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Trabecular bone recovers from mechanical unloading primarily by restoring its mechanical function rather than its morphology

Engin Ozcivici [a,*], Stefan Judex [b]

[a] Department of Mechanical Engineering, Izmir Institute of Technology, Urla, Izmir, Turkey
[b] Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY 11794, USA

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ABSTRACT

Upon returning to normal ambulatory activities, the recovery of trabecular bone lost during unloading is limited. Here, using a mouse population that displayed a large range of skeletal susceptibility to unloading and reambulation, we tested the impact of changes in trabecular bone morphology during unloading and reambulation on its simulated mechanical properties. Female adult mice from a double cross of BALB/cByJ and C3H/HeJ strains (n = 352) underwent 3 wk of hindlimb unloading followed by 3 wk of reambulation. Normally ambulating mice served as controls (n = 30). As quantified longitudinally by in vivo μCT, unloading led to an average loss of 43% of trabecular bone volume fraction (BV/TV) in the distal femur. Finite element models of the μCT tomographies showed that deterioration of the trabecular structure raised trabecular peak Von-Mises (PVM) stresses on average by 27%, indicating a significant increase in the risk of mechanical failure compared to baseline. Further, skewness of the Von-Mises stress distributions (SVM) increased by 104% with unloading, indicating that the trabecular structure became inefficient in resisting the applied load. During reambulation, bone of experimental mice recovered on average only 10% of its lost BV/TV. Even though the addition of trabecular tissue was small during reambulation, PVM and SVM as indicators of risk of mechanical failure decreased by 56% and 57%, respectively. Large individual differences in the response of trabecular bone, together with a large sample size, facilitated stratification of experimental mice based on the level of recovery. As a fraction of all mice, 66% of the population showed some degree of recovery in BV/TV while in 89% and 87% of all mice, PVM and SVM decreased during reambulation, respectively. At the end of the reambulation phase, only 8% of the population recovered half of the unloading induced losses in BV/TV while 50% and 49% of the population recovered half of the unloading induced deterioration in PVM and SVM, respectively. The association between morphological and mechanical variables was strong at baseline but progressively decreased during the unloading and reambulation cycles. The preferential recovery of trabecular micromechanical properties over bone volume fraction emphasizes that mechanical demand during reambulation does not, at least initially, seek to restore bone's morphology but its mechanical integrity.

Introduction

Loss of weight-bearing in skeletal extremities reduces tissue mass as mechanical loads provide critical anabolic and anti-catabolic signals [1–3]. The accompanying deterioration of bone's estimated [4] and actual [5] mechanical properties, combined with decreases in muscle strength [6,7] and postural stability [8] increase the risk of traumatic and non-traumatic fractures, particularly upon returning to regular weight bearing activities [9–11]. Daily bouts of exercise [12] or high-frequency motions [13–15] can partially alleviate the reduction in bone quantity, morphology and mechanical properties during unloading. However, once mechanical loads are reintroduced to skeletal extremities during reambulation, recovery of bone mass is often slow and incomplete. If full recovery is accomplished, it requires several times the duration of unloading [16–18]. During the initial phase of reambulation, bone mass may even continue to deteriorate in humans [16,19] and rodents [20], further compromising bone's structural integrity at a time when mechanical support is critical to withstand reloading of the skeleton.

To more accurately assess fracture risk of eroded skeletal sites than by dual X-ray absorptiometry (DXA) or quantitative computed tomography (QCT) alone, mechanical testing of bone can be performed non-invasively by simulating the application of mechanical loads to the imaged bone structure with the finite element (FE) method [21–24]. Simulations can be performed for specific sub-regions of the scanned images and filtered for different features. For instance, a digital structure from which non load-bearing trabeculae were digitally

* Corresponding author at: Department of Mechanical Engineering, Rm 110, Izmir Institute of Technology, Urla, Izmir, 35430, Turkey. Fax: +90 232 750 6701.
E-mail address: enginozcivici@iyte.edu.tr (E. Ozcivici).

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removed better predicts mechanical strength than a structure containing load-bearing and non load-bearing elements [25]. Further, the high spatial resolution of the FE method at the micron-scale can aid in understanding the risk of mechanical failure, and changes in it, through the detection of those locations in which peak stresses occur as well as by testing whether the microstructural arrangement of trabeculae allows for efficient load transfer without overstressing individual elements [26].

FE analysis of skeletal sites in astronauts indicated a 5% loss in proximal femoral strength per month [4], at least twice the rate previously suggested by methods based on bone density and mass [2,27,28]. A similar discrepancy between the loss of trabecular bone quantity and its mechanical properties has been observed in rodent unloading models [14,29]. As a result, a panel recently recommended the integration of FE analysis with QCT to more effectively evaluate astronaut bone health in future studies [3]. While FE analysis has been used to describe the deterioration of bone's mechanical properties during spaceflight and ground-based analogs, much less is known about changes in bone's mechanical properties during reambulation. After long-duration missions, QCT based estimates of bone strength suggested that recovery of bone mass was incongruent with recovery of its estimated mechanical properties [16]. While it is clear that during altered loading environments, bone's mechanical properties cannot be fully predicted from its morphology, little is known of how bone's simulated mechanical properties change across individuals with distinct mechano-sensitivity and which trabecular architectural variable(s) play a dominant role in modulating bone mechanics.

To address this question, we quantified longitudinal changes in simulated mechanical parameters of trabecular bone during unloading and reambulation using a 2nd generation (F2) genetically heterogeneous mouse population bred for producing a large range of responses to altered mechanical demand. For this experiment, the association of specific chromosomal regions with the magnitude of trabecular deterioration/recovery during unloading/reambulation has been reported for morphology [30] and estimated mechanical properties [31]. Here, we asked the following questions: (1) Are changes in simulated mechanical properties similar in magnitude and variability to morphologic changes during unloading and reambulation? (2) To what extent do architectural and mechanical traits recover in trabecular bone during reambulation across a population with a large range of mechano-responses? (3) Which architectural variables determine changes in bone's simulated mechanical properties during unloading/reambulation?

Materials and methods

Experimental design

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Stony Brook University. A total of 352 female adult (16 wk old) mice from a 2nd generation (F2) cross of BALB/cByJ (BALB — high response to mechanical unloading) and C3H/HeJ (C3H — low response to mechanical unloading) inbred strains were used [32]. At baseline (n = 436), mice were μCT-scanned in vivo (vivaCT40, Scanco Medical, Switzerland) at the distal femoral metaphysis under isoflurane inhalation. Immediately following the scans, both hindlimbs were unloaded for 3 wk [33]. Upon completing the unloading phase, μCT scans were taken (n = 359) and mice were released for 3 wk of normal cage activity (reambulation). Following reambulation (n = 352), mice were μCT scanned again (22 wk old). Sample sizes were not identical at the three μCT scan time points primarily because of scheduling conflicts for the time consuming in vivo scans (mice that could not be scanned were sacrificed). A small number of mice did not adapt to hindlimb unloading and in accordance to the IACUC protocol, mice with deteriorating health and/or weight losses were terminated. Non-suspended age-matched control mice (n = 30) were allowed regular cage activities throughout the 6 wk experimental period and received μCT scans at identical time points as experimental mice. Reconstructed μCT tomographies were directly converted into finite element (FE) models and subjected to uniaxial compression for the evaluation of trabecular micro-mechanical properties.

Micro-computed tomography

The distal femoral metaphysis was assessed at an isometric voxel size of 17.5 μm. The analyzed region comprised 1500 μm (85 slices) with the most distal slice of 600 μm (35 slices) proximal from the growth plate [32]. Trabecular bone was separated from cortical bone using an image processing language algorithm [34] followed by 3D Gaussian blurring with "sigma" and "support" values of 0.3 and 1. The threshold value that segmented calcified tissue from background was set at 29.5% of the maximum voxel intensity for all mice and time points. For trabecular bone, bone volume fraction (BV/TV), connectedness (Conn.D), number (Tb.N) thickness (Tb.Th) and the degree of anisotropy (D.A) were determined.

Finite element modeling

Changes in mechanical properties induced by altered mechanical demand were evaluated in the femoral metaphysis (as defined above) comprising both trabecular and cortical bone. Every voxel representing trabecular or cortical bone was directly translated to a FE brick element with an isotropic size of 17.5 μm (ScancoFE, Scanco Medical). A frictionless compression test in the longitudinal (dominant habitual loading) direction was applied to all FE models [14]. Linear elastic properties were assigned to trabecular and cortical bone with an elastic modulus (E) of 25 GPa and Poisson's ratio (υ) of 0.3 [35–37]. FE solutions were completed via an iterative FE-solver [38] with a force (N) and displacement (mm) tolerance of 1×10^{-4} (ScancoFE, Scanco Medical). During post-processing, voxels pertaining to cortical bone were excluded and simulated mechanical properties were analyzed only for trabecular bone. Thus, in contrast to a test in which only a trabecular core is subjected to a simulated compression test, here, trabecular bone was compressed in a more physiologic manner with force transfer from both endplates as well as from trabecular struts connected to the cortical shell [14].

Apparent stiffness values for trabecular bone were calculated as the ratio of input force to resultant displacement. To simplify the stress representation while including all stress components arising in normal and shear directions, Von-Mises (VM) stresses, a derived stress value that is closely related to failure in trabecular bone [39], were calculated for all time points. For all models, calculated VM stress values were normalized to the axial boundary force magnitude to ensure that all models were subjected to a 1 N compressive force. To prevent local artifacts and singularities, peak VM (PVM) stresses were calculated as the 95th percentile of the VM stress distribution [14]. Trabecular bone of F2 mice that showed higher PVM values under the same loading conditions was assumed to be more prone to mechanical failure.

Skewness of the VM stress distribution (SVM) was calculated for each individual's VM stress histograms to quantify the non-uniformity of the stress histogram as an indicator of trabecular efficiency in load bearing [26]. When the VM stress histogram is normally distributed, stress transfer is assumed to be efficient as most of the trabecular elements are subject to intermediate stresses. As the trabecular structure changes its morphology, the stress histogram may change its distribution. For instance, a left leaning distribution with a long tail to its right indicates a mechanically less efficient structure because the bulk of the trabecular elements is subjected to lower stresses while a relatively small number of elements on the right tail become disproportionally overstressed to support the applied load (Fig. 1).

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