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Knee loading dynamically alters intramedullary pressure in mouse femora

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Abstract

Dynamic mechanical loads have been known to stimulate bone formation. Many biophysical factors such as number of daily loading cycles, bone strain, strain-induced interstitial fluid flow, molecular transport, and modulation of intramedullary pressure have been considered as potential mediators in mechanotransduction of bone. Using a knee loading modality that enhances anabolic responses in mouse hindlimb, we addressed a question: Do oscillatory loads applied to the knee induce dynamic alteration of intramedullary pressure in the femoral medullary cavity? To answer this question, mechanical loads were applied to the knee with a custom-made piezoelectric loader and intramedullary pressure in the femoral medullary cavity was measured with a fiber optic pressure sensor. We observed that in response to sinusoidal forces of 0.5 Hz and 10 Hz, pressure amplitude increased up to 4-N loads and reached a plateau at 130 Pa. This amplitude significantly decreased with a loading frequency above 20 Hz. To confirm alteration of intramedullary pressure, real-time motion of microparticles in a glass tube inserted to the femoral medullary cavity *ex vivo* was visualized. Taken together, these data reveal that knee loading dynamically alters intramedullary pressure as a function of loading intensities and frequencies.

Keywords: Mechanical stimulation; Knee joint; Medullary cavity; Pressure; Microparticles

Introduction

A long bone such as a femur or a tibia constitutes a mechanosensitive system [1,2], and dynamic loading through walking and jogging are well-known to enhance its remodeling [3–5]. To devise effective mechanical intervention for promoting bone formation [6], treating osteoporosis [7], or accelerating fracture healing [8], elucidation of the mechanism underlying load-driven remodeling is indispensable [9]. Biophysical mediators for mechanotransduction in bone have been searched for, and conceivable candidates include strain magnitude or rate [10,11], number of daily loading cycles [12], interstitial fluid flow [13,14], transport of nutrient and/or waste molecules [15–17], and intramedullary pressure [18,19]. Among them, intramedullary pressure has been shown to enhance bone formation, where a turkey ulna *ex vivo* was linked to an external pressure source for oscillatory modulation of the ulnar intramedullary pressure [20,21]. Although load-driven alteration in intramedullary pressure is considered as a potential cause for anabolic responses, it is unclear whether pressure alteration in the turkey study is actually inducible in a medullary cavity *in vivo* with any existing loading modality.

Thus, the present study addressed the following questions: Is intramedullary pressure in a medullary cavity *in vivo* altered synchronous to dynamic loading? And if so, is the amplitude of modulation affected by loading intensities and frequencies? In order to examine those questions, a relatively novel loading method, "knee loading," was employed using a mouse femur as a model system [22]. Knee loading belongs to a joint loading modality, in which lateral loads are applied to the epiphysis for enhancing bone formation throughout a long bone. In our previous mouse studies, knee loading was shown to increase formation of cortical bone in tibiae [22,23]. Furthermore, elbow

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loading, the other form of the joint loading modality, was shown to elevate bone formation in mouse ulnae [24].

Joint loading apparently stimulates anabolic responses in cortical bone with *in situ* strain < 100 μstrain at the site of bone formation. One hypothesis for explaining the mechanism of anabolic effects is that dynamic loads to the joint induce modulation of intramedullary pressure throughout a long bone. In this study, we examined this hypothesis by detecting minute alteration of intramedullary pressure in the femoral medullary cavity with knee loading. Our hypotheses were (a) intramedullary pressure is altered synchronous to dynamic loading, and (b) the amplitude of pressure oscillation depends on loading intensities and loading frequencies.

Materials and methods

Animal use

Femora of C57BL/6 mice (female, ~16 weeks old) with a body weight of ~20 g were used in the experiments. The procedures for use of mice were approved by the Institutional Animal Care and Use Committee at Indiana University School of Medicine. The mice were anesthetized with 2% isoflurane and the periosteal surface of femora was exposed. For *in vivo* pressure calibration, a pair of holes (~0.3 mm in diameter) was generated with a blunt drill bit at the site ~2 mm and ~8 mm to the distal femoral end. The former was used for connecting to a water column and the latter for inserting a cannula linked to a pressure sensor.

For *in vivo* measurements of intramedullary pressure, a single hole at ~8 mm to the distal end was created. For *ex vivo* monitoring of microparticles in the femur, the hole at the same location was generated and a glass tube was inserted. The femur for the *ex vivo* experiment was immediately harvested and maintained in the αMEM culture medium supplemented with 10% fetal calf serum.

Calibration of a pressure sensing system

Prior to measuring intramedullary pressure in response to knee loading, the pressure sensing system was calibrated using the glass tube filled with a saline solution as well as the femur *in vivo* (Fig. 1). The sensing system consisted of a fiber optic pressure sensor (FOP-MIV, FISO Technologies Inc., Ontario, Canada), a fiber optic cable, and a pressure monitor (PM-250, FISO Technologies Inc., Ontario, Canada). The sensor was designed to measure the relative pressure with thermal sensitivity of ≤±1%/°C. The sensor tip was 0.95 mm in diameter, and it is connected to a fiber optic cable. We confined the sensor tip and a part of the cable into a 3-ml syringe filled with a saline solution containing 10 units/ml of heparin, and inserted a 23GTW cannula (Becton Dickinson and Co., Franklin Lakes, NJ) into the femoral medullary cavity. In order to minimize pressure alterations due to insertion of the cannula, we made a shallow indentation on the cortical surface and inserted the cannula into the cavity without releasing the medullary pressure outside the cannula. The height of the water column was adjustable in the range up to 2000 Pa (20 cm H_2O). The optical signal from the pressure sensor was relayed through a fiber optic cable and converted into a voltage signal with the optic pressure monitor. The voltage signal was transmitted to a PC through the BNC-2110 interface.

The baseline intramedullary pressure was measured as difference between an ambient atmospheric pressure and the pressure inside the medullary cavity. The cannula was inserted into the femoral medullary cavity and the sensor system was equilibrated for 30 min. The pressure in the cannula was abruptly released to the ambient pressure by opening a dilator switch (Pinnacle 15–725, Terumo Medical Co., MD), which was connected to the syringe. We employed three mice and conducted three measurements for each of the three mice.

In vivo pressure measurements with knee loading

The custom-made piezoelectric mechanical loader was used to apply lateral loads to the knee (Fig. 2). Intramedullary pressure in the femoral medullary cavity was measured using 3 animals. The same animals were used for knee loading and sham loading. The loading signal was sinusoidal, and we employed the actuator voltage from 10 to 100 V (peak-to-peak). Since the applied loads were calibrated to 0.05 N per actuator voltage, the actual force applied to the knee was estimated from 0.5 N (10 V) to 5 N (100 V). For sham loading, the piezoelectric loader was activated without contacting the knee to the loading rod. To evaluate the frequency responses, the knee was loaded at varying frequencies ranging from 0.05 Hz to 50 Hz. Note that the pressure sensor was zeroed prior to measurements, and therefore the observed signal represented the pressure alteration from the unloaded state.

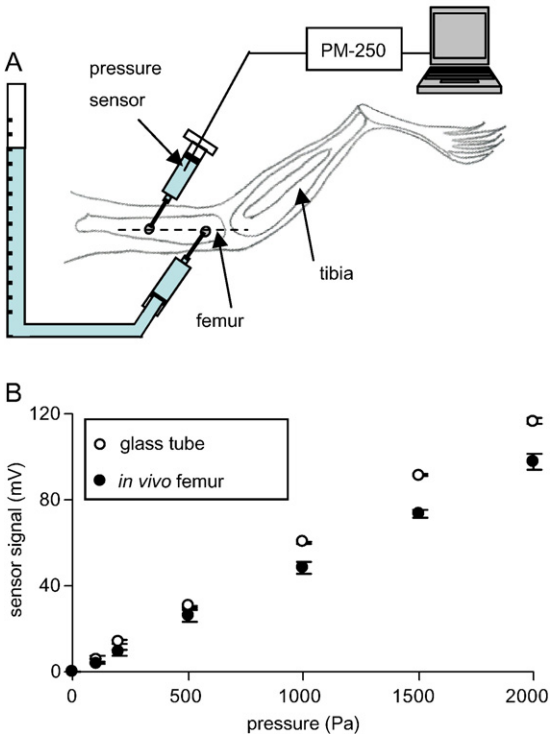

Fig. 1. Pressure calibration. (A) Schematic illustration of an experimental setup for pressure calibration using a femur *in vivo*. Two surgical holes were generated for connecting a pressure sensor and a water column to a femoral cavity. Pressure calibration was conducted using the femur *in vivo* and a glass tube filled with a saline solution. (B) Relationship between the applied pressure in Pa and the sensor signal in mV. The best fit regression line is $y=0.057x+2.236$ ($r^2=0.99$; water column), and $y=0.049x-0.488$ ($r^2=99$; *in vivo* femur).

Ex vivo monitoring of microparticle motions with knee loading

The real-time motion of microparticles (3 μm in diameter, Polysciences Inc., Warrington, PA) was monitored using the femur *ex vivo*. The bone sample was immobilized in the sample holder of the *ex vivo* piezoelectric loader using super glue (Henkel Loctite Co.), and the loading rod was placed at the distal epiphysis. The loader was mounted on a NIKON microscope (E600 eclipse, Kanagawa, Japan), and a cylindrical glass pipette (340 μm in internal diameter, and 6 mm in length; Drummond Scientific Co., Broomall, PA) was inserted into the femoral bone cavity. This tube was filled with a saline solution containing 10 units/ml of heparin and 0.01 g/ml of microparticles. Note that this *ex vivo* loader was previously used to monitor molecular transport with a "fluorescence recovery after photobleaching technique (FRAP)" [16]. The rod was slid by the piezoelectric actuator (LPD12060X, Megacera Inc.), which was regulated with a voltage amplifier (PZD700 M/S, TREK) through a BNC-2110 interface (National Instruments). We employed 4 N force with the loading frequency at 0.5 Hz. Using a 10× objective, the real-time motion of microparticles inside the glass tube was