Bone 43 (2008) 1093–1100

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

The effects of frequency-dependent dynamic muscle stimulation on inhibition of trabecular bone loss in a disuse model

Hoyan Lam, Yi-Xian Qin *

Department of Biomedical Engineering, Stony Brook University, 350 Psychology A Bldg., Stony Brook, NY 11794-2580, USA

ARTICLE INFO

Article history:
Received 12 April 2008
Revised 24 July 2008
Accepted 30 July 2008
Available online 13 August 2008

Edited by: D. Burr

Keywords:
Electrical muscle stimulation
Bone fluid flow
Loading frequency
Bone adaptation
Functional disuse
Osteopenia
Skeletal adaptation

ABSTRACT

Clinical electrical muscle stimulation has been shown to alleviate muscle atrophy resulting from functional disuse, yet little is known about its effect on the skeleton. The objective of this study is to evaluate the potential of dynamic muscle stimulation on disused trabecular bone, and to investigate the importance of optimized stimulation frequency in the loading regimen. Fifty-six skeletally mature Sprague–Dawley rats were divided into seven groups for the 4-week experiment: baseline control, age-matched control, hindlimb suspended (HLS), and HLS with muscle stimulation at 1 Hz, 20 Hz, 50 Hz, and 100 Hz. Muscle stimulation was carried out for 10 min per day for 5 days per week, total of 4 weeks. The metaphyseal and epiphyseal trabecular regions of the distal femurs were analyzed with microcomputed tomography and histomorphometry methods. HLS alone for 4-week resulted in a significant amount of trabecular bone loss and structural deterioration. Muscle contraction at 1 Hz was not sufficient to inhibit trabecular bone loss and resulted in similar amount of loss to that of HLS alone. Bone quantity and structure were significantly improved by applying muscle stimulation at mid-frequency (20 Hz and 50 Hz). Dynamic stimulation at 50 Hz demonstrated the greatest preventive effect on the skeleton against functional disused alone animals (up to +147% in bone volume fraction, +38% in trabecular number and −36% in trabecular separation). Histomorphometric analysis showed that the stimulation, regardless of its frequency, did not have an effect on the bone formation indices, such as mineral apposition rate and bone formation rate. Overall, the data demonstrated the potentials of frequency-dependent dynamic muscle contraction in regulating skeletal adaptive responses under disuse conditions. Dynamic muscle stimulation, with a specific regimen, may be beneficial to future orthopedic research in developing a countermeasure for disuse osteopenia and osteoporosis.

Published by Elsevier Inc.

Introduction

Conditions associated with disuse osteoporosis are often identified as decreased bone mass and deterioration of the skeletal micro-architecture. Disuse osteoporosis is a common skeletal disorder in the elderly, in patients subjected to prolonged immobility or bed-rest, e.g., fracture and spinal injury, and in astronauts who participate in long-duration spaceflight. In addition to bone loss, functional disuse and microgravity can cause muscle atrophy. Taken together, these physiological changes generate additional health complications, including increased risk of falls and fracture, and poor long-term recovery. Thus, there is a great need to develop a clinically applicable intervention for the prevention of progressive muscle atrophy and osteopenia.

Analyses of spinal cord injury (SCI) patients showed significant reduction in bone mineral density (BMD) in the disused limbs[1–3] and higher incidence of fracture [4,5]. More than 1 year after spinal cord injury, 30% to 40% of demineralization was observed in the femoral neck, distal femur and proximal tibia [2]. It has been reported that SCI induced osteopenia/osteoporosis reached the fracture threshold (BMD of 1 g/cm^2) 1 to 5 years after the injury, with a fracture frequency of 5% to 34% [4,6–8]. Similarly, analyses from space missions of 4 to 12 months duration have demonstrated that weightlessness can induce 1% to 2% BMD loss per month in the spine, 1.6% BMD loss in the hip, and 1.2% in the lower extremities [9,10]. In addition, the reduction in trabecular BMD in both hip and femur regions was greater than 2% per month, while there was only minimal decrease in the cortical bone [11].

Clinical muscle stimulation has been examined extensively in SCI patients to strengthen skeletal muscle and alleviate muscle atrophy with promising outcomes [12,13]. A few physical training studies further investigated this electrical stimulation technique to determine the effect on osteopenia. These studies showed mixed results in bone density data [14–16]. Using dual energy X-ray absorptiometry (DXA), BeDell et al. found no change in BMD of the lumbar spine and femoral neck regions after functional electrical stimulation-induced cycling exercise, while Mohr et al. illustrated a 10% increase in BMD in the

* Corresponding author. Fax: +1 631 632 8577.
E-mail address: Yi-Xian.Qin@sunysb.edu (Y.-X. Qin).

8756-3282/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.bone.2008.07.253

proximal tibia after 12 months of similar training [14,16]. In a 24-week study of SCI patients in whom 25 Hz electrical stimulation was applied to the quadriceps muscles daily, Belanger and colleagues reported a 28% recovery of BMD in the distal femur and proximal tibia, along with increased muscle strength [15].

In order to explore the mechanism of disuse osteoporosis and to develop new interventions, animal models have been used to study skeletal adaptation, mimicking results from human disuse. Exposure of rats to 14 days of microgravity demonstrated an 11% reduction of trabecular BMD in the distal femur region [17]. Data from COSMOS flights (14–40 days) showed similar outcomes, with decreased mineralized tissue mass, trabecular number and thickness, and increased resorption activity [18–20]. Ground-based hindlimb suspension (HLS) rodent models can induce significant 10% to 30% BMD reduction in both femur and tibia [21–23]. Histomorphometric analyses revealed a 66% decrease in trabecular osteoblast surface, and up to a 70% decrease in bone formation rate (BFR), while there was minimal change seen in resorptive activity [22,24].

Electrical muscle stimulation with disuse animal models also demonstrated effects on the musculoskeletal system. Several investigators have shown that muscle contraction can prevent muscle atrophy to varying degrees, depending on the experimental regimen [25,26]. Other changes included transition between type I and II muscle fibers and increased resistance to fatigue [27–29]. When studying bone adaptive response to electrical stimulus, results were mixed. Zerath et al. found that electrical stimulation increased osteoblast activity after 3-weeks disuse, yet Midura et al. reported partial preventive effect on osteopenia [30,31]. One explanation for these discordant outcomes observed in both clinical and *in vivo* studies might be the selection of the applicable signals.

Our group has demonstrated previously that various mechanical stimuli frequencies can generate different level of surface strain and intramedullary pressure in long bone [32,33]. Here, we hypothesized that daily induced dynamic muscle contraction can inhibit bone loss and maintain the trabecular network during a 4-week study using a functional disuse model, and that the adaptive response is dependent on the stimulation frequency. To further explore the potential of electrical stimulation as a non-invasive approach to the skeletal system, our objective was to investigate the effect of dynamic electrical muscle contraction on disused trabecular bone. In particular, we examined the importance of the stimulation frequency in the inhibition of osteopenia in a hindlimb suspension (HLS) animal model.

Materials and methods

Experimental design

All experimental procedures were approved by the Laboratory Animal Use Committee at Stony Brook University. Fifty-six 6-months-old female Sprague–Dawley retired breeder rats (Taconic, NY) were used to investigate the effects of frequency-dependent dynamic muscle stimulation (MS) on skeletal adaptation under disuse environment. They were housed individually in 18″×18″×24″ (L×W×H) stainless steel HLS cages in a temperature-controlled room with a 12:12 h light:dark cycle, and were provided standard rodent chow and water ad libitum. Animals were transferred to these cages one week prior to the experiment start date in order to acclimate them to their environment. Animals were randomly assigned to seven groups with $n=8$ per group: (1) baseline control, (2) age-matched control, (3) HLS, (4) HLS+1 Hz MS, (5) HLS+20 Hz MS, (6) HLS+50 Hz MS, and (7) HLS+100 Hz MS. Functional disuse was induced by HLS, setup modified from Morey–Holton and Globus [34]. Briefly, animal's tail was cleaned with 70% alcohol and lightly coated with tincture of benzoin. Once the tail was dried and sticky, a tail harness was attached to the tail with a piece of surgical tape. The tape was secured with two strips of elastic adhesive bandage; one over the end of the tape at the base of the tail and the other about half-way up to the end of the tail. The tail harness was then attached to a swivel apparatus suspended from the top of the cage. An approximately 30° head-down tilt was set to prevent contact of the animal's hindlimbs with the cage bottom. The animal's forelimbs were allowed full access to the entire cage bottom. The body weight of each animal was weighted three times per week throughout the study.

Electrical MS protocol

For the four experimental groups, dynamic MS was applied in conjunction with HLS for 4 weeks. For the daily stimulation, animals were anesthetized and remained suspended on a counter-top with the experimental set-up. Muscle contraction was induced with two disposable needle-size electrodes (L-type guage #3, Seirin, Weymouth, MA); one electrode was placed at the right lateral proximal quadriceps, ~5 mm away from the greater trochanter, and the other was placed at the lateral distal quadriceps, above the condyles. The electrodes were then connected to a 100 MHz arbitrary waveform generator (Model 395, Wavetek) to transmit a 1 ms square pulse with various stimulation frequencies (1 Hz, 20 Hz, 50 Hz and 100 Hz) for 10 min per day, 5 days per week, for a total of 4 weeks. Age-matched and HLS animals were also subjected to anesthesia for the same amount of time per day as the experimental animals to account for any potential effect due to isoflurane inhalation. A rest-insertion period (2 s contraction followed by 8 s rest) was added in the MS regimen to avoid muscle fatigue.

Microcomputed tomography (μCT)

After 4 weeks of study, animals were euthanized, and the right femurs were harvested and preserved in 70% ethanol. Using a high resolution μCT scanner (μCT-40, SCANCO Medical AG, Bassersdorf, Switzerland), the distal portion of the femur was scanned with a spatial resolution of 15 μm. All images were evaluated using Gaussian filter, with specific sigma, support and threshold values of 0.5, 1, and 347, respectively. Three consecutive 750 μm regions of trabecular bone (M1, M2 and M3) were analyzed in the distal metaphysis, immediately proximal to the growth plate (Fig. 1). M1 is the section closest to the diaphysis, M2 is the middle section between M1 and M3, and M3 is the section closest to the growth plate. One 750 μm region of trabecular bone was also analyzed in the distal epiphysis of each femur (Fig. 1). Values for bone volume fraction (BV/TV, given as %), connectivity

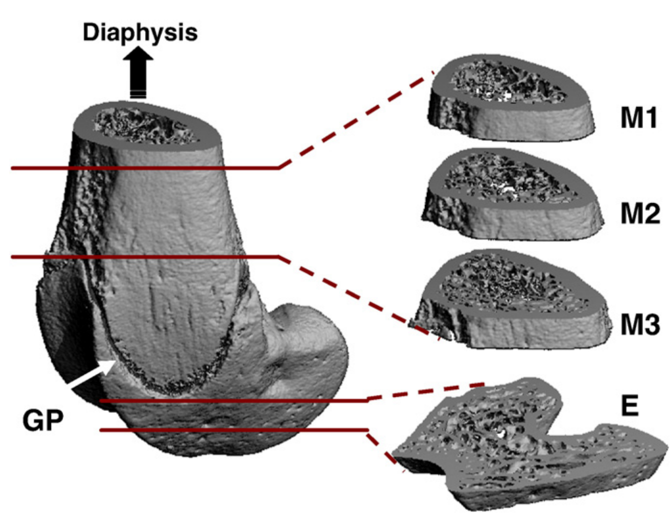

Fig. 1. Trabecular bone at three metaphyseal sections and one epiphyseal region of the distal femurs was evaluated using microcomputed tomography. GP=growth plate (arrow); M=metaphysis; E=epiphysis.