Bone loss due to disuse and electrical muscle stimulation

Hiroyuki Tamaki1*, Kengo Yotani2, Futoshi Ogita2, Hikari Kirimoto1, Hideaki Onishi1 and Norikatsu Kasuga3

1 Institute for Human Movement and Medical Sciences, Niigata University of Health and Welfare, 1398 Shimami, Kita-ku, Niigata 950-1398, Japan
2 National Institute of Fitness and Sports in Kanoya, 1 Shiromizu, Kanoya, Kagoshima 891-2393, Japan
3 Aichi University of Education, 1 Hiroswa, Igaya, Kariya, Aichi 448-8542, Japan

Received: June 14, 2016 / Accepted: July 8, 2016

Abstract The mass and structure of bone tissue adapt to the mechanical loads imparted by gravity and movement, and are controlled by the balance between bone formation and bone resorption. The primary adaptations of bone to disuse are demineralization and loss (thinning) of trabecular and cortical bone. Exercise training and electrical muscle stimulation (ES) induce adaptive changes in bone that improve bone strength and inhibit bone loss. ES has been generally applied to patients undergoing physical rehabilitation to maintain and/or recover muscle mass and force-generating capacity in disused muscles. ES-induced muscle contraction of disused muscle can also ameliorate deleterious post-disuse adaptation of bone. The mechanical effects of ES-induced muscle contraction are essential for the maintenance of bone mass and strength, which are achieved through the cooperative functions of osteocytes, osteoblasts, and osteoclasts. The effects of ES, however, are dependent on the stimulation paradigm, including the intensity, frequency, and number of stimuli and the duration of the intervention. This review summarizes the literature on the effects of ES-induced muscle contraction on disuse osteopenia.

Keywords : atrophy, muscle-bone interaction, denervation, mechanical stress

Introduction

Bone mass is regulated by a dynamic balance between bone formation and bone resorption. The mass and structure of bone tissue adapt to the mechanical loads imparted by gravity and movement. Limb disuse due to denervation, suspension, or immobilization causes musculoskeletal atrophy accompanied by a large reduction in bone mass and changes in cortical and trabecular architecture.

Some clinical studies have found that direct electrical stimulation (ES) to denervated muscles increased muscle mass and average fiber diameter1,2. In addition, there are some reports of increased bone mass after ES-induced muscle contractions in patients with spinal cord injury (SCI)3,4. Electrical muscle stimulation affects muscles and bones through various pathways, including mechanical, circulatory, and humoral pathways. ES causes a muscle contraction that results in mechanical loads applied to bones through the tendon-bone interface. In addition, muscle contraction induced by ES or exercise facilitates cytokine (myokine) production5 and increases bone blood flow and capillary vascularity6,7. One or more of these factors may explain the effects of ES on the reduction of bone loss due to disuse. Furthermore, the effects of ES on bone and muscle atrophy are influenced by the type of disuse model and the nature of the experimental regimen, such as the intensity, frequency, and number of contractions8-11. Here, we describe the effects of ES-induced muscle contraction on bone tissue, review the data supporting these effects, and suggest possible mechanisms underlying these effects. We begin by describing bone adaptation to disuse, and then discuss bone responses to ES-induced muscle contraction.

Bone adaptation to disuse

Bone atrophy is characterized by a reduction in bone volume and bone mineral density and alterations in bone architecture such as reductions in cortical and trabecular bone mass, impairment of bone architecture, and deteriorations of bone strength and material level properties as a result of early increases in bone resorption and prolonged decreases in bone formation. Complete disuse of the limbs, such as in SCI, induces relatively...
dramatic bone loss. By contrast, hindlimb suspension induces relatively modest bone loss. Denervation induced by sciatic neurectomy causes paralysis and atrophy in denervated hindlimb muscles and is one of the methods used to model disuse osteopenia\(^1\). Nerve-freezing methods can be used to immobilize innervated muscles by paralyzing nerve function for certain periods, thus creating a disuse model with temporary damage that also allows experimental observation of the post-atrophy recovery process\(^3-13\). There are some differences in the bone-loss process between the sciatic neurectomy and nerve-freezing models\(^24\). The bone-loss process occurred more gradually in the nerve-freezing model than in the sciatic neurectomy model in the early stage of disuse atrophy, that is, for 2 - 3 weeks after denervation (DN); but there was no significant difference in trabecular bone loss at 3 - 4 weeks, when the lowest trabecular bone volume (BV/TV) level was reached.

**Changes in bone tissue after denervation.** It has been widely demonstrated by previous studies\(^2,12,16-18\) that there are two phases of long-term hindlimb disuse: an early phase, which consists of a rapid decrease of bone volume, and a later phase, in which there is a slowdown of the atrophic process and the bone mass and microstructure seem to be stable. Trabecular and cortical microstructural parameters (bone volume fraction, trabecular thickness, trabecular number, connection density, cortical area, cortical thickness, and bone mineral density) in rat tibiae or femurs significantly decrease during the first 1 - 2 weeks of hindlimb disuse. Trabecular bone loss during hindlimb disuse tended to be greater in magnitude and preceded cortical bone loss, owing to the higher turnover and metabolic activity of trabecular bone\(^19,20\). Trabecular bone loss and morphological changes due to denervation started in the first week after surgery and gradually decreased over the next 3 - 10 weeks\(^16\).

It is far more detrimental to the mechanical competency of the bone to lose a trabecular connection than to have overall thinning of the trabecular network\(^21\). Alterations of trabecular architecture during hindlimb disuse are characterized by trabecular bone thinning and fragmentation. In rats, the decrease in trabecular thickness (trabecular thinning) mainly occurs in the early stage of disuse atrophy, and the fragmentation of trabeculae occurs after 5 - 6 weeks of hindlimb disuse. The patterns of trabecular alteration are quite different between trabecular bone loss induced by mechanical factors such as hindlimb disuse and that induced by a hormonal factor such as estrogen deficiency. In a rat-hindlimb-disuse model, trabeculae thinning was observed in the whole area of the secondary spongiosa, and there was little difference between the central and peripheral areas. On the contrary, in estrogen-deficient rats, trabeculae thinning was identified primarily in the central area of the secondary spongiosa\(^22\). In general, osteoclasts dig out a cavity approximately 50 - 60 μm deep called a resorption pit, and remove old bone tissue at remodeling sites. Trabeculae thinning and excessive osteoclast activity result in the fragmentation of trabeculae.

**Bone responses to ES-induced muscle contraction**

**Clinical studies.** The level of physical activity plays a key role in determining bone and muscle mass\(^33\). Performance of physical activity or exercise training requires muscle contraction, but ES has been utilized for patients as a therapeutic intervention and a functional substitute for voluntary muscle contraction. Therapeutic ES improves the neuromuscular functional condition by strengthening muscles, increasing motor control, reducing spasticity, decreasing pain, and increasing range of motion\(^24\). Functional ES induces muscle contraction and produces functionally useful movement during stimulation\(^26\). Transcutaneous ES is traditionally applied to patients undergoing physical rehabilitation to maintain and/or recover mass and force-generating capacity in disused muscles. Some clinical reports have found that direct ES to denervated muscles in patients with SCI increased muscle mass and average fiber diameter\(^2\). Some findings from animal experiments lend further support to the notion that ES helps to limit denervation-induced muscle atrophy and improve muscle force-generating capacity and recovery\(^25-27\), whereas other studies have generated contradictory results\(^28-30\). The effects of ES on muscle atrophy appear to be influenced by the type of disuse model and the nature of the experimental regimen, such as the intensity, frequency, and number of contractions\(^8,9\).

Muscle contraction confers mechanical load upon bone tissue. In patients with SCI, there are some reports of increased bone mass after ES-induced muscle contractions\(^31-34\), and one report that osteopenia of the distal femur and proximal tibia and loss of quadriceps strength was partly reversed by training assisted by ES at 25 Hz for 24 weeks\(^3\). However, several other studies have demonstrated no effect of ES strengthening or cycle ergometry on measures of bone health\(^31-33\). These contradictory reports of the effects of ES-induced muscle contraction on bone mineral density may be due to different ES regimens and/or the length of time post-SCI.

**Experimental animal studies.** In animal models, maintenance of ES-induced muscle contraction within cast-immobilized limbs resulted in less bone loss than in non-stimulated immobilized limbs\(^35\). Muscle mass influences the stimulation delivered to the bone tissue through ES-induced muscle contraction. Although an ES-induced muscle contraction might deliver relatively low stimuli to bone tissue, it helps to prevent or reduce disuse-induced osteopenia because mechano-sensitivity and the speed of mechanical loading, i.e., strain rate, are major influences on bone volume and architecture\(^36-37\). Static loads have no effect on bone remodeling activity, whereas the effects
of dynamic loading can be profound. In addition, the osteogenic effect of loading appears to be greatest when the strains and strain rates are high and the strain distributions unusual\(^{38,39}\). Some studies have demonstrated that even low-magnitude mechanical stimuli increase bone and muscle mass in humans and other animals\(^{40-42}\).

Dynamic muscle stimulation with mid-, and high-frequency ES regimens (20, 50, and 100 Hz) for 10 min per day for 4 weeks inhibited trabecular bone loss of the femur (bone volume fraction, trabecular number, trabecular separation, and connection density) determined by three dimensional micro-computed tomography in a suspension disuse model\(^{35,36}\). The notion that a higher frequency of ES increases muscle contraction force is conceivable because of the summation of twitch contraction forces\(^{33}\). Stimulation frequency is also an important factor in the effect of ES on bone tissue. Qin et al. reported that ES from 1 Hz to 100 Hz generated various nonlinear bone stresses and fluid pressures in bone, and that maximal bone strain was observed with ES at 10 Hz, and mitigated bone loss\(^{43,45}\). It is important to explore the potential of ES at 10 Hz for the reduction of muscle and bone loss due to disuse.

In tail-suspended rats, low-frequency-ES-induced contractions of the hindlimb muscles did not prevent the decrease in bone mass parameters (bone volume fraction, trabecular thickness, and trabecular number), but did ameliorate the decrease in bone formation parameters (osteoid surface, osteoblast surface, and mineralizing surface) and elevated periosteal and cancellous bone formation rates.\(^{46}\). The daily chronic muscle stimulation was achieved using a 10-Hz bipolar rectangular current with an intensity of 0.2 - 8 mA for 3 weeks.

Recent studies using low-frequency ES of 10 Hz (incomplete tetanus) have reported delayed trabecular bone and muscle loss during the early stage of musculoskeletal atrophy in disused rats\(^{31,47}\). In these reports, the tibialis anterior (TA) muscle was stimulated directly with current of 4, 8 or 16 mA at a frequency of 10 Hz, for 30 min per day, 6 days per week, for 1 or 3 weeks. The ES regimen was carried out with 2-s stimulation followed by 6-s rest. Muscle contraction induced by ES of 16 mA reduced trabecular bone loss (bone volume fraction, trabecular thickness, and connection density) and decreased osteoid thickness and osteoid-osteocyte numbers; but ES of 4 or 8 mA did not reduce bone loss.

**ES-induced muscle contraction force as a means of mechanically loading bone.** ES-induced muscle contraction has multiple effects, including generation of muscle contraction force and resulting mechanical load on bone tissue, enhancement of blood flow and fluid flow in bone, and secretion of muscle-derived factors that might influence bone metabolism.

Mechanical loading is one of the major factors affecting bone remodeling\(^{45,56}\). Muscle contraction force delivers mechanical stress to bone in vivo, similar to the stress delivered during physical exercise, loading, and vibration. Maintaining muscle volume to maintain the force-generating capacity of the muscle is of importance for bone health. In general, a higher intensity/frequency of ES causes higher muscle tension because of the recruitment of muscle fibers and/or summation of twitch contractions; this is then more likely to deliver adequate mechanical stimuli to bone. Tetanic tension caused by higher ES frequencies may reduce disuse-induced osteopenia. This is supported by the observation that muscle stimulation at 20 - 100 Hz for 4 weeks inhibited trabecular bone loss in the disused rat femur\(^{40}\).

Bone strain is a direct modulator of mechano-adaptive osteogenesis. The relationships between peak dynamic load and an increase/decrease in bone mass in the cortical and trabecular regions are essentially linear\(^{36}\). Sugiyama et al. reported that peak strain at the tibial mid-shaft could be determined in vivo during walking (~300 με), jumping (~600 με), and the application of a dynamic load (0 - 14 N) by strain gauges attached to the bone surface\(^{54}\). In the tibia of a neurectomized limb, a peak dynamic load of 2 N resulted in a similar peak strain to that observed during walking. The minimum effective strain required to maintain BV/TV has been reported to be around 1000 με (6.6 N)\(^{56}\) in mouse and rat tibiae\(^{48}\). Caulkins et al. estimated that an ES-induced muscle contraction force of 1 N produced approximately 200 με in resultant maximal cortical strain in a rabbit proximal tibia\(^{79}\).

However, even a relatively small muscle force or mechanical load can reduce disuse-induced osteopenia. Low-magnitude mechanical stimuli increased bone formation in animal models of disuse\(^{54,50}\). Bone strains as small as 5 με applied at 30 Hz or 90 Hz for 20 min per day over 1 year or 4 weeks induced a 34% increase in proximal femur trabecular bone density and increased bone formation rates in sheep and hindlimb-suspended rats. Moreover, daily ES at 10 Hz for 2 s followed by 6 s of rest, repeated for 225 cycles a day, which generated a muscle contraction force of approximately 20 - 30% of maximal contraction force, reduced trabecular bone loss during the early stage of musculoskeletal atrophy due to disuse\(^{41,47}\). With these parameters, the ES caused little summation of twitch contraction\(^{11}\), therefore, it is estimated that 4500 repetitions of a mechanical stimulus (10 Hz × 2 s × 225 repetitions) at 10 Hz a day would have been applied to the bone.

In addition, this type of ES-induced muscle force reduced trabecular bone loss and the decrease in osteoid and osteocyte numbers embedded in the osteoid area that occurs following denervation\(^{77}\). Streptomycin treatment did not induce bone loss, but attenuated the ES-induced reduction in the loss of disused bone\(^{47}\). Streptomycin treatment resulted in approximately a 70 - 90% reduction in the bone anabolic response to ES-induced muscle contraction. Many studies have reported that streptomycin may be a stretch-activated ion channel blocker\(^{51,52}\), and
stretched-activated ion channels are included in osteoblasts and osteocytes. The resulting osteoblastic osteogenesis maintained by ES-induced muscle contraction might be caused in part through activation of mechanosensors in bone tissue. Mechanical loading induced by ES might have a major influence on the mass and structure of bone tissue.

**ES-induced muscle contraction as a means to enhance circulatory and humoral factors.** In addition to the mechanical effects of ES, circulatory and humoral factors can also affect bone mass. Exercise training such as treadmill walking (15 m/min, 60 min/day for 12 weeks) and swimming (5 days/week for 8 weeks) increased capillary vascularity and bone blood flow in the femur and tibia of aged rats. Transcutaneous ES-induced muscle contraction also increased cortical microcirculatory flow, bone blood flow, and bone mineral content in the tibia. ES-induced muscle contraction is an effective tool to enhance fluid flow in bone. ES-induced muscle contraction enhances venous and arterial blood flow, which subsequently increases intramedullary pressure and fluid flow in bone. Oscillatory fluid flow induced shear stress, decreased osteoclastogenesis, and stimulated osteoblast proliferation and differentiation. Shear stress acting on bone cells owing to blood circulation–driven interstitial fluid flow seems to play an important role in bone formation and resorption.

Mechanotransduction converts physical forces into biochemical signals that are then integrated into cellular responses. During mechanical signal transmission, osteoblasts, osteocytes, and cells that line bone might act as sensors. These cells also produce growth factors that might signal osteogenic factors to differentiate into osteoblasts and promote osteoblast activity. Mechanical loading promotes bone formation, osteoblastic differentiation and activity, and bone lining cell (BLC) reactivation, and inhibits the expression of osteoclast differentiation factor and osteoclast numbers. Mechanical stimuli of osteoblasts induce the secretion of growth factors including insulin-like growth factor (IGF), vascular endothelial growth factor, transforming growth factor-β, and the bone morphogenetic protein, which are considered to be the principal local regulators of osteogenesis.

The role of muscle-derived growth factors in bone formation has recently been discussed. Muscle seems to be an important local source of growth factors for bone tissue, yet the cellular and molecular mechanisms linking muscle and bone tissues are not well understood. Exercise and muscle contraction alter the secretion of several myokines that appear to affect bone metabolism. Some authors have reported that secretion of myostatin (growth differentiation factor-8) in muscle inhibits bone formation, whereas IGF-1 and fibroblast growth factor-2 stimulate bone formation in vivo and in vitro. Myokine secretion induced by muscle contraction might be one mechanism by which muscle contraction stimulates bone formation. However, molecular and cellular pathways by which muscle contraction affects osteoblasts, osteoclasts, and osteocytes in bone tissue are poorly understood.

It should be noted that skeletal muscle is a resource for generating mechanical, circulatory, and humoral factors. An ES training regimen that reduces structural and functional damage in denervated muscle fibers may potentiate the anabolic activity of bone tissue, but the dilemma regarding optimal stimulation parameters remains. For example, in one study that assessed the optimal stimulation intensity for the recovery of skeletal muscle and bone after denervation, direct ES (16 mA) retarded denervated muscle and bone atrophy and up-regulated IGF-I mRNA expression. However, this intensity of ES might adversely affect the regeneration of nerve terminals and/or the membrane systems involved in excitation-contraction coupling, which is the physiological process of converting an electrical stimulus to mechanical activation of the contractile myofibrils. Conversely, relatively low-intensity (4 and 8 mA) ES regenerated nerve terminals and membrane systems involved in excitation-contraction coupling, but did not retard denervated muscle and bone atrophy. These results indicate the importance of establishing the optimal stimulation intensity according to the specific structural and functional profiles that are targeted for improvement. Similarly, human studies on ES regimens aimed at preventing or reducing muscle and bone loss induced by disuse are important for the development of clinical interventions.

**Conclusion**

Clinical and experimental studies have demonstrated that muscle can function as a generator of mechanical load to bone and as an endocrine and a paracrine organ. The effects of ES-induced muscle contraction on atrophying bone are influenced by the type of disuse model and the nature of the ES regimen, including the intensity, frequency, and number of stimuli. Oстеogenesis maintained by ES-induced muscle contraction might be caused in part through activation of mechanosensors in bone tissue. Therefore, elucidation of the complex nature of muscle-bone interactions may lead to the advancement of therapeutic strategies to ameliorate disuse-induced osteopenia.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this article.

**Acknowledgments**

This work was supported by JSPS KAKENHI (grant nos. 25350829, 25282163, 16K13021).
References


31) Bloomfield SA, Mysiw WJ and Jackson RD. 1996. Bone mass and endocrine adaptations to training in spinal cord in-


