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Cortical and trabecular bone adaptation to incremental load magnitudes using the mouse tibial axial compression loading model

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ABSTRACT

The mouse tibial axial compression loading model has recently been described to allow simultaneous exploration of cortical and trabecular bone adaptation within the same loaded element. However, the model frequently induces cortical woven bone formation and has produced inconsistent results with regards to trabecular bone adaptation. The aim of this study was to investigate bone adaptation to incremental load magnitudes using the mouse tibial axial compression loading model, with the ultimate goal of revealing a load that simultaneously induced lamellar cortical and trabecular bone adaptation. Adult (16 weeks old) female C57BL/6 mice were randomly divided into three load magnitude groups (5, 7 and 9 N), and had their right tibia axially loaded using a continuous 2-Hz haversine waveform for 360 cycles/day, 3 days/week for 4 consecutive weeks. *In vivo* peripheral quantitative computed tomography was used to longitudinally assess midshaft tibia cortical bone adaptation, while *ex vivo* micro-computed tomography and histomorphometry were used to assess both midshaft tibia cortical and proximal tibia trabecular bone adaptation. A dose response to loading magnitude was observed within cortical bone, with increasing load magnitude inducing increasing levels of lamellar cortical bone adaptation within the upper two thirds of the tibial diaphysis. Greatest cortical bone adaptation was observed at the midshaft where there was a 42% increase in estimated mechanical properties (polar moment of inertia) in the highest (9 N) load group. A dose response to load magnitude was not clearly evident within trabecular bone, with only the highest load (9 N) being able to induce measureable adaptation (31% increase in trabecular bone volume fraction at the proximal tibia). The ultimate finding was that a load of 9 N (engendering a tensile strain of 1833 με on medial surface of the midshaft tibia) was able to simultaneously induce measurable lamellar cortical and trabecular bone adaptation when using the mouse tibial axial compression loading model in 16 week old female C57BL/6 mice. This finding will help plan future studies aimed at exploring simultaneous lamellar cortical and trabecular bone adaptation within the same loaded element.

Introduction

Animal models wherein controlled loads are introduced to the skeleton have been instrumental in advancing understanding of the response of bone to mechanical stimuli. As a result of studies utilizing animal models, we now know bone preferentially responds to dynamic rather than static stimuli, only short durations of loading are required to initiate an adaptive response, and bone cells accommodate to unique mechanical loading environments [1]. With the sequencing of the mouse genome and subsequent generation of transgenic mice, attention has shifted to exploration of molecular pathways underlying the skeletal response to loading. For instance, recent work utilizing transgenic animal models has demonstrated important mechanotransductive roles for molecules within the Wnt signaling pathway, including low-density lipoprotein receptor-related protein 5 and sclerostin [2,3].

The rodent ulna axial compression loading model has evolved as a useful model to investigate the response of bone to mechanical loading [4,5]. It allows controlled mechanical loads to be introduced both non-invasively and unilaterally, enabling bone responses to be explored in the absence of trauma and with the contralateral side serving as an internal control site. These features represent an advance on alternative loading models, such as jump training and treadmill running which load the skeleton bilaterally, and the rodent 4-point loading model which causes traumatic periosteal woven bone formation (see Robling et al. [6] for review). However, the ulna axial compression loading model does not readily allow investigation of trabecular bone responses to loading due to the principally cortical bone structure of the rodent ulna with virtual lack of trabeculae.

The mouse tibial axial compression loading model has recently been described to enable simultaneous exploration of cortical and trabecular bone adaptation to mechanical loading within a single bone [7,8]. The

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model involves axially loading the tibia through a flexed knee and dorsiflexed ankle. Because of the natural curvature of the tibia, the compressive load applied to the bone is converted into bending with peak compressive strain engendered at the posterolateral border and peak tensile strain at the anteromedial surface of the tibial diaphysis [9–11]. Studies utilizing the model have generally demonstrated a loading benefit on trabecular bone volume fraction (bone volume [BV]/total volume [TV]) within the proximal tibia [7,8,10,12–17,19–23]. However, BV/TV decreased in some studies depending on experimental conditions [7,9,24,25], and a dose response to load magnitude on BV/TV within the proximal tibia has yet to be clearly demonstrated [9,16,17,19]. In terms of cortical bone, previous work using the mouse tibial axial compression loading model has demonstrated a clear dose response to load magnitude at the tibial diaphysis [7,9,16,19]; however, woven bone formation on the periosteal surface is often evident [9,10,17,19–23,26]. As woven bone apposition is considered a pathological response to excessive load and subsequent damage accumulation [27], its frequent observation limits the ability of the mouse tibial axial compression loading model to allow simultaneous exploration of non-pathological (i.e. lamellar) cortical and trabecular bone adaptation to mechanical loading.

To further the understanding of the mouse tibial axial compression loading model, the current study aimed to investigate combined cortical and trabecular bone adaptation to incremental load magnitudes. The ultimate goal was to reveal a load magnitude that simultaneously induced lamellar cortical and trabecular bone adaptation.

Methods

Animals

Forty female C57BL/6 mice were purchased (Jackson Laboratories, Bar Harbor, ME) and acclimated until 16 weeks of age. Animals were housed under standardized conditions with *ad libitum* access to standard mouse chow and water. All procedures were performed with prior approval from the Institutional Animal Care and Use Committee of Indiana University.

Strain gauge measurement

Four mice were randomly selected for a load–strain calibration study. The right hindlimbs were harvested after euthanasia and stored at −20 °C. Limbs were allowed to warm to room temperature over several hours on the day of strain gauge measurement and the medial surface of the mid-diaphysis was minimally exposed. A single element strain gauge (EA-06-015DJ-120; Measurements Group, Inc., Raleigh, NC) was bonded with cyanoacrylate (M-Bond 200; Measurements Group, Inc., Raleigh, NC) to the middle of the medial surface of the midshaft tibia. The leg was axially loaded at a frequency of 2 Hz and peak loads of 3, 5, 7 and 9 N using the same loading system as used for experimentation (see *In vivo* axial tibial loading). The strain gauge voltage signal was routed through a signal conditioning amplifier (Model 2210; Measurements Group, Inc., Raleigh, NC), and the peak-to-peak voltage measured on a digital oscilloscope. Voltage was converted to strain using a calibration factor derived from measured and calculated (using beam theory) strains collected using an aluminum cantilever.

In vivo axial tibial loading

Thirty-six mice were randomly divided into three load magnitude groups—5, 7 and 9 N. The right leg was fixed between molded knee and foot cups on a computer-controlled electromagnetic mechanical actuator (Enduratec ELF 3200; Bose Corporation, Eden Prairie, MN), with the animal under isoflurane anesthesia (Abbott Laboratories, North Chicago, IL). The tibia was axially loaded across a near fully flexed knee and dorsiflexed ankle. Loading was applied with a continuous 2-Hz haversine waveform for 360 cycles/day, 3 days/week for 4 consecutive weeks. Left legs were not loaded, with left tibiae serving as internal controls. A recent study using the tibial axial compression loading model found loading effects were isolated to loaded bones [20]. Normal cage activity was allowed between loading sessions.

In vivo pQCT

Cortical bone adaptation to mechanical loading was assessed *in vivo* using peripheral quantitative computed tomography (Stratec XCT Research SA+; Stratec Medizintechnik GmbH, Pforzheim, Germany). Animals were positioned on a custom scanning platform at baseline and following the 4-week loading regime, with the animal under isoflurane anesthesia (Abbott Laboratories, North Chicago, IL). Each leg was centered in the machine gantry and a scout scan of the tibia performed for tomographic scan localization. A tomographic scan was performed at the midshaft tibia using a 0.46-mm collimation and 70 μm voxel size. This voxel size is relatively large compared to the cortical thickness of the mouse tibial midshaft increasing the potential for partial volume effects. However, good agreement has previously been shown between pQCT, micro-computerized tomography (μCT) and histological measures of cortical bone properties in mice [28]. Analyses were restricted to cortical bone using contour mode 1 with a threshold of 400 mg/cm^3 within the Stratec software (version 6.20C; Stratec Medizintechnik GmbH, Pforzheim, Germany). Total bone mineral content (Tt.BMC; mg/cm), total bone area (Tt.Ar; mm^2), and cortical area (Ct.Ar; mm^2) were recorded for each bone, and the minimum (I_{MIN}; mm^4) and maximum second moments of area (I_{MAX}; mm^4) derived according to Gere and Timoshenko [18]. Medullary area (Me.Ar; mm^2) was derived as Tt.Ar minus Ct.Ar.

Ex vivo μCT

Animals were euthanized following the 4-week loading regime, and the right and left tibias dissected free and placed in 10% neutral buffered formalin for 48 hours before being stored in 70% alcohol. A desktop micro-computerized tomography machine (μCT-20; Scanco Medical AG, Auenring, Switzerland) scanning with an isotropic voxel size of 9 μm was used to assess cortical bone geometry at 1 mm increments along the entire tibial diaphysis. Tomographic images were imported into ImageJ (National Institutes of Health, MD) wherein the polar moment of inertia (I_P) was derived as the sum of the I_{MAX} and I_{MIN} measurements, with I_{MAX} and I_{MIN} being determined using standard and custom macros. The same desktop micro-computerized tomography machine was also used to assess trabecular bone properties

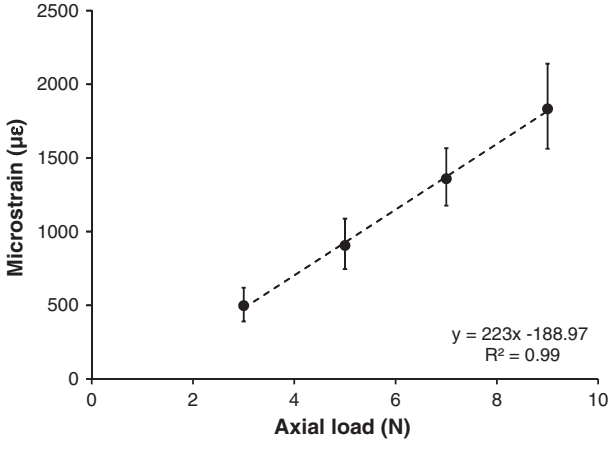

Fig. 1. Strain engendered on the medial surface of the midshaft tibia in response to incremental external load magnitudes. Error bars indicate 95% confidence intervals.