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Constrained Tibial Vibration in Mice: A Method for Studying the Effects of Vibrational Loading of Bone

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Abstract

Background—Vibrational loading can stimulate the formation of new trabecular bone, or maintain bone mass. Studies investigating vibrational loading have often used whole-body vibration (WBV) as their loading method. However, WBV has limitations in small animal studies because transmissibility of vibration is dependent on posture. In this study we propose constrained tibial vibration (CTV) as an experimental method for vibrational loading of mice under controlled conditions.

Method of Approach—In CTV the lower leg of an anesthetized mouse is subjected to vertical vibrational loading while supporting a mass. The setup approximates a one degree-of-freedom mass-spring system where the leg acts as the spring. Accelerometers were used to measure the transmissibility of vibration through the lower leg (foot to knee) in CTV at frequencies from 20–150 Hz. First, the frequency response of transmissibility was quantified *in vivo*, and dissections were performed to remove one component of the mouse lower leg (the knee joint, foot, or soft tissue) in order to investigate the contribution of each component to the frequency response of the intact leg. Next, a finite element model of a mouse tibia-fibula was used to estimate the deformation of the bone during CTV. Finally, strain gages were used to determine the dependence of bone strain on loading frequency.

Results—The *in vivo* mouse leg in the CTV system had a resonant frequency at approximately 60 Hz for ± 0.5 G vibration (1.0 G peak-to-peak) with a moving mass of 125 g. Removing the foot caused the natural frequency of the system to shift from 60 to 70 Hz; removing the soft tissue caused no change in natural frequency; and removing the knee changed the natural frequency from 60 to 90 Hz. Using the FE model, maximum tensile and compressive strains during CTV were estimated to be on the cranial-medial and caudo-lateral surfaces of the tibia, respectively, and the peak transmissibility and peak cortical strain occurred at the same frequency. Strain gage data confirmed the relationship between peak transmissibility and peak bone strain indicated by the FE model, and showed that the maximum cyclic tibial strain during CTV of the intact leg was $330 \pm 82 \mu\epsilon$ and occurred at 60–70 Hz.

Conclusions—This study presents a comprehensive mechanical analysis of CTV, a loading method for studying vibrational loading under controlled conditions. This model will be used in future *in vivo* studies, and has the potential to become an important tool for understanding the response of bone to vibrational loading.

Key Terms

Vibration; bone; osteoporosis; transmissibility; strain; WBV

Introduction

Studies that have investigated the osteogenic effect of vibrational loading have primarily focused on whole-body vibration (WBV) [1–9], although some studies have used other vibrational loading methods [10,11]. One limitation for mouse studies is that vibration at a skeletal site is difficult to control since the animal is free to move and thus the posture can change. Mice are commonly used in studies of skeletal biology, especially when investigating genetic or molecular factors.

In this study we propose constrained tibial vibration (CTV) as a new method for controlled loading of the murine skeleton. In CTV the lower leg of an anesthetized mouse is subjected to vibrational loading while supporting a mass that is free to move vertically (in the direction of the long axis of the tibia/fibula, Fig. 1). CTV is not dependent on posture and applies vibration to only one leg of a subject, thereby allowing for use of the contralateral limb as an internal control, and factors such as vibration magnitude, frequency, and moving mass can be adjusted in order to investigate the effects of these loading parameters. Additionally, CTV applies vibration more directly to a skeletal site of interest, the proximal tibia, which is our focus because it is one site where age-related bone loss is observed in mice [12], and in a previous study we reported that the proximal tibia had a larger bone formation response to WBV than other skeletal sites [1].

We characterized the vibrational behavior of the mouse leg loaded by CTV in a series of three studies. First, we investigated the frequency response of transmissibility of acceleration through a mouse lower leg during CTV, and examined the individual contributions of various structures of the mouse leg (the knee, foot, or soft tissue) to the dynamic response. Second, we used an elastic finite element model of the tibia/fibula to estimate the spatial distribution of longitudinal strain, as well as to compare the frequency responses of transmissibility and cortical strain. Finally, strain gages were used to validate the relationship between transmissibility and strain indicated by the finite element model, and to determine the absolute magnitudes of cortical bone strain as a function of input frequency.

Methods and Results

General Methods

Vibrational Loading System—The loading system for CTV was developed by modifying a previously described system for WBV [1] that was based on a system designed by Fritton et al. [13]. For CTV loading, an acetal plastic (Delrin®) loading stage was attached to the vibration platform of the system (Fig. 1). The bottom portion of the loading stage consists of a small plate with a vertical post (3/8" diameter) extending upward. The top of this post has a hemispherical depression (17/64" radius, approximately 3/16" depth at the center) that holds the flexed knee of the mouse during loading. The top portion of the loading stage consists of another plastic plate that is free to glide vertically on two guide rods (ceramic coated aluminum) via linear bearings. A vertical post extends downward from the top platform and the heel of the mouse fits into a hemispherical depression on the bottom of this post. Two accelerometers (PCB Piezotronics Model #333B52, Depew, NY) were used to measure acceleration at the loading stage and at the upper platform. The total moving mass of the upper platform and post is 124 g (132 g with accelerometer).

Animal Studies—All mice used in this study were male C57Bl/6, approximately 6 months old (approximately 30 g body mass; Harlan Sprague Dawley, Indianapolis, IN). *In vivo* studies utilized a total of 7 mice under anesthesia (isoflurane: 1.5–2.0% in air, 1.5 L/min, delivered via nosecone). For CTV loading, each mouse was placed in the prone position on a platform that extended beyond the sides of the CTV device and was stationary during loading. The right leg of the mouse extended off the side of the platform, with the lower leg constrained between the two posts of the CTV loading stage. Another 16 mice were used for *post mortem* studies after euthanasia by CO₂ asphyxiation. All methods were consistent with NIH guidelines for the care and use of laboratory animals, and were approved by the Washington University Animal Studies Committee.

Measurement of Transmissibility—Accelerometers were used to measure transmissibility of acceleration. Accelerometer data were acquired with a signal conditioner (PCB Piezotronics Model #482A16, Depew, NY) and data acquisition system (National Instruments Model #SCXI-1301, Austin, TX). Data were collected and filtered using Labview software (National Instruments, 2000 Hz sampling rate; 6th order low-pass Butterworth filter, cutoff frequency 400 Hz). Transmissibility (T) was determined at each frequency of interest (20–150 Hz, 5 Hz increments) from filtered acceleration data as the acceleration magnitude at the top platform, $X(t)$, divided by the acceleration magnitude at the bottom platform, $Y(t)$, averaged over five cycles. Acceleration was approximately centered at zero, therefore an acceleration magnitude of “0.5 G” (where 1.0 G = acceleration due to gravity) actually oscillates between ± 0.5 G (1.0 G peak-to-peak). For each change in frequency, the vibration magnitude was lowered to the minimum, then the frequency was changed, and the magnitude slowly increased to the desired magnitude. Phase shift (φ , expressed in degrees) was also determined from raw acceleration data using the equation:

$$\varphi = \frac{360 f_I n}{f_s} \quad (1)$$

where f_I is the vibrational loading frequency, n is the average number of data points between the zero-intercept of the input and output accelerations, and f_s is the sampling frequency.

The natural frequency of the system (assuming a one degree-of-freedom vibrational system as a first-order approximation, Fig. 1) was determined as the loading frequency corresponding to the highest value of the imaginary (out of phase) component of transmissibility, calculated from the equation:

$$T_i = T \sin \varphi \quad (2)$$

where T is the experimentally determined transmissibility at the frequency of interest, and φ is the experimentally determined phase shift. Plots of transmissibility and phase shift were also used to qualitatively confirm natural frequencies.

The accelerometer on the bottom platform of the CTV device (input) had an average signal-to-noise ratio (SNR) of 34.1 dB for 0.5 G vibration magnitude. For lower vibration magnitudes, the SNR decreased accordingly, reaching 18.3 dB for 0.1 G vibration.

Study 1: Effect of Components of the Mouse Lower Leg on Transmissibility

Methods

Transmissibility, phase shift, and imaginary component of transmissibility were calculated for CTV loading of a mouse lower leg *in vivo* for loading frequencies from 20–150 Hz at input magnitudes from 0.1–0.5 G acceleration magnitude during vibration. Two complete sets of data were collected for each mouse ($n = 7$ mice) on separate days. For each trial, the natural frequency and the transmissibility at this frequency were determined; these values were averaged across the two trials per mouse. Data were analyzed by one way ANOVA in order to determine statistically significant changes in the natural frequency and transmissibility magnitude.

To determine the individual vibrational contribution of components of the lower leg, we also compared the frequency response of the mouse leg following removal of either the foot, soft tissue, or knee. Mice ($n = 9$) were killed, and then immediately dissected to remove one of the components of the lower leg: the foot, the soft tissue, or the knee (the lower leg was disarticulated from the upper leg at the knee joint). Data were then collected on the partially dissected limb for an input magnitude of 0.5 G maximum acceleration. A magnitude of 0.5 G was used to provide the greatest input signal-to-noise ratio based on the *in vivo* results. Three mice were used for each dissection; two sets of data were collected consecutively for each mouse and averaged. The final condition (bone only) includes results for all 9 mice. Data were analyzed by one way ANOVA to determine statistically significant changes ($p < 0.10$).

Results

For CTV loading of mice *in vivo*, we observed changes in the frequency response as a function of input vibrational magnitude. Loading at 0.1 G did not produce a singular peak in imaginary component of transmissibility. At magnitudes of 0.2–0.3 G, the average natural frequency of the system was approximately 70 Hz, while at magnitudes of 0.4–0.5 G, the average natural frequency shifted to approximately 60 Hz ($p < 0.05$). Figure 2b depicts the frequency response of imaginary component of transmissibility (*in vivo*) for a loading magnitude of 0.5 G.

For nearly all dissections, removing a component of a mouse lower leg resulted in a change in the natural frequency of the system (Fig. 2c–e). The *in vivo* system had a natural frequency of 60 Hz and a peak transmissibility magnitude of 2.0 ± 0.2 . In contrast, the system with a fully dissected tibia-fibula (bone only) had a natural frequency of 120–130 Hz and a peak transmissibility magnitude of 3.0 ± 1.0 . Removing the foot resulted in a change of natural frequency from 60 to 70 Hz ($p = 0.034$) with no change in peak transmissibility compared to the *in vivo* system. Removing the soft tissue had no effect on the natural frequency or the peak transmissibility magnitude compared to the *in vivo* system. Removing the knee resulted in the largest change of the frequency response, changing the natural frequency of the system to 85–90 Hz ($p < 0.001$) and increasing the peak transmissibility magnitude to 2.4 ± 0.3 ($p = 0.088$).

Study 2: Finite Element Modeling of the Mouse Lower Leg

Methods

A previously-described finite element model of a mouse tibia-fibula [14] was used to estimate both the distribution of longitudinal strain on the tibia and the frequency response of transmissibility and strain due to CTV. Tetrahedral elements (15-noded) were used throughout. A 132 g mass was rigidly tied to the distal end of the bone model (X elements) to simulate the fully dissected tibia-fibula (bone only) configuration. Material properties of bone were chosen in order to match (within 5 Hz) the first natural frequency of the model to experimental data (125 Hz for “bone only” condition): $E_{\text{cortical}} = 9.3$ GPa, $E_{\text{trabecular}} = 0.25$ GPa). Circular elastic

components (axial springs) were added at the proximal end of the model, as well as between the distal end of the model and the mass (Fig. 3a) to simulate the apparent dynamic behavior of the femur/knee and foot/ankle joints, respectively. By including only one of these components, it was possible to approximate the “no foot” and “no knee” dissections. Material properties of the elastic components were determined by matching the natural frequency of the model to the experimentally determined natural frequency for each configuration (60 Hz for in vivo and “no tissue”, 70 Hz for “no foot”, 90 Hz for “no knee”). The distal component (X elements) had a 0.6 mm radius and 0.3 mm thickness, and a modulus of elasticity of 25.0 MPa. The proximal component (X elements) had a 0.9 mm radius and a 0.3 mm thickness, and a modulus of elasticity of 4.6 MPa. Gravity was not included in the model. (Subsequent analysis indicated that inclusion of gravitational effects did not affect the natural frequency of the model.) Simulations were performed with ABAQUS software (SIMULIA, Providence, RI) on a Dell Optiplex GX270 (2.79 GHz processor, 1.00 GB RAM), and took approximately 10 minutes each.

Results

The peak tensile strains estimated by the model were located on the cranial-medial surface of the tibia, approximately equidistant from the tibial plateau and the distal tibiofibular junction for all frequencies examined (Fig. 3b). The peak compressive strains were located on the caudo-lateral surface of the tibia, at approximately the same proximodistal location. The magnitudes of both bone strain and transmissibility were strongly frequency dependent, with both reaching their maximum values at the natural frequency of the model (60 Hz, Fig. 3d).

Study 3: Direct Measurement of Bone Deformation by Strain Gage

Methods

Semiconductor strain gages (Micron Instruments Model #SS-080-050-500P-S1, Simi Valley, CA) were used to quantify tibial strain during CTV loading in order to validate the frequency response of bone strain estimated by the finite element model, and to find absolute values of cyclic cortical strain. Data were processed using a signal conditioner (National Instruments Model #SCXI-1321, Austin, TX) and isolation amplifier (National Instruments Model #SCXI-1121), with 3.33 V excitation and $100 \times$ gain with a quarter bridge configuration. Data were collected using Labview software (National Instruments, 1200 Hz sampling rate).

Mice ($n = 7$) were killed, then gages were immediately attached with cyanoacrylate (Loctite #4471, Rocky Hill, CT) to the cranial-medial surface of the tibia at the location of maximum tensile strain estimated by the FE model (Fig. 3b). Mice were loaded in CTV at frequencies from 20–150 Hz (5 Hz increments) at 0.5 G maximum input acceleration. Average cyclic (peak-to-peak) strain was determined at each loading frequency from raw strain data as the difference between the maximum strain value and the minimum strain value, averaged over five cycles. Two complete data sets were collected consecutively, and the results were averaged.

The SNR for strain gage recordings was dependent on the loading frequency. The maximum SNR (at 70 Hz loading) was 35.9 dB, while the minimum SNR (at 150 Hz) was 20.8 dB.

Results

Preload by the 132 g mass produced a tibial strain of $228 \pm 41 \mu\epsilon$. The magnitude of cyclic cortical strain was highly dependent on loading frequency during CTV (Fig. 4a). Strain values were strongly correlated ($R^2 = 0.83$) with values for transmissibility of acceleration for the intact *in vivo* mouse leg (Fig. 4b). At the natural frequency of the system (60–70 Hz) the peak-to-peak strain had an average magnitude of $330 \pm 82 \mu\epsilon$.

Discussion

In this study, constrained tibial vibration (CTV) was developed as a new method to apply controlled vibrational loading to the murine skeleton. We found that the frequency response resembles a 1-degree-of-freedom vibrational system that depends on input vibrational magnitude. We also found that components of the mouse leg such as the knee joint and the foot contribute to the vibrational behavior of the *in vivo* mouse lower leg in the CTV system, although the knee joint contributes most significantly. A finite element model was used to estimate the locations of peak strain during CTV, as well as to examine the frequency dependence of cortical strain magnitude. Finally, strain gages were used to quantify absolute strain values during CTV, as well as to confirm that peak strain strains occur at the same loading frequency as the peak transmissibility as indicated by the FE model.

For a 1-DOF vibrational system, the natural frequency is an indication of the apparent stiffness of the deformable element (the mouse leg) and the mass in motion (the moving mass). The magnitude of transmissibility is an indication of how near the driving frequency is to the natural frequency (resonance) and the damping in the system [15]. The natural frequency of the system was significantly higher for the dissected tibia/fibula (bone only) than for the intact leg, indicating that the apparent stiffness of the dissected tibia/fibula is higher. Similarly, the magnitude of the imaginary component of transmissibility was also significantly higher for the dissected tibia/fibula, indicating that it has less damping than the intact leg. Removing the foot from a mouse leg caused a small increase in apparent stiffness, with no change in damping. Conversely, removing the soft tissue of the lower leg led to a non-significant loss of damping in the system, but no change in apparent stiffness. Removing the knee had the most noticeable effect on the response, with a large increase in stiffness and a non-significant decrease in damping. These changes indicate that motion at the knee has a large effect on the vibrational behavior of the intact leg.

Results of the FE model of the tibia indicated that during CTV, the tibia will experience the largest deformation at the natural frequency of the *in vivo* mouse leg (60 Hz). The material properties of bone, which were chosen in order to match the first natural frequency of the model with experimental data for the “bone only” configuration, were lower than previously reported values for cortical bone [16]. This difference could be attributed to several factors. First, the model was based on the geometry of a tibia from a 4-month SAMP6 mouse rather than a 6-month C57BL/6 mouse. The two strains have some similar bone dimensions, although the SAMP6 tibia has a 30% thicker cortex and 15% greater bone area at the tibial midshaft, while the C57BL/6 has a 34% larger medullary area (data not shown). Second, the boundary conditions of the model may have been more highly constrained than those of the “bone only” condition. When the FE model was less rigidly constrained, the observed natural frequency was lower. Despite these limitations, the strain profile determined by the model was similar to that estimated for quasi-static end-loading of the mouse tibia (data not shown).

The frequency response of cortical strain determined by strain gage measurements closely mirrored the response of transmissibility for a mouse leg *in vivo*. This validates the concept of using transmissibility of acceleration to non-invasively predict the frequency at which peak strains occur during vibrational loading. The maximum dynamic (peak-to-peak) strain experienced by a mouse tibia during loading in our CTV device was $330 \pm 82 \mu\epsilon$. The strain magnitude produced by CTV is dependent not only on the mechanical properties of the mouse limb, but also on input vibrational parameters (frequency and magnitude) and the magnitude of the moving mass (data not shown). Thus, future studies can examine bone responses to vibrational loading across a range of strains.

Conclusions

This study presents a comprehensive mechanical analysis of constrained tibial vibration (CTV), a model for studying the skeletal effects of vibrational loading under controlled conditions. Vibrational frequency and magnitude, along with the magnitude of the moving mass, could be altered to control the amount of bone strain experienced during CTV. This loading model will be used in future *in vivo* studies, and may become an important tool for understanding the response of bone to vibration.

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Figure 1.

(a) Picture of a mouse in the constrained tibial vibration (CTV) loading device. The mouse is anesthetized with isoflurane and placed on a stationary platform. The right lower leg of the mouse is constrained by the two posts of the CTV device, with the knee contacting the bottom post (vibration source) and the foot supporting the weight of the top platform (moving mass) which is free to move vertically. (b) MicroCT image of the mouse lower leg in the CTV device. Both the distal femur and the foot are in series with the tibia-fibula and contribute to the vibrational behavior of the mouse leg in the CTV system. (c) Diagram (illustrating the approximate position of the accelerometers used to determine transmissibility) relating the CTV device to a one degree-of-freedom lumped-parameter vibrational system. Using this model, the mass of the system (M) represents the combined mass of the upper platform of the CTV device (the mass of the leg is neglected), while k and c represent the apparent stiffness and damping of the leg, respectively. Transmissibility is calculated as the magnitude of acceleration at the top platform $X(t)$ (output) divided by the magnitude of acceleration at the bottom platform $Y(t)$ (input). Acceleration magnitude was determined by averaging the absolute magnitudes of five maximum acceleration values and five minimum acceleration values (acceleration was approximately centered at zero).

Figure 2.

(a) Schematics showing the various dissections used to determine the effect of each component of the mouse leg on the vibrational behavior of the system. (b) Frequency responses of imaginary component of transmissibility with standard deviation error bars for loading magnitude of 0.5 G. The “bone only” configuration had very large standard deviations for loading frequencies ≥ 100 Hz, however all bones tested had peak values for imaginary component of transmissibility between 110–130 Hz. (c–e) Graphs showing average transmissibility (c), phase shift (d), and imaginary component of transmissibility (e) of a mouse leg loaded in CTV after the removal of one component of the lower leg ($n = 3$ mice per dissection). *In vivo* and “bone only” data are also included for comparison.

Figure 3.

Finite element model of a mouse tibia-fibula in CTV. (a) Assembly of the FE model, showing the end mass, which simulates the top platform of the CTV device, as well as the proximal and distal elastic elements that simulate the elastic contributions of the knee joint and the foot, respectively. (b) Profile of peak longitudinal strain produced during CTV loading. The picture on the left shows the cranial-medial surface of the tibia (the location of peak tensile strain). The picture on the right is a cross-section of the tibia showing the relative distribution of peak tensile and compressive strain. (c) Graph showing the frequency dependence of transmissibility (of displacement) and longitudinal strain as estimated by the FE model. Near the natural frequency of the model, both the transmissibility and the longitudinal strain approach infinity since the model does not have a damping component.

Figure 4.

Average frequency response of cyclic cortical strain on the tibia of an intact mouse leg subjected to CTV loading, compared to the frequency response of transmissibility of acceleration (0.5 G maximum acceleration input magnitude). Strain gages were placed at the location of peak tensile strain (determined by the FE model). The average peak strain value was $330 \pm 82 \mu\epsilon$, and occurred at 60–70 Hz. Error bars represent the standard deviation of dynamic strain magnitude recorded at each loading frequency of interest. Strain and transmissibility values were strongly correlated ($R^2 = 0.83$). Strain data and transmissibility data were recorded from different animals.