Bone Formation Induced by a Novel Form of Mechanical Loading on Joint Tissue

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Abstract

Because of insufficient mechanical loading, exposure to weightlessness in space flight reduces bone mass. In order to maintain bone mass in a weightless condition, we investigated a novel form of mechanical loading - joint loading. Since some part of gravity-induced loading to our skeletal system is absorbed by viscoelastic deformation of joint tissues, we hypothesized that deformation of joint tissues would generate fluid flow in bone and stimulate bone formation in diaphyseal cortical bone. In order to test the hypothesis, we applied directly oscillatory loading to an elbow joint of mice and conducted bone histomorphometry on the diaphysis of ulnae. Using murine femurs ex vivo, streaming potentials were measured to evaluate a fluid flow induced by joint loading. Bone histomorphometry revealed that compared to no loading control, elbow loading increased mineralizing surface, mineral apposition rate, and bone formation rate 3.2-fold, 3.0-fold, and 7.9-fold, respectively. We demonstrated that joint loading generated a streaming potential in a medullar cavity of femurs. The results support a novel mechanism, in which joint loading stimulates effectively bone formation possibly by generating fluid flow, and suggest that a supportive attachment to joints, driven passively or actively, would be useful to maintain bone mass of astronauts during an exposure to weightlessness.

Keywords: mechanical loads, mice, bone, ulna, elbow, streaming potential

Introduction

The goal of a current study is to evaluate the role of joint loading in maintenance of bone mass under weightlessness. In a critical path roadmap developed by NASA, the need for the exercise regimen during exposure to weightlessness is addressed. In order to minimize decreases in bone mass in space, one of the critical questions raised in the critical path roadmap is whether impact bone loading is indispensable.

In this report, we addressed a question whether gentle loading applied to joints would enhance new bone formation in the diaphysis. During a routine exercise under earth’s gravity, loads transmitted to diaphyseal cortical bone are dissipated at least in part through deformation of viscoelastic joint tissues. The deformation of bone is known to generate strain-induced viscous fluid flow (Piekarski et al., 1977; Tate et al., 2000), and flow-driven shear stress is considered to stimulate proliferation and differentiation of osteoblasts (Pavalko et al., 1998; Reich et al., 1990; Ryder et al., 2001) and osteocytes (Ajubi et al., 1999; Klein-Nulend et al., 1995). Since some part of bone deformation is generated in joints, we investigated a possibility that direct deformation of joints without any strain in cortical bone would stimulate bone formation in diaphyseal cortical bone.

In the current study, a piezoelectric mechanical loader (Tanaka et al., 2003) was employed to apply mechanical loading to a murine elbow joint. Streaming potentials were measured in a medullar cavity of murine femurs ex vivo in order to evaluate load-induced fluid flow in cortical bone. Here, we demonstrate that joint loading induced streaming potentials and increased new bone formation.

Materials and Methods

Mechanical Loading

Twenty female C57BL/6 mice (14 weeks old) with a body weight of approximately 20 g were used for this study. Ten mice were used for a bone histomorphometric analysis and the other ten mice were used for the measurement of streaming potentials. The mouse was mask-anesthetized using 2% isoflurane. The loading was applied with the piezoelectric loader (Tanaka et al., 2003) for 3 minutes per day for 3 consecutive days to an elbow of right arm through a lateral-medial direction (Fig. 1A). A left arm was used as non-loading control. The loading force was sinusoidal at 2 Hz with a peak-to-peak force of 0.5 N. In order to avoid local stress concentrations between the joint and the loader, the surface of the loader was covered with a silicon rubber sheet.
Bone Formation by Joint Loading

Histomorphometry

The joint-loaded mice were given an injection of 0.05 ml saline containing 1% calcein 2 and 6 days after the last loading, and the ulnae were harvested 13 days after the loading. The harvested ulnae were fixed in 10% formalin for 2 days. After dehydration by immersion in a series of ethanol solutions, the samples were embedded in methyl methacrylate. Transverse sections of 50 µm in thickness were cut at 2.5 mm distal from the elbow using a diamond wire saw, and ground with a sand paper (#400) to about 20 µm in thickness. The sections were examined with a Nikon fluorescence microscope. Using the Bioquant semiautomatic digitizing system (R&M Biometrics, Nashville, TN), three morphometric parameters such as mineralizing surface (MS/BS, %), mineral apposition rate (MAR, µm/day), and bone formation rate (BFR/BS, µm²/µm²/year) were determined on a periosteal surface, where MS = sum of the length of double-labeled perimeter and half of single-labeled perimeter, BS = total length of perimeter, MAR = average radial distance between the two labels per day, and BFR/BS = MS/BS x MAR x 3.65 (Hsieh et al., 2001).

Streaming Potential

To reveal existence of fluid flow induced by joint loading, we measured streaming potentials in a medullar cavity of murine femurs ex vivo. The periosteal surface of femurs was dissected free of muscle and was kept moist with a saline solution (PBS). Two holes were drilled on the anterior surface of the midshaft at a 5-mm interval. Bone marrow was flashed out and replaced with PBS through the holes. Electrodes (silver-plated copper wires with a diameter of 0.1 mm) were inserted into the two holes, which were sealed with glue (M-Bond 200, Measurement Group Inc., NC, USA). The electrodes were connected to a windows-based computer via a 16-bit data acquisition board (PCI-6052E, National Instruments Co., TX, USA). Using the piezoelectric loader, sinusoidal loads with 5.8 N peak-to-peak were applied to the femoral distal epiphysis at 2 Hz in the lateral-medial direction. Simultaneously, streaming potential generated between the electrodes was recorded at a 250-msec interval. Fast Fourier Transform was conducted to extract the streaming potential corresponding to the loading frequency at 2 Hz. The spectrums obtained from fifty loadings were accumulated and averaged.

Data Analysis

In order to examine statistical significance in the histomorphometric data, we conducted paired t-tests (StatView, Version 5.0, SAS Institute Inc., Cary, NC) with a significance level at p < 0.05.

Results and Discussion

Formation of periosteal bone by elbow loading

Bone histomorphometry with fluorescent calcein-labeling revealed that elbow loading significantly stimulated bone formation. The cross section of the diaphysis, 2.5 mm distal (16% of ulnar length) to the elbow, clearly showed double labeling on the periosteal surface.

Fig. 1. Schematic diagram of elbow loading and ulna loading. (A) Elbow loading with a piezoelectric loader. (B) Conventional bone loading (ulna bending). The ulna is exposed to bending moment because of its natural curvature.

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with sinusoidal loading for 3 min per day for 3 days on the elbow (Fig. 2). Three bone morphometric parameters such as MS/BS, MAR, and BFR/MS were significantly increased by elbow loading (Fig. 3). The increase in MS/BS, MAR, and BFR/MS was 3.2-fold, 3.0-fold, and 7.9-fold, respectively.

Streaming potential

Streaming potential, induced by a coupling between ion flux and fluid movement, is a good indicator of fluid flow in bone (Beck *et al.*, 2002). In response to joint loading at 2 Hz with 5.8-N force (Fig. 4A), we observed a spike of potentials at 2 Hz on the spectrum (Fig. 4B). The peak of streaming potential was 43 μV higher than a background level. Assuming a linear relationship between applied loads and streaming potentials, the streaming potential inducible by 0.5-N joint-loading was estimated as 3.7 μV.

The current study examined the role of joint loading in bone formation - a novel loading modality. Our histomorphometric data demonstrated that joint loading required a lower magnitude of loading force (0.5 N peak-to-peak) to induce bone formation than ulna bending (Fig. 1B), which required over 1 N peak-to-peak force (Robling *et al.*, 2002). Our results suggest that joint loading is an effective loading regimen to maintain bone mass under weightlessness in space.

![Fig. 2. Calcein-stained ulnae with elbow loading at 2 Hz for 3 min for 3 days. The section of ulnae, 2.5 mm distal to an elbow joint, is shown. (A) Control ulna (no loading) with very faint staining. (B) Loaded ulna with strong staining. (C) Magnified periosteal bone, a circled region in (B), with double calcein-labeling representing a newly formed bone between two calcein injections.](image)

![Fig. 3. Histomorphometric parameters representing the effects of elbow loading at 2 Hz for 3 min for 3 days. (A) MS/BS (mineralizing surface). (B) MAR (mineral apposition rate). (C) BFR/BS (bone formation rate). The data represent the mean ± the standard error (n = 10). The asterisk indicates significant difference (p < 0.05) from the non-loading control (left ulna).](image)
of murine femurs excitation with 5.8-N peak-to-peak force at 2 Hz was given to the epiphysis. The dashed line shows the spectrum of no-loading control. The solid line represents the spectrum of the loaded femurs, and the potential was measured in the medullar cavity of femurs under the loading. The solid line represents the spectrum of the loaded femurs, and the dashed line shows the spectrum of no-loading control.

![Fig. 4](image_url)

**Fig. 4.** Streaming potentials by joint loading. (A) Input force. Sinusoidal excitation with 5.8-N peak-to-peak force at 2 Hz was given to the epiphysis of murine femurs *ex vivo*. (B) Frequency spectrum of streaming potentials. The potential was measured in the medullar cavity of femurs under the loading. The solid line represents the spectrum of the loaded femurs, and the dashed line shows the spectrum of no-loading control.

The results we obtained support the novel role of joints as an inducer of fluid flow and a stimulator of bone formation. Conventionally a joint has been considered as a shock absorber that protects bone from impact loading (Voloshin *et al.*, 1981). In our study, however, joint loading showed a significant increase in bone formation on periosteal surface of diaphyseal cortical bone. It has been well-recognized that the osteogenic responses can be induced by bone strain *in vivo* (Rubin *et al.*, 1985; Turner *et al.*, 1994) and fluid flow *in vitro* (You *et al.*, 2000). Since streaming potential is a good indicator of fluid flow in bone, our data on streaming potentials and bone histomorphometry support that joint loading can induce fluid flow and stimulate bone formation without causing strain in cortical bone.

The current study also contributes to design a potential joint supporter to prevent bone loss. The portable joint attachment, driven by a small electric actuator or routine joint motions, could generate fluid flow actively or passively in bone and stimulate bone formation. Such supporters can be useful for astronauts to maintain bone mass.

**Acknowledgements**

The authors thank Dr. Charles H. Turner and Dr. Imranul Alam for mechanical loading and Dr. Keith Condon for bone histomorphometry. The study was in part supported by Whitaker Foundation and NIHRO1EB001019.

**References**


