Pressure and Shear Stress in Trabecular Bone Marrow During Whole Bone Loading

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Abstract

Skeletal adaptation to mechanical loading is controlled by mechanobiological signaling. Osteocytes are highly responsive to applied strains, and are the key mechanosensory cells in bone. However, many cells residing in the marrow also respond to mechanical cues such as hydrostatic pressure and shear stress, and hence could play a role in skeletal adaptation. Trabecular bone encapsulates marrow, forming a poroelastic solid. According to the mechanical theory, deformation of the pores induces motion in the fluid-like marrow, resulting in pressure and velocity gradients. The latter results in shear stress acting between the components of the marrow. To characterize the mechanical environment of trabecular bone marrow *in situ*, pore pressure within the trabecular compartment of whole porcine femurs was measured with miniature pressure transducers during stress-relaxation and cyclic loading. Pressure gradients ranging from 0.013 to 0.46 kPa/mm were measured during loading. This range was consistent with calculated pressure gradients from continuum scale poroelastic models with the same permeability. Micro-scale computational fluid dynamics models created from computed tomography images were used to calculate the micromechanical stress in the marrow using the measured pressure differentials as boundary conditions. The volume averaged shear stress in the marrow ranged from 1.67 to 24.55 Pa during cyclic loading, which exceeds the mechanostimulatory threshold for mesenchymal lineage cells. Thus, the loading of bone through activities of daily living may be an essential component of bone marrow health and mechanobiology. Additional studies of cell-level interactions during loading in healthy and disease conditions will provide further insight into marrow mechanobiology.

Introduction

Skeletal adaptation to mechanical loads is controlled by cells that sense the local and spatially averaged deformation of the tissue, and express signaling molecules to surrounding cells that affect the addition or removal of bone tissue. Through this process, bone mineral density decreases during extended periods of inactivity, such as bed rest (LeBlanc et al., 1990) and space flight (LeBlanc et al., 2000), and increases when frequent load-unload cycles are applied through, for example, intense physical activity (Courteix et al., 1998; Tournaire et al., 2007). Osteocyte mechanotransduction is the primary controller of bone adaptation and repair (Bonewald, 2006; Schaffler et al., 2014; You et al., 2008). However, cells in the marrow are also mechanically sensitive (Luu et al., 2009; Qin, 2003; Soves et al., 2014). Indeed, mechanical loading of mouse limbs *in vivo* induces mechanical signaling between marrow cells (Soves et al., 2014) and increased proliferation and osteogenic gene expression in marrow stromal cells (Li et al., 2004; Mantila Roosa et al., 2011; Ozcivici et al., 2010).

Bone marrow is a highly vascularized and cellular soft tissue located in the central cavity of long and axial bones, and in the pore spaces of trabecular bone (Tavassoli and Yoffey, 1983). Bone marrow is the niche for multipotent mesenchymal stromal cells (MSCs), which are progenitors for connective tissue cells capable of differentiating into osteogenic, chondrogenic, and adipogenic lineages (Rosen and Bouxsein, 2006), and hematopoietic stem cells from which

both blood and immune cells derive, including macrophages such as osteoclasts. Marrow cells respond to mechanical stimuli. For example, steady and oscillatory shear stress both affect MSC differentiation to the osteogenic lineages (Castillo and Jacobs, 2010; Li et al., 2004), as well as nitric oxide (NO) and prostaglandin production in marrow-derived pre-osteoclast (McAllister et al., 2000).

Loading of whole bones increases the pressure in the bone marrow. In human femurs tested *in vitro*, the pressure reached 1 to 2 kPa during physiologic loading (Downey et al., 1988). The pressure was higher in the head than the neck, and increased with loading rate. Impact loading of ovine tibia resulted in pressures as high as 35 kPa in the trabecular pore space (Bryant, 1983). These pressures may be too low to provide substantial load-bearing effects (Arramon and Cowin, 1997; Bryant, 1983; Kafka, 1983; Ochoa et al., 1991), and are below previously measured limits for mechanotransduction in MSCs (Castillo and Jacobs, 2010) and hematopoietic lineage cells, such as pre-osteoclasts (Kwon et al., 2012). However, the pore deformation forces motion of the fluid-like marrow, which is resisted by shear stress between the marrow components. Since marrow is composed primarily of cells, this shear stress would be sustained by intercellular interactions.

We hypothesized that the shear stress in the trabecular bone marrow during loading reaches a sufficient magnitude to affect cell mechanobiology. As such, the goal of this study was

to measure pressure and pressure gradients in whole bones under quasi-physiologic loading and determine the associated marrow kinetics and kinematics. Specifically, we 1) measured pressures at four locations in the neck of fresh porcine femurs during stress relaxation and cyclic loading; 2) parametrically investigated the interaction between strain rate and pore pressure gradients using continuum poroelastic models; and 3) calculated the fluid flow using high-resolution finite element models to determine the associated shear stress distributions and velocity gradients in the marrow.

Methods

Nine freshly harvested porcine femurs were taken from animals 6-8 months of age (approximately 115 kg). The bones were obtained from a local abattoir (Martin's Custom Butchering, Wakarusa, IN) at the time of slaughter. Testing was completed within 8 hours post-mortem, as bone marrow properties are highly sensitive to storage (Metzger et al., 2014). Miniature pressure transducers (Model 060 (1.5 x 0.3 mm), Precision Measurements Inc., Ann Arbor, MI) were used to measure the pore pressure *in situ* during cyclic and stress-relaxation loading. Four holes approximately 5 mm apart and 150 mm deep were prepared along the curvature of the femoral neck using a 2.38-mm drill bit (Fig. 1A). The pressure transducers were inserted through cannulae (Harvard Apparatus, Holliston, MA), which were press-fit approximately 147 mm into the holes, with the transducers approximately 2 mm exposed from

the cannulae (Fig. 1A-inset). The cannulae were subsequently backfilled with tap water, and sealed with epoxy to avoid air bubbles (Fig. 1A). Two strain gauge rosettes were fixed to the anterior and posterior aspects of the lesser trochanter (Fig. 1A). The surface of the bone was sanded to remove the periosteum and degreased with ethanol prior to strain gauge attachment with cyanoacrylate glue, with the gauge leads sealed with polyurethane (Viceconti et al., 1992).

A custom fixture was designed to maintain the temperature and hydration of the bone during testing (Fig. 1B). The bones were tested while submerged in water, and a circulating bath was used to maintain the water temperature at 37° C, as bone marrow viscosity depends strongly on temperature (Bryant et al., 1989; Metzger et al., 2014). The distal femur was potted in PMMA and fixed to the bottom of the water tank. Compressive loading was applied to the femoral head through a hemispherical loading platen.

Three bones were subjected to stress-relaxation loading. A 2 mm displacement was applied to the femoral head at a rate of approximately 0.5 mm/s and held for 600 seconds. The remaining six bones were subjected to sinusoidal cyclic compression from approximately 600 to 2000 N at 1 Hz. Pressure, strain, and load data were monitored at 100 Hz (LabVIEW, National Instruments, Austin, TX). The data were filtered using a 5 Hz third-order Butterworth filter. Pressure differentials between the three pairs of adjacent transducers in each bone specimen were calculated for the first full load cycle based on the 3-D transducer spacing determined from μ -

computed tomography (μ -CT) imaging as described subsequently. Average pressure gradients were determined by taking the absolute value of the pressure gradients over the first 5 sec of loading for the stress-relaxation specimens and over the first, full 1 sec cycle of the cyclically loaded specimens (Table 1).

Following testing, the bone specimens were imaged twice with computed tomography. The first image was taken at 80 μ m to measure the transducer spacing (Fig. 2A). The femurs were then sectioned and 20 μ m resolution images of the metaphysis were obtained at 70 kVp and 114 mA (Scanco μ CT-80, Brüttisellen, Switzerland) (Fig. 2B). The high-resolution μ -CT images of the trabecular bone between each pair of pressure sensors were used to create a finite element model of the bone marrow within a 4 mm cubic region (Birmingham et al., 2013; Coughlin and Niebur, 2012). Briefly, images were Gaussian filtered. The marrow regions were converted to tetrahedral finite element meshes having from 3,669,000 to 6,988,000 tetrahedral elements using the marching cubes algorithm in Visualization Toolkit (VTK, Kitware, Clifton Park, NY). A 200 μ m layer of fluid was added around the entire boundary of the region of interest to allow pressure boundary conditions to be applied uniformly to the superior and inferior faces. A no-slip condition was applied to this layer of fluid on the perpendicular faces of the models (Fig 2C-inset).

Continuum poroelastic models were created to estimate the trabecular strain rates required to induce the pressure gradients found experimentally. Five 4 x 4 x 4 mm cubes were modeled with one thousand hexahedral elements (ADINA, Watertown, MA) (Fig. 3). The bone was assigned a uniform isotropic modulus of 500 MPa and Poisson's ratio of 0.3 (Keaveny et al., 2001). Orthotropic permeability tensors were computed from micro-scale models of the marrow. Briefly, finite element models of the marrow space were analyzed with a Newtonian fluid property and a viscosity of 0.5 Pa•s. A pressure of 80 Pa was applied to one face with a 0 Pa pressure assigned to the opposite face (Fig. 3A). The remaining faces had free-flow boundary conditions. Three such models, with pressure applied along three orthogonal directions, allowed the three columns of the Darcy permeability tensor to be calculated assuming a linear relationship between the pressure gradient and flow rate (Fig. 3B). The calculated permeability (Table 2) was consistent with those previously found experimentally (Arramon and Nauman, 2001; Nauman et al., 1999). The permeability was applied assuming a marrow viscosity of 85 Pa•s.

The continuum models were analyzed at five strain rates from 0.1% s⁻¹ to 1.0% s⁻¹ in order to cover the range of strains potentially experienced within the trabecular bone of the femoral neck. Zero pore pressure was prescribed on the proximal face of the cube, while fluid flux was constrained on the distal and lateral faces (Fig. 3C). To understand the role of the

constrained flow conditions on the pore pressure, a second solution for each model was obtained with zero pore pressure on the loaded face as well as the lateral faces, allowing the fluid to flow freely through all but the distal face (Fig. 3D). Pore pressure was analyzed at the distal face of the loading for both flow conditions to determine the resulting pressure gradient as a function of loading rate.

Computational fluid dynamics models were used to calculate the shear stress distribution of the bone marrow within the trabecular pore space of each sample to obtain a detailed understanding of the effects on the marrow mechanical environment. In these models, the fluid elements were assigned non-Newtonian fluid properties with a density of 0.95 g/cm³. The viscosity was assigned a power-law relationship of $\eta = 85.5 (\dot{\gamma})^{-1}$, where η is viscosity (Pa•s) and $\dot{\gamma}$ is the shear rate (s⁻¹). For numerical stability, the model incorporates a maximum viscosity, which was set to 100 Pa•s. We varied this maximum viscosity from 47.5 to 1500 Pa•s to determine the sensitivity. This model was based on our previous measurements in fresh porcine bone marrow using a parallel plate rheometer (Metzger et al., 2014). Because the exponent in the relationship is -1, this constitutive law has similar characteristics to Herschel-Bulkley model with a yield stress of 85 Pa and dynamic viscosity of 0 (Metzger et al., 2014). The marrow was assumed to be a homogenous fluid (Bryant et al., 1989; Coughlin and Niebur, 2012; Dickerson et al., 2007; Metzger et al., 2015; Ochoa and Hillberry, 1992). The bone-

marrow interface was assigned a rigid no-slip boundary condition, assuming that the adjacent cells were attached to the bone matrix by integrins (Birmingham et al., 2013; Coughlin and Niebur, 2012; Dickerson et al., 2007; Metzger et al., 2015). The experimentally measured time-dependent pressure differentials were prescribed to the proximal face of the bone models and zero pressure was prescribed to the distal face. Fluid flow into and out of the perpendicular faces was constrained (Fig. 2C).

A transient, dynamic solution was performed using ADINA-F® (Watertown, MA). The spatial distribution of shear stress within the marrow cavity was analyzed to describe the results. A 3.4 x 3.4 x 3.4 mm³ sub-region in the center of the sample was selected for post-processing to avoid artifacts due to boundary conditions. The volumetric mean shear stress and velocity in the marrow were calculated using volume integration in Paraview (Paraview, Kitware, Clifton Park, NY). The volumetric averages were further time-averaged at 10 Hz over the first 1 sec load cycle for cyclic specimens and 10 Hz over the first 5 sec of loading for stress-relaxation specimens.

Results

Trabecular pore pressure increased during stress-relaxation loading and dissipated during stress-relaxation (Fig. 4A). On average, the peak pressure was 1.7 ± 1.3 kPa (mean $\pm$ S. D.) and ranged from 0.07 to 5 kPa. The mean pressure gradient was 0.125 ± 0.11 kPa/mm during the

first 5 s of loading across all the samples. The pressure varied with the load during cyclic loading (Fig. 4B). The peak pressures reached as high as 2.5 kPa. The mean pressure gradient was 0.17 ± 0.11 kPa/mm during cyclic loading averaged over 1 cycle. The pore pressure gradients did not depend on trabecular bone volume fraction (BV/TV) between adjacent transducers (Fig. 5). The strain rate on the cortical surface was approximately $0.02\% \text{ s}^{-1}$ during stress-relaxation and $0.12\% \text{ s}^{-1}$ during cyclic loading.

Parametric analysis using poroelastic models indicated that the measured pressure gradients were consistent with deformation-driven fluid motion. The constrained flow model developed pressure gradients from 0.029 ± 0.027 kPa/mm when loaded at a rate of $0.1\% \text{ s}^{-1}$ to 0.302 ± 0.272 kPa/mm at $1.0\% \text{ s}^{-1}$ (Fig. 6A). The free-flow model developed pressure gradients an order of magnitude lower than the constrained flow model under similar loading conditions (Fig. 6B).

When the measured pressure gradients were applied across the detailed marrow models, the volumetric mean marrow shear stress was 7.1 ± 6.2 Pa during stress-relaxation (Fig. 7A) and 9.6 ± 6.9 Pa when averaged over the 1-second cycle and across all regions analyzed (Fig. 7B). The volumetric mean shear stress increased with the measured pressure differential during both stress-relaxation (Fig. 7A, $R^2=0.73$, $p<0.005$) and cyclic loading (Fig. 7B, $R^2=0.23$, $p<0.05$).

The volumetric mean marrow velocity relative to the bone averaged 0.006 ± 0.005 mm/s across the stress-relaxation models over the first second of loading, and was positively correlated to the measured pressure gradient ($p < 0.005$). During cyclic loading, the volumetric mean marrow velocity averaged 0.01 ± 0.008 mm/s for the six samples over the first cycle, but was not correlated to measured pressure gradient ($p > 0.2$). The peak volumetric mean velocity gradient magnitude within each sample during the first second of stress-relaxation averaged 0.07 ± 0.06 s⁻¹ across the three samples and 0.11 ± 0.08 s⁻¹ during the first full cycle across the six cyclically loaded specimens.

The average marrow shear stress was not sensitive to the maximum marrow viscosity in the models, as sufficient shear stress must develop in the marrow to resist the applied pressure gradient. Rather, the velocity gradients, and hence the maximum velocity of the marrow are altered by changing the viscosity in the models. When the maximum viscosity in the power-law model was set to 47.5 Pa•s, the marrow velocity magnitude increased from 0.0194 to 0.0456 mm/s, while a higher limit of 1500 Pa•s decreased the average velocity to 0.0013 mm/s.

Discussion

While osteocytes are the primary mechanosensors in bone, mechanical stimulation in the marrow cell population may also play an important role in the adaptive response to skeletal loads. Using complementary experiments, imaging, and computational models, this study

demonstrates that deformation of whole bones induces motion, and consequently shear stress, in the bone marrow that could induce a mechanobiological response. The measured pressure was small relative to the applied loads and to the reported mechanobiological response threshold. However, when the pressure gradients were applied in micromechanical fluid dynamics models, the shear stress exceeded the reported threshold levels for mechanobiological response of a number of cell lineages (Table 3). As such, the intercellular motion and deformation of the cells in the marrow space could stimulate mechanobiological pathways in skeletal progenitors directly (Mantila Roosa et al., 2011) or through paracrine signaling from surrounding cells in the marrow (Soves et al., 2014). This mechanobiological signaling could play a role in bone marrow adaptation with aging (Rosen and Bouxsein, 2006), which would have a subsequent effect on bone adaptation, or it could directly affect bone formation (Birmingham et al., 2014; Webster et al., 2015).

The *in situ* micromechanical environment of bone marrow has not been characterized previously. As such, the principle strength of this study was quantification of the marrow micromechanical environment during physiologic loading. In particular, this study quantified the motion and shear stress in trabecular bone marrow within real trabecular pore geometries driven by measured pressure gradients. Although direct measurement of these quantities in whole, intact femurs is not possible with present technology, indirect quantification was achieved

by combining experiments with computational models. While poroelasticity based on Darcy permeability provides a macroscopic view of marrow mechanics, it can only provide a volume-averaged estimate of the fluid environment based on assumed pore geometry and orientation (Arramon and Nauman, 2001; Kohles et al., 2001). The use of micro-scale models provides additional insight into the distribution of shear stress within the pore space. Taken together, a complementary experimental and numerical approach such as the one used here, combining measured pressure and trabecular geometry provided a strategy to assess the marrow mechanics. Computational models at a continuum scale offered insights into the role of strain rate on the measured pressure gradients, while micromechanical models allowed direct computation of the shear stress within the marrow.

Although this study provides new insights into the biomechanics of trabecular bone marrow during whole bone loading, several limitations should be considered when interpreting the data. The primary limitation is the use of porcine femora, which differ from human bones in geometry and loading. Porcine bones were used because it was possible to perform the experiments within a short time post mortem, which is essential because of the rapid changes in bone marrow properties during storage (Metzger et al., 2014). For similar reasons, the applied loading was simplified, with no muscle loading on the greater trochanter, which would have required inserting screws and breaching the cortical shell (Ochoa et al., 1997). However, the

study validates the concept of pressure gradients in the trabecular bone, and the methods could be used to extract marrow mechanics from previous or future experiments in human bone under various loading conditions based on multi-scale poroelastic models. A second limitation is the assumption that bone marrow is a homogenous fluid. Bone marrow might be more appropriately characterized as a colloidal suspension of cells. In the continuum poroelastic models, the relationship between pressure gradient and fluid motion is assumed to be linear, which is reasonable for small ranges of fluid velocity (Metzger et al., 2015). In the micro-scale models we modeled bone marrow as a non-Newtonian fluid, as is common with colloidal suspensions of high concentrations (Rutgers, 1962). Capturing the true behavior of the marrow would require multi-scale modeling that incorporates cell-cell adhesion, cell-fluid interaction and multiple cell geometries (Forgacs, 1995; Preziosi et al., 2010; Vaughan et al., 2015).

The role of marrow mechanobiology in diseases remains an open question. A number of diseases could affect or be affected by marrow mechanical properties, including osteoporosis (Justesen et al., 2001), diabetes, cancer metastasis (Lynch et al., 2013), and marrow aplasia (Naveiras et al., 2009), where the cell population may be altered. However, we are not aware of any measurements of altered marrow mechanical properties due to diseases. These same diseases are often accompanied by changes in the trabecular bone volume fraction, which affects the permeability, the stiffness, and the resulting strains. Hence, the cumulative effects on bone

marrow shear stress are not easily predictable, as our data for pressure gradient vs. permeability demonstrate (Fig. 6). Higher loading rates, or potentially inertial loading regimes, are the only certain method to increase marrow shear stress in a given disease condition, and these have shown some positive effects in experimental (Judex et al., 2007; Webster et al., 2015; Xie et al., 2006) and clinical studies (Gilsanz et al., 2006).

The results complement previous reports on the pressure developed within trabecular bone marrow during whole bone loading. The pressure in proximal human femurs averaged 3.3 kPa when loaded to 1200 N at 1.0 s^{-1} and depended on strain rate (Downey et al., 1988). Interestingly, similar pressure increases were measured in an *in vivo* study in the rat femur during muscle activation (Kumar et al., 1979). The pressure also varied throughout the metaphysis of the ovine tibia during impact loading (Bryant, 1983), but the magnitude was much higher, consistent with the rate dependence of poroelastic materials.

Increased bone marrow pressure increases bone formation, although the underlying mechanobiology is not known. Dynamic compression of mouse tibiae induced pressure increases of about 2 kPa, and were associated with new bone formation (Hu et al., 2012). Increased intraosseous pressure in a similar range caused by venous ligation also resulted in metaphyseal bone formation in goats (Welch et al., 1993). In rats (Bergula et al., 1999) and mice (Stevens et al., 2006a) increased marrow pressure due to venous ligation, also in the 1 to 2 kPa

range, induced bone formation only in the case of unloaded limbs, suggesting venous ligation compensates for normal marrow pressurization due to mechanical loading. The bone formation was related to increased fluid flow from the marrow into the bone and depended on COX-2 and NOS-3 gene expression (Bergula et al., 1999; Stevens et al., 2006b). The effects of such osseous hypertension appear to depend on non-physiologic pressures. However, the pressures we measured were of similar magnitude and could play some role in normal bone or bone marrow maintenance.

The combination of the periosteal strain measurements and poroelastic modeling provided support for the measured pressure gradients found experimentally. The measured periosteal bone strains were approximately 2% higher than those found in *in vivo* human femurs during physiologic loading (Aamodt et al., 1997). Previous computational models found the strain in the trabecular bone of the human femoral neck was approximately 0.3% for similar levels of cortical bone strain (Keyak et al., 1993; Keyak et al., 1990; Lotz et al., 1991; Lotz et al., 1990). Using this as an estimate of the trabecular bone strain over a 1 s period in these experiments, 2/3 of the experimentally measured pressure gradients fell within the range calculated from the continuum poroelastic models with constrained flow, while the unconstrained boundary conditions under-predicted the measured pressure gradients for most samples (Fig. 6). As such, the constrained flow boundary condition is an upper bound on the

pressure gradient, while the free flow is a lower bound. The constrained case provided a better estimate, which led us to apply a nearly constrained flow condition in the micro-scale models.

The use of multiple modeling scales provides a powerful means for investigating marrow mechanics. Fluid-structure interaction models using the same marrow properties (Metzger et al., 2014) resulted in similar pressure gradients to those found in the constrained flow poroelastic models when a strain rate of $0.1\% \text{ s}^{-1}$ was applied (Metzger et al., 2015). This suggests that poroelastic models can be used to determine trabecular pore pressure and pressure gradients, while micro-scale models enable spatial quantification of shear stress.

The concept of hydraulic stiffening and strengthening of whole bone has been controversial (Arramon and Cowin, 1997; Bryant, 1983; Kafka, 1983; Ochoa et al., 1991). In contrast, the inverse question of the role of poroelasticity in marrow mechanobiology has received little attention. Our results demonstrate that interaction between the trabecular bone and marrow generates significant cellular-level motion eliciting mechanical stress and strain within the marrow and its cellular constituents. As such, we propose that the role of bone marrow in resisting mechanical loads during activities of daily living is secondary to the effects these loads play in normal bone marrow mechanobiology, which in turn is essential to human health.

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468 **Table Captions**

469 **Table 1.** Average of the absolute value of the measured pressure gradients over the first 5 sec of
470 loading for the stress-relaxation specimens and over the first, full 1 sec cycle of the cyclically
471 loaded specimens. Proximal represents the gradients between adjacent transducers closest to the
472 femoral head (PT1-PT2) and distal represents the gradient between adjacent transducers most
473 distal to femoral head (PT3-PT4).

474 **Table 2.** The orthotropic intrinsic permeability was determined from image-based fluid flow
475 models of 4 x 4 x 4 mm trabecular bone images from five of the cyclically loaded femurs, and
476 applied to continuum poroelastic analyses. Samples span the entire range of permeability
477 measured across all specimens studied.

478 **Table 3.** Measured mechanostimulatory shear stress thresholds for resident marrow cell
479 populations found in literature.

Figure Captions

Figure 1: A) Four holes were drilled along the femoral neck to provide insertion points for miniature pressure transducers. Inset - Transducers were fed through cannulae, which were press-fit into the holes (scale bar = 5 mm). One arm of the cannula was used to pass the transducer leads, while the other was used to backfill the hole with water. Both were sealed with epoxy. Strain gauge rosettes were attached to the anterior and posterior greater trochanter. B) The femora were tested while submerged in a water bath. The distal end of the femur was potted in PMMA and fixed to the water filled tank. A circulating heater was used to maintain the water at 37 °C throughout testing.

Figure 2: A) Femora were imaged using micro-CT at locations between pressure transducers at a resolution of 20 μ m. B) A 4 mm³ cubic region was selected from between adjacent pressure transducers and C) the marrow space was meshed with approximately 1 million tetrahedral finite elements. The measured pressure differentials were applied to the proximal face of models with zero pressure applied to the distal face. Inset – A no-slip boundary condition was applied on the lateral faces of the fluid models. D) Computational fluid dynamics models were solved to find the marrow shear stress, which is depicted on streamlines in this image.

Figure 3: A) The orthotropic permeability was determined for five porcine specimens by applying a pressure gradient along each coordinate axis, with 80 Pa applied on one face of the fluid and 0 Pa on the opposing face. A thin layer of fluid surrounded the entire model to allow uniform boundary conditions (not shown for clarity). B) The Darcy permeability tensors were calculated as the ratio of flow-rate to the calculated pressure gradient along all three coordinate axes. Five finite element models with one thousand hexahedral elements were created with orthotropic permeability corresponding to the principal values from the tensors calculated in each micro-scale model, a uniform isotropic modulus of 500 MPa, and Poisson's ratio of 0.3 (Keaveny et al., 2001). The models were solved for boundary conditions where the fluid was constrained to only flow through the superior surface (C and D) or through the superior and lateral surfaces (E and F).

Figure 4: A) Bone marrow pressure increased when a static load was applied. The average peak pressure was 1.7 ± 1.3 kPa (mean $\pm$ std). Pressure dissipated during the holding period, but had little effect on the applied load. The strain rate on the anterior cortex was approximately $0.02\% \text{ s}^{-1}$. B) The pressure varied sinusoidally with load during cyclic loading, with an average pressure differential of 0.73 ± 0.57 kPa during the first full loading cycle across all of the

specimens. The strain rate on the anterior cortex was approximately $0.12\% \text{ s}^{-1}$ during cyclic loading. Representative sample depicted.

Figure 5: Time-averaged pressure gradients were calculated with transducer spacing determined from the micro-CT images. Pressure gradients did not correlate to trabecular bone volume fraction during A) stress-relaxation or B) cyclic loading. Each color represents the three samples from a single femur.

Figure 6: A) Constrained and B) free-flow boundary conditions were applied to continuum poroelastic analysis under five loading conditions. Fluid flow was restricted to one face of the model in constrained models, while fluid flow was unrestricted to all faces except one in the free-flow model. Pore pressure gradients decreased with increasing intrinsic permeability. The permeability in the z-direction was chosen for each of the models. Markers represent time-averaged pressure gradients measured experimentally during cyclic loading, with each color representing one femur.

Figure 7: A) The volumetric- and time-averaged mean shear stress ranged from 0.311 to 16.42 Pa during stress-relaxation and increased with the time-averaged pressure gradient. B) The volumetric- and time-averaged shear stress during cyclic loading ranged from 2.14 to 17.57 Pa and increased with the time-averaged pressure gradient. Each color represents the three samples from a single femur. Dashed lines are $\pm 95\%$ C.I.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

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Figure 7

Table 1. Average of the absolute value of the measured pressure gradients over the first 5 sec of loading for the stress-relaxation specimens and over the first, full 1 sec cycle of the cyclically loaded specimens. Proximal represents the gradients between adjacent transducers closest to the femoral head (PT1-PT2) and distal represents the gradient between adjacent transducers most distal to femoral head (PT3-PT4).

	Pressure Gradient (kPa/mm)		
	Proximal	Middle	Distal
<i>Stress-Relaxation</i>			
Specimen 1	0.013	0.061	0.072
Specimen 2	0.052	0.079	0.071
Specimen 3	0.322	0.236	0.219
<i>Cyclic</i>			
Specimen 1	0.124	0.124	0.103
Specimen 2	0.069	0.090	0.017
Specimen 3	0.143	0.138	0.221
Specimen 4	0.265	0.266	0.213
Specimen 5	0.148	0.462	0.243
Specimen 6	0.131	0.140	0.164

Table 2. The orthotropic intrinsic permeability was determined from image-based fluid flow models of 4 x 4 x 4 mm trabecular bone images from five of the cyclically loaded femurs, and applied to continuum poroelastic analyses. Samples span the entire range of permeabilities measured across all porcine bone specimens studied.

	Permeability (m ²)		
	Medial-Lateral	Anterior-Posterior	Proximal-Distal
Sample 1	4.77•10 ⁻¹⁰	2.98•10 ⁻¹⁰	8.64•10 ⁻¹⁰
Sample 2	1.17•10 ⁻⁹	1.65•10 ⁻⁹	2.64•10 ⁻⁹
Sample 3	1.32•10 ⁻⁹	2.56•10 ⁻⁹	4.03•10 ⁻⁹
Sample 4	3.36•10 ⁻⁹	8.35•10 ⁻⁹	7.82•10 ⁻⁹
Sample 5	5.61•10 ⁻⁹	3.11•10 ⁻⁹	1.32•10 ⁻⁸

Table 3. Measured mechanostimulatory shear stress thresholds for resident marrow cell populations found in literature.

	Threshold (Pa)	Source
Mesenchymal stem cells	0.90	(Yourek et al., 2010)
Osteoblasts	2.0	(Kapur et al., 2002)
Osteoclasts	0.5-2.0	(Cui et al., 2012)
Megakaryocytes	1-2	(Soves et al., 2014)