Images were recorded immediately prior to paralysis (Day 0) and 12 and 28 d following paralysis. Two dimensional transverse slices from the tibia mid-shaft (A) illustrate the clear atrophy of soft tissue (ST) surrounding the fibula (F) and tibia (T). Higher resolution of the proximal tibia metaphysis (B, 10.5 m voxel) demonstrate the profound degradation of trabecular bone that is induced by transient muscle paralysis.

reduced bone deformation during locomotion (i.e., peak normal strains) underlies the profound loss of bone mass induced by transient muscle paralysis. Specifically, we examined whether proximal tibia bone loss induced by calf paralysis would be mitigated by electrical muscle stimulation (EMS) of the calf or quadriceps muscle groups. Although Botox inhibits acetylcholine transfer in motor neurons, muscle contraction is still possible via EMS. Using separate calibration mice, we used strain gages to determine EMS protocols that, when applied to either the quadriceps or calf muscles, would induce similar peak normal strains on the periosteal anterior medial tibia cortex (n=3 mice; mean±s.e. normal strain induced by calf EMS: 144±22 με; quadriceps EMS: 117±13 με). The magnitude of these peak strains are approximately 40% of those induced in the tibia during walking, as estimated by inverse dynamics combined with finite element analysis (unpublished data). We then randomized 22 female C57B6 mice (20 wk of age) into 4 groups: 1) Saline+calf EMS (S EMS; n=3), 2) Botox of calf+no EMS (Botox; n=6), 3) Botox of calf+calf EMS (B EMS C; n=8), and 4) Botox of calf+quadriceps EMS (B EMS Q; n=6). At day zero, all mice had micro-CT scans of the proximal tibia, and received IM injections of Botox (10 μl of 2.0 unit/100 g) or equal volume saline (10 μl) in the right calf while anesthetized. Beginning on day zero, mice were anesthetized and positioned supine with knee and foot supported at 90°, with the foot secured. For the EMS stimulated mice, two electrodes were positioned on the mid and distal bare calf or quadriceps for EMS which was performed 5 d/wk, 3000 cycles per day using a 4 Hz square wave at 10 V peak. The only difference between calf and quadriceps EMS was the impulse length (calf: 1000 μs; quadriceps: 600μs). Final imaging of all mice was performed at 21 d with primary outcome measures of calf and quadriceps wet muscle weight and proximal tibia metaphyseal trabecular bone morphology.

EMS of the calf did not alter muscle mass in saline control mice (S EMS: 3.7±2.2% vs. contralateral calf) nor the amount of muscle atrophy observed in any of the Botox treated groups (Figure 2). Likewise, the diminishment of quadriceps muscle mass in mice whose calf muscles were transiently paralyzed was not altered by EMS stimulation of either the calf or quadriceps. We observed that Botox negative control mice demonstrated a -51.5±7.2% degradation of trabecular bone volume fraction (BV/TV) in 21 d. This degradation of trabecular bone was not significantly altered by calf EMS (-41.3±7.0%) or quadriceps EMS (-49.1±8.6%). This preliminary study clearly has limitations. First, it is not clear how accurately 3000 contractions approximates overall daily gait activity in caged mice (and, further, whether its implementation in a single 11 minute bout of loading is sufficient to substitute for daily locomotion-induced loading). The study was also limited in sample size and certainly not exhaustive in terms of potential EMS signal regimens or techniques for inducing muscle contraction (e.g., surface electrodes may not be sufficient for this goal;[109]). Interestingly, however, while EMS has been shown to be effective in mitigating bone loss during hindlimb suspension[110,111], EMS has had minimal success in preventing SCI-induced bone loss in humans[112-114]. Two reasonable explanations for these data arise, both of which clearly require additional study. First, it may simply be that greater EMS-induced mechanical stimuli are required to inhibit muscle paralysis-induced bone loss. Al-

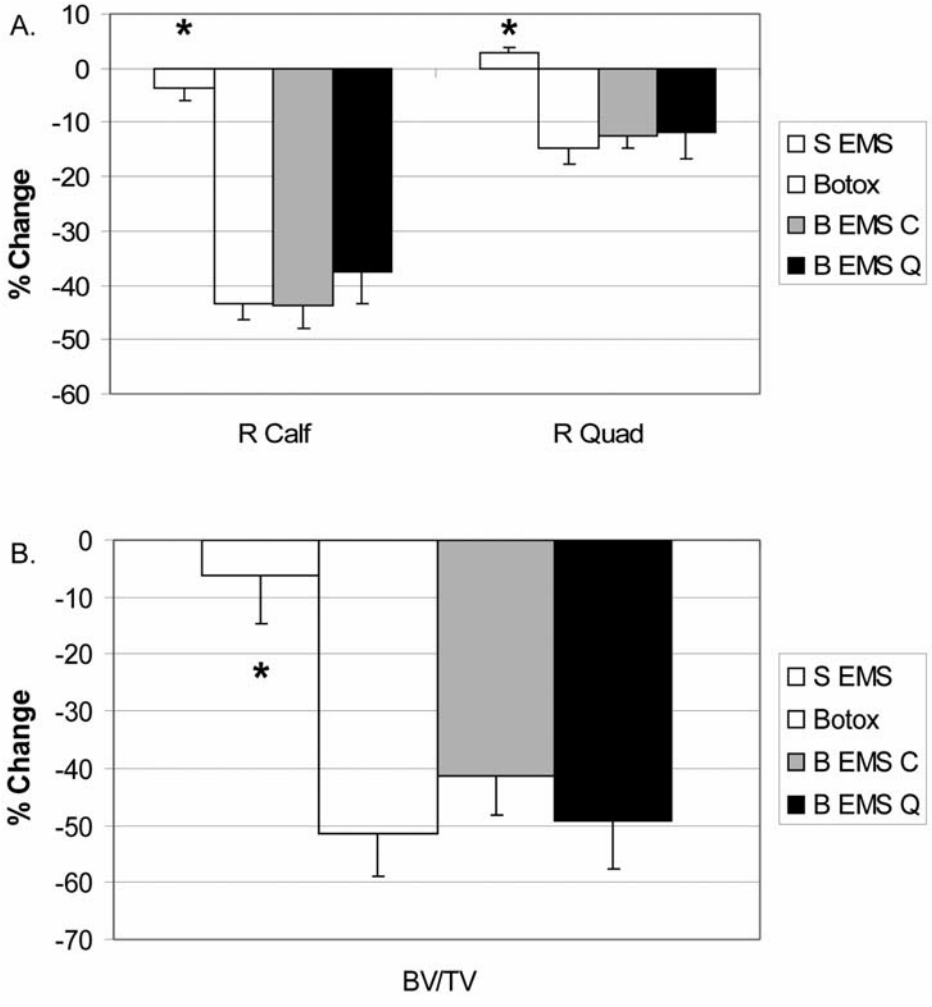

Figure 2. Muscle wet weight alterations (% vs contralateral limb) demonstrated the the EMS interventions did not alter muscle atrophy following transient muscle paralysis (A). Similarly, the EMS interventions did not diminish the loss of BV/TV induced by transient muscle paralysis (B, *; $p<0.001$ vs all other groups).

ternatively, gait-induced bone deformations may not mediate the signaling pathway responsible for surge of osteoclastic resorption precipitated by acute muscle dysfunction. If so, we believe that attempts to use large scale deformations of bone to inhibit acute bone loss due to SCI or focal muscle paralysis will be only minimally successful.

A view forward

Based on this review, we believe that it could be speculated that profound reductions in bone deformation concomitant with SCI and/or muscle pathologies are not the only causal event for the rapid and severe bone degradation that follows. We and others have now shown that intramuscular injection of Botox rapidly, profoundly, and focally degrades muscle mass and bone mass in mice. The acute loss of bone arising from transient muscle paralysis substantially exceeds that observed via hindlimb suspension, but is similar in scope and magnitude to that observed after sciatic neurectomy. Interestingly, such profound osteoclastic activation is associated with what appears to be a lesser reduction in large magnitude locomotion-induced bone deformation compared to hindlimb suspension. In this context, however, it is clear that we understand very little about the ramifications of focally lost neural connectivity within the musculoskeletal system. Currently, neuronal influences on skeletal remodeling are perhaps best understood for the sympathetic nervous system, which, when stimulated, has been shown to inhibit bone formation and stimulate bone resorption[115,116]. Paradoxically, however, sympathetic nerve activity is markedly decreased after SCI[117,118], which should inhibit resorption, increase bone formation and lead to increases in bone mass, which clearly does not occur. In our view, models that enable exploration of such focal signaling pathways hold potential to clarify the cellular and molecular mechanisms that underlie the co-dependence of muscle and bone in achieving and maintaining tissue homeostasis.

Acknowledgements

This work was supported, in part, via funding from NIAMS (AR45665), The Christopher and Dana Reeve Foundation, the Sigvard T. Hansen, Jr. Endowed Chair, and the Zimmer Fracture Biology Professorship.

References

1. Pope MH. Giovanni Alfonso Borelli—the father of biomechanics. Spine (Phila Pa 1976) 2005;30:2350-5.
2. Des Chene D. Mechanisms of life in the seventeenth century: Borelli, Perrault, Regis. Stud Hist Philos Biol Biomed Sci 2005;36:245-60.
3. Frost HM, Schonau E. The "muscle-bone unit" in children and adolescents: a 2000 overview. J Pediatr Endocrinol Metab 2000;13:571-90.
4. Roux W. Der zuchtende Kampf der Teile, oder die "Teilauslese im Organismus (Theorie der 'funktionellen Anpassung')". Leipzig: Wihelm Engelmann; 1881.
5. Roesler H. The history of some fundamental concepts in bone biomechanics. J Biomech 1987;20:1025-34.
6. Frost HM. An approach to estimating bone and joint loads and muscle strength in living subjects and skeletal remains. Am J Hum Biol 1999;11:437-55.
7. Turner CH. Muscle-bone interactions, revisited. Bone 2000;27:339-40.
8. Thompson D. On Growth and Form (abridged edition, original edition: 1917). Cambridge: University Press; 1961.
9. Stockdale FE, Nikovits W, Jr., Christ B. Molecular and cellular biology of avian somite development. Dev Dyn 2000;219:304-21.
10. Bailey P, Holowacz T, Lassar AB. The origin of skeletal muscle stem cells in the embryo and the adult. Curr Opin Cell Biol 2001;13:679-89.
11. Zanchetta JR, Plotkin H, Alvarez Filgueira ML. Bone mass in children: normative values for the 2-20-year-old population. Bone 1995;16(4Suppl.):393S-9S.
12. Rauch F, Bailey DA, Baxter-Jones A, Mirwald R, Faulkner R. The 'muscle-bone unit' during the pubertal growth spurt. Bone 2004;34:771-5.
13. Zofkova I. Hormonal aspects of the muscle-bone unit. Physiol Res 2008;57(Suppl.1):S159-69.
14. Seeman E, Hopper JL, Young NR, Formica C, Goss P, Tsalamandris C. Do genetic factors explain associations between muscle strength, lean mass, and bone density? A twin study. Am J Physiol 1996;270(2Pt.1):E320-7.
15. Kuh D, Hardy R, Butterworth S, et al. Developmental origins of midlife grip strength: findings from a birth cohort study. J Gerontol A Biol Sci Med Sci 2006;61:702-6.
16. Sayer AA, Syddall H, Martin H, Patel H, Baylis D, Cooper C. The developmental origins of sarcopenia. J Nutr Health Aging 2008;12:427-32.
17. Judex S, Carlson KJ. Is bone's response to mechanical signals dominated by gravitational loading? Med Sci Sports Exerc 2009;41:2037-43.
18. Xu L, Nicholson P, Wang Q, Alen M, Cheng S. Bone and muscle development during puberty in girls: a seven-year longitudinal study. J Bone Miner Res 2009;24:1693-8.
19. Parfitt AM. The attainment of peak bone mass: what is the relationship between muscle growth and bone growth? Bone 2004;34:767-70.
20. West-Eberhard MJ. Developmental plasticity and the origin of species differences. Proc Natl Acad Sci USA 2005;102(Suppl.1):6543-9.
21. Wapniarz M, Lehmann R, Reincke M, Schonau E, Klein K, Allolio B. Determinants of radial bone density as measured by PQCT in pre- and postmenopausal women: the role of bone size. J Bone Miner Res 1997;12:248-54.
22. Schiessl H, Frost HM, Jee WS. Estrogen and bone-muscle strength and mass relationships. Bone 1998;22:1-6.
23. Rubin CT, McLeod KJ, Bain SD. Functional strains and cortical bone adaptation: epigenetic assurance of skeletal integrity. J Biomech 1990;(23Suppl.1):43-54.
24. Schoenau E. From mechanostat theory to development of the "Functional Muscle-Bone-Unit". J Musculoskelet Neuronal Interact 2005;5:232-8.
25. Sun X, Lei SF, Deng FY, et al. Genetic and environmental correlations between bone geometric parameters and body compositions. Calcif Tissue Int 2006;79:43-9.
26. Jones HH, Priest JD, Hayes WC, Tichenor CC, Nagel DA. Humeral hypertrophy in response to exercise. J Bone Joint Surg Am 1977;59:204-8.
27. Haapasalo H, Kontulainen S, Sievanen H, Kannus P, Jarvinen M, Vuori I. Exercise-induced bone gain is due to enlargement in bone size without a change in volumetric bone density: a peripheral quantitative computed tomography study of the upper arms of male tennis players. Bone 2000;27:351-7.
28. Kontulainen S, Kannus P, Haapasalo H, et al. Good maintenance of exercise-induced bone gain with decreased training of female tennis and squash players: a prospective 5-year follow-up study of young and old starters and controls. J Bone Miner Res 2001;16:195-201.
29. Baxter-Jones AD, Kontulainen SA, Faulkner RA, Bailey DA. A longitudinal study of the relationship of physical activity to bone mineral accrual from adolescence to young adulthood. Bone 2008;43:1101-7.
30. Lewis SJ, Smith PE. Osteoporosis prevention in myasthenia gravis: a reminder. Acta Neurol Scand 2001;103:320-2.
31. Deconinck N, Dan B. Pathophysiology of duchenne muscular dystrophy: current hypotheses. Pediatr Neurol 2007;36:1-7.
32. Larson CM, Henderson RC. Bone mineral density and fractures in boys with Duchenne muscular dystrophy. J Pediatr Orthop 2000;20:71-4.
33. Hsu JD. Skeletal changes in children with neuromuscular disorders. Prog Clin Biol Res 1982;101:553-7.
34. Shaw NJ, White CP, Fraser WD, Rosenbloom L. Osteopenia in cerebral palsy. Arch Dis Child 1994;71:235-8.
35. Tasdemir HA, Buyukavci M, Akcay F, Polat P, Yildiran A, Karakelleoglu C. Bone mineral density in children with cerebral palsy. Pediatr Int 2001;43:157-60.

36. Grimby G, Broberg C, Krotkiewska I, Krotkiewski M. Muscle fiber composition in patients with traumatic cord lesion. Scand J Rehabil Med 1976;8:37-42.
37. Castro MJ, Apple DF Jr, Staron RS, Campos GE, Dudley GA. Influence of complete spinal cord injury on skeletal muscle within 6 mo of injury. J Appl Physiol 1999;86:350-8.
38. Kern H, Hofer C, Modlin M, et al. Stable muscle atrophy in long-term paraplegics with complete upper motor neuron lesion from 3- to 20-year SCI. Spinal Cord 2008; 46:293-304.
39. Shields RK, Dudley-Javoroski S. Musculoskeletal plasticity after acute spinal cord injury: effects of long-term neuromuscular electrical stimulation training. J Neurophysiol 2006;95:2380-90.
40. Shields RK, Chang YJ, Dudley-Javoroski S, Lin CH. Predictive model of muscle fatigue after spinal cord injury in humans. Muscle Nerve 2006;34:84-91.
41. Shields RK, Dudley-Javoroski S. Musculoskeletal adaptations in chronic spinal cord injury: effects of long-term soleus electrical stimulation training. Neurorehabil Neural Repair 2007;21:169-79.
42. Rittweger J, Gerrits K, Altenburg T, Reeves N, Maganaris CN, de Haan A. Bone adaptation to altered loading after spinal cord injury: a study of bone and muscle strength. J Musculoskelet Neuronal Interact 2006;6:269-76.
43. Frey-Rindova P, de Bruin ED, Stussi E, Dambacher MA, Dietz V. Bone mineral density in upper and lower extremities during 12 months after spinal cord injury measured by peripheral quantitative computed tomography. Spinal Cord 2000;38:26-32.
44. Wilmet E, Ismail AA, Heilporn A, Welraeds D, Bergmann P. Longitudinal study of the bone mineral content and of soft tissue composition after spinal cord section. Paraplegia 1995;33:674-7.
45. Mazess RB, Whedon GD. Immobilization and bone. Calcif Tissue Int 1983;35:265-7.
46. Garland DE, Stewart CA, Adkins RH, et al. Osteoporosis after spinal cord injury. J Orthop Res 1992;10:371-8.
47. Garland DE, Lewonowski K, Adkins RH, Stewart CA. Visual versus quantified digital radiographic determination of bone density. Contemp Orthop 1993;26:591-5.
48. Zehnder Y, Luthi M, Michel D, et al. Long-term changes in bone metabolism, bone mineral density, quantitative ultrasound parameters, and fracture incidence after spinal cord injury: a cross-sectional observational study in 100 paraplegic men. Osteoporos Int 2004;15:180-9.
49. de Bruin ED, Dietz V, Dambacher MA, Stussi E. Longitudinal changes in bone in men with spinal cord injury. Clin Rehabil 2000;14:145-52.
50. Modlesky CM, Majumdar S, Narasimhan A, Dudley GA. Trabecular bone microarchitecture is deteriorated in men with spinal cord injury. J Bone Miner Res 2004;19:48-55.
51. Slade JM, Bickel CS, Modlesky CM, Majumdar S, Dudley GA. Trabecular bone is more deteriorated in spinal cord injured versus estrogen-free postmenopausal women. Osteoporos Int 2005;16:263-72.
52. Kiratli BJ, Smith AE, Nauenberg T, Kallfelz CF, Perkash I. Bone mineral and geometric changes through the femur with immobilization due to spinal cord injury. J Rehabil Res Dev 2000;37:225-33.
53. de Bruin ED, Herzog R, Rozendal RH, Michel D, Stussi E. Estimation of geometric properties of cortical bone in spinal cord injury. Arch Phys Med Rehabil 2000;81:150-6.
54. Modlesky CM, Slade JM, Bickel CS, Meyer RA, Dudley GA. Deteriorated geometric structure and strength of the midfemur in men with complete spinal cord injury. Bone 2005;36:331-9.
55. Eser P, Frotzler A, Zehnder Y, et al. Relationship between the duration of paralysis and bone structure: a pQCT study of spinal cord injured individuals. Bone 2004;34:869-80.
56. Shields RK, Dudley-Javoroski S, Boaldin KM, Corey TA, Fog DB, Ruen JM. Peripheral quantitative computed tomography: measurement sensitivity in persons with and without spinal cord injury. Arch Phys Med Rehabil 2006;87:1376-81.
57. Giangregorio LM, Craven BC, Webber CE. Musculoskeletal changes in women with spinal cord injury: a twin study. J Clin Densitom 2005;8:347-51.
58. Hsieh-Li HM, Chang JG, Jong YJ, et al. A mouse model for spinal muscular atrophy. Nat Genet 2000;24:66-70.
59. Shanmugarajan S, Swoboda KJ, Iannaccone ST, Ries WL, Maria BL, Reddy SV. Congenital bone fractures in spinal muscular atrophy: functional role for SMN protein in bone remodeling. J Child Neurol 2007;22:967-73.
60. Shanmugarajan S, Tsuruga E, Swoboda KJ, Maria BL, Ries WL, Reddy SV. Bone loss in survival motor neuron (Smn(-/-) SMN2) genetic mouse model of spinal muscular atrophy. J Pathol 2009;219:52-60.
61. Hamrick MW. Increased bone mineral density in the femora of GDF8 knockout mice. Anat Rec A Discov Mol Cell Evol Biol 2003;272:388-91.
62. Hamrick MW, Pennington C, Byron CD. Bone architecture and disc degeneration in the lumbar spine of mice lacking GDF-8 (myostatin). J Orthop Res 2003;21:1025-32.
63. Morse L, Teng YD, Pham L, et al. Spinal cord injury causes rapid osteoclastic resorption and growth plate abnormalities in growing rats (SCI-induced bone loss in growing rats). Osteoporos Int 2008;19:645-52.
64. Liu D, Zhao CQ, Li H, Jiang SD, Jiang LS, Dai LY. Effects of spinal cord injury and hindlimb immobilization on sublesional and supralesional bones in young growing rats. Bone 2008;43:119-25.
65. Jee WS, Yao W. Overview: animal models of osteopenia and osteoporosis. J Musculoskelet Neuronal Interact 2001;1:193-207.
66. Simske SJ, Greenberg AR, Luttges MW. Effects of suspension-induced osteopenia on the mechanical behaviour of mouse long bones. J Mater Sci Mater Med 1991;2:43-50.
67. Sakata T, Sakai A, Tsurukami H, et al. Trabecular bone turnover and bone marrow cell development in tail-suspended mice. J Bone Miner Res 1999;14:1596-604.

68. Tuukkanen J, Wallmark B, Jalovaara P, Takala T, Sjogren S, Vaananen K. Changes induced in growing rat bone by immobilization and remobilization. Bone 1991;12:113-8.
69. Kingery WS, Offley SC, Guo TZ, Davies MF, Clark JD, Jacobs CR. A substance P receptor (NK1) antagonist enhances the widespread osteoporotic effects of sciatic nerve section. Bone 2003;33:927-36.
70. Thompson DD, Rodan GA. Indomethacin inhibition of tenotomy-induced bone resorption in rats. J Bone Miner Res 1988;3:409-14.
71. Shaker JL, Fallon MD, Goldfarb S, Farber J, Attie MF. WR-2721 reduces bone loss after hindlimb tenotomy in rats. J Bone Miner Res 1989;4:885-90.
72. Ijiri K, Ma YF, Jee WS, Akamine T, Liang X. Adaptation of non-growing former epiphysis and metaphyseal trabecular bones to aging and immobilization in rat. Bone 1995;17(4Suppl.):207S-12S.
73. Morey-Holton ER, Globus RK. Hindlimb unloading rodent model: technical aspects. J Appl Physiol 2002; 92:1367-77.
74. Colleran PN, Wilkerson MK, Bloomfield SA, Suva LJ, Turner RT, Delp MD. Alterations in skeletal perfusion with simulated microgravity: a possible mechanism for bone remodeling. J Appl Physiol 2000;89:1046-54.
75. McCarty R, Kvetnansky R, Kopin IJ. Plasma catecholamines in rats: daily variations in basal levels and increments in response to stress. Physiol Behav 1981; 26:27-31.
76. Kamiya H, Sasaki S, Ikeuchi T, et al. Effect of simulated microgravity on testosterone and sperm motility in mice. J Androl 2003;24:885-90.
77. Wimalawansa SM, Wimalawansa SJ. Simulated weightlessness-induced attenuation of testosterone production may be responsible for bone loss. Endocrine 1999; 10:253-60.
78. Halloran BP, Bikle DD, Cone CM, Morey-Holton E. Glucocorticoids and inhibition of bone formation induced by skeletal unloading. Am J Physiol 1988;255(6Pt.1):E875-9.
79. Marenzana M, De Souza RL, Chenu C. Blockade of beta-adrenergic signaling does not influence the bone mechanoadaptive response in mice. Bone 2007;41:206-15.
80. Alford EK, Roy RR, Hodgson JA, Edgerton VR. Electromyography of rat soleus, medial gastrocnemius, and tibialis anterior during hind limb suspension. Exp Neurol 1987;96:635-49.
81. Clarke KA, Still J. Gait analysis in the mouse. Physiol Behav 1999;66:723-9.
82. Zeng QQ, Jee WS, Bigornia AE, et al. Time responses of cancellous and cortical bones to sciatic neurectomy in growing female rats. Bone 1996;19:13-21.
83. Sievanen H, Heinonen A, Kannus P. Adaptation of bone to altered loading environment: a biomechanical approach using X-ray absorptiometric data from the patella of a young woman. Bone 1996;19:55-9.
84. Allen MR, Hogan HA, Bloomfield SA. Differential bone and muscle recovery following hindlimb unloading in skeletally mature male rats. J Musculoskelet Neuronal Interact 2006;6:217-25.
85. Suzuki N, Motohashi N, Uezumi A, et al. NO production results in suspension-induced muscle atrophy through dislocation of neuronal NOS. J Clin Invest 2007;117:2468-76.
86. Fujita N, Fujimoto T, Tasaki H, Arakawa T, Matsubara T, Miki A. Influence of muscle length on muscle atrophy in the mouse tibialis anterior and soleus muscles. Biomed Res 2009;30:39-45.
87. Thomason DB, Booth FW. Atrophy of the soleus muscle by hindlimb unweighting. J Appl Physiol 1990;68:1-12.
88. Shao C, Liu M, Wu X, Ding F. Time-dependent expression of myostatin RNA transcript and protein in gastrocnemius muscle of mice after sciatic nerve resection. Microsurgery 2007;27:487-93.
89. Liu M, Zhang D, Shao C, Liu J, Ding F, Gu X. Expression pattern of myostatin in gastrocnemius muscle of rats after sciatic nerve crush injury. Muscle Nerve 2007;35:649-56.
90. Batt J, Bain J, Goncalves J, et al. Differential gene expression profiling of short and long term denervated muscle. Faseb J 2006;20:115-7.
91. Amblard D, Lafage-Proust MH, Laib A, et al. Tail suspension induces bone loss in skeletally mature mice in the C57BL/6J strain but not in the C3H/HeJ strain. J Bone Miner Res 2003;18:561-9.
92. Akhter MP, Cullen DM, Pedersen EA, Kimmel DB, Recker RR. Bone response to *in vivo* mechanical loading in two breeds of mice. Calcif Tissue Int 1998;63:442-9.
93. Poliachik SL, Threet D, Srinivasan S, Gross TS. 32 wk old C3H/HeJ mice actively respond to mechanical loading. Bone 2008;42:653-9.
94. Kodama Y, Umemura Y, Nagasawa S, et al. Exercise and mechanical loading increase periosteal bone formation and whole bone strength in C57BL/6J mice but not in C3H/Hej mice. Calcif Tissue Int 2000;66:298-306.
95. Judex S, Garman R, Squire M, Busa B, Donahue LR, Rubin C. Genetically linked site-specificity of disuse osteoporosis. J Bone Miner Res 2004;19:607-13.
96. Mizoguchi F, Mizuno A, Hayata T, et al. Transient receptor potential vanilloid 4 deficiency suppresses unloading-induced bone loss. J Cell Physiol 2008;216:47-53.
97. Kostenuik PJ, Halloran BP, Morey-Holton ER, Bikle DD. Skeletal unloading inhibits the *in vitro* proliferation and differentiation of rat osteoprogenitor cells. Am J Physiol 1997;273(6Pt.1):E1133-9.
98. Jiang SD, Jiang LS, Dai LY. Spinal cord injury causes more damage to bone mass, bone structure, biomechanical properties and bone metabolism than sciatic neurectomy in young rats. Osteoporos Int 2006;17:1552-61.
99. Kondo H, Nifuji A, Takeda S, et al. Unloading induces osteoblastic cell suppression and osteoclastic cell activation to lead to bone loss via sympathetic nervous system. J Biol Chem 2005;280:30192-200.
100. Warner SE, Sanford DA, Becker BA, Bain SD, Srinivasan S, Gross TS. Botox induced muscle paralysis rapidly degrades bone. Bone 2006;38:257-64.

101. Poliachik SL, Bain SD, Threet D, Huber P, Gross TS. Transient muscle paralysis disrupts bone homeostasis by rapid degradation of bone morphology. Bone 2009.
102. Warner SE, Huber P, Threet D, Srinivasan S, Gross TS. RANKL inhibition prevents the loss of bone volume and bone strength caused by Botox induced muscle paralysis. Trans Orthop Res Soc 2006;31:193.
103. Ausk BJ, Huber P, Srinivasan S, Gross TS. Focal bone loss in mice with transient muscle paralysis. J Bone Miner Res 2007;22(S1):S358.
104. Poliachik SL, Bain SD, Srinivasan S, et al. Significant trabecular bone degradation occurs within five days of muscle paralysis. J Bone Miner Res 2008;23(S1):S71.
105. Chappard D, Chennebault A, Moreau M, Legrand E, Audran M, Basle MF. Texture analysis of X-ray radiographs is a more reliable descriptor of bone loss than mineral content in a rat model of localized disuse induced by the Clostridium botulinum toxin. Bone 2001;28:72-9.
106. Grimston SK, Silva MJ, Civitelli R. Bone loss after temporarily induced muscle paralysis by Botox is not fully recovered after 12 weeks. Ann N Y Acad Sci 2007;1116:444-60.
107. Blouin S, Gallois Y, Moreau MF, Basle MF, Chappard D. Disuse and orchidectomy have additional effects on bone loss in the aged male rat. Osteoporos Int 2007;18:85-92.
108. Libouban H, Blouin S, Moreau MF, Basle MF, Audran M, Chappard D. Effects of risedronate in a rat model of osteopenia due to orchidectomy and disuse: densitometric, histomorphometric and microtomographic studies. Micron 2008;39:998-1007.
109. Kim SJ, Roy RR, Zhong H, et al. Electromechanical stimulation ameliorates inactivity-induced adaptations in the medial gastrocnemius of adult rats. J Appl Physiol 2007;103:195-205.
110. Lam H, Qin YX. The effects of frequency-dependent dynamic muscle stimulation on inhibition of trabecular bone loss in a disuse model. Bone 2008;43:1093-100.
111. Swift JM, Nilsson MI, Hogan HA, Sumner LR, Bloomfield SA. Simulated Resistance Training During Hindlimb Unloading Abolishes Disuse Bone Loss and Maintains Muscle Strength. J Bone Miner Res 2009.
112. BeDell KK, Scremin AM, Perell KL, Kunkel CF. Effects of functional electrical stimulation-induced lower extremity cycling on bone density of spinal cord-injured patients. Am J Phys Med Rehabil 1996;75:29-34.
113. Eser P, de Bruin ED, Telley I, Lechner HE, Knecht H, Stussi E. Effect of electrical stimulation-induced cycling on bone mineral density in spinal cord-injured patients. Eur J Clin Invest 2003;33:412-9.
114. Clark JM, Jelbart M, Rischbieth H, et al. Physiological effects of lower extremity functional electrical stimulation in early spinal cord injury: lack of efficacy to prevent bone loss. Spinal Cord 2007;45:78-85.
115. Elefteriou F, Ahn JD, Takeda S, et al. Leptin regulation of bone resorption by the sympathetic nervous system and CART. Nature 2005;434:514-20.
116. Yirmiya R, Goshen I, Bajayo A, et al. Depression induces bone loss through stimulation of the sympathetic nervous system. Proc Natl Acad Sci U S A 2006;103:16876-81.
117. Stjernberg L, Blumberg H, Wallin BG. Sympathetic activity in man after spinal cord injury. Outflow to muscle below the lesion. Brain 1986;109 (Pt 4):695-715.
118. Jeon JY, Steadward RD, Wheeler GD, Bell G, McCargar L, Harber V. Intact sympathetic nervous system is required for leptin effects on resting metabolic rate in people with spinal cord injury. J Clin Endocrinol Metab 2003;88:402-7.