Mechanical Loading Causes Detectable Changes in Morphometric Measures of Trabecular Structure in Human Cancellous Bone

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The relationships between mechanical loads and bone microstructure are of interest to those who seek to predict bone mechanical properties from microstructure or to predict how organization of bone microstructure is driven by mechanical loads. While strains and displacements in the material are inherently responsible for mechanically caused changes in the appearance of the microstructure, it is the morphometric measures of microstructural organization that are often available for assessment of bone quality. Therefore, an understanding of how strain history is reflected in morphometric measures of bone microstructure has practical implications in that it may provide clinically measurable indices of mechanical history in bone and improve interpretation of bone mechanical properties from microstructural information. The objective of the current study was to examine changes in morphometric measures of cancellous bone microstructure in response to varying levels of continuum level strains. The experimental approach included stereologic analysis of microcomputed tomography (μ CT) images of human cancellous bone samples obtained at sequentially increasing levels of strain in a custom-made loading apparatus mounted in a μ CT scanner. We found that the degree of anisotropy (DA) decreased from baseline to failure and from failure to postfailure. DA partially recovered from postfailure levels upon unloading; however, the final DA was less than at failure and less than at baseline. We also found that average trabecular thickness (Tb.Th.Av) increased with displacements at postfailure and did not recover when unloaded. Average trabecular number decreased when the specimens were unloaded. In addition, the heterogeneity of Tb.Th as measured by intra-specimen standard deviation (Tb.Th.SD) increased and that of trabecular number (Tb.N.SD) decreased with displacements at postfailure. Furthermore, the intraspecimen coefficient of variation of trabecular number decreased at postfailure displacements but did not recover upon unloading. Finally, the coefficient of variation of trabecular separation at unload was less than that at baseline. These measures can be developed into image-based indices to estimate strain history, damage, and residual mechanical properties where direct analysis of stresses and strains, such as through finite element modeling, may not be feasible. It remains to be determined how wide a time interval can be used to estimate strain history before remodeling becomes an overriding effect on the trabecular architecture. [DOI: 10.1115/1.4024136]

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Introduction

A large portion of bone quality and fracture risk is accounted for by bone mass; however, bone microstructure is also important. Numerous studies examined the relative contribution of bone microstructure to bone strength and fracture risk (eg. [1–7]). However, the extent to which changes in the microstructural organization of cancellous bone can be used as a means for estimating loading history and the rate at which failure progresses are not well understood. It is intuitively obvious that displacements can cause changes in the microstructural organization of the bone; however, the nature of these changes and the extent to which they are reflected in measures of microstructure that are extractable from image analysis is not clear. Due to advances in imaging modalities, more detailed *in vivo* information on the microstructure of bone is increasingly available. An understanding of the relationship between mechanical loads and changes in bone microstructure may allow for the development of tools that utilize bone texture for estimating the loading history in that bone (by texture, we refer to any recognizable pattern in the appearance of the bone including those described by statistical and morphometric methods). Together with an appropriate model, information on the subject-specific mechanical environment of a bone could allow for prediction of the residual fatigue life in that bone. Such information could also be used to enhance understanding of a bone's adaptive response in the period following the imaging. Using samples from a whale bone, previous work presented qualitative evidence that deformations in cancellous bone may be such that they may be reflected in changes in morphometric measures of trabecular structure [8]. The objective of the current study was to quantitatively examine the changes in the microstructure of human cancellous bone at various levels of compressive deformations using a combination of μ CT and mechanical loading. More recent studies point to a potentially important role of cancellous bone heterogeneity in assessment of bone quality that may not be achieved by bone density or average measures of trabecular architecture [6,9–11]. Therefore, a particular emphasis was put on the heterogeneity of the trabecular architecture.

Methods

Seven fresh frozen femoral and tibial cancellous bone cylinders from human cadavers were used. Baseline measurements of each specimen served as its own control; therefore no effort was made to control for age, gender, or anatomic site. The specimens were mounted in a custom-built loading device placed in the μ CT system, allowing for a series of scans without the need to remove the samples during mechanical testing [12,13]. The details of the μ CT system have been described in a previous report [12]. Scans (35 μ m voxel size) were made at baseline (zero strain), within the preyield (elastic; 0.004 strain) deformation, at postyield (failure; 0.01 strain), at postfailure (beyond strain to maximum load; 0.1 strain) and, finally, after unloading to the no-load state. The scans were performed at 50 kV and 0.2 mA with 400 views over 360 deg, frame averaging of 128 per view (1/30 s each), and the reconstructions used a sinc filter. The microstructural analyses were performed using previously described 3D stereology methods [3,6]. Baseline images were segmented using an established global thresholding method [14,15]. The method calculates the local maxima and minima of gray levels by comparing the voxel under question with three others in all directions and records them if they are higher (for maxima) or lower (for minima) than predefined tolerance values. The qualifying local minima are averaged to obtain a global minimum and the qualifying local maxima are averaged to obtain a global maximum. These values are then averaged to obtain a global threshold value. Subsequent image sets of the same sample were thresholded by adjusting the global threshold so as to match bone volume (BV) with that of the baseline image to avoid microstructure variations caused by thresholding inaccuracies. The volume of interest was selected to include the entire specimen. In addition to the volume averages of bone

volume fraction (BV/TV.Av), trabecular thickness (Tb.Th.Av), number (Tb.N.Av), and separation (Tb.Sp.Av), the within-specimen standard deviation (BV/TV.SD, Tb.Th.SD, Tb.N.SD, and Tb.Sp.SD) and coefficient of variation (BV/TV.CV, Tb.Th.CV, Tb.N.CV, and Tb.Sp.CV) of these parameters were calculated as measures of microstructural heterogeneity [6]. To calculate heterogeneity, stereology measurements were performed in each plane perpendicular to the direction of compression. The DA and connectivity density ($-\#Euler/Vol$) were also calculated using the whole 3D image volume [6]. Degree of anisotropy was calculated using the star volume method [16].

Differences between deformation stages within a sample were examined using a mixed model ANOVA with subject as random and strain level as a fixed effect. Each specimen was treated as a subject. Post hoc analyses were conducted using paired t-tests. Similar to analyses of damage progress in fatigue loading [17], baseline measurements were compared with increasing levels of strain until significance is detected ($p < 0.05$). Once a difference was detected, the new strain level was chosen for further comparison. If significant recovery from postfailure levels was observed, the unloaded level was again compared to the baseline. Statistical analysis was performed using JMP (Version 7.0.2, SAS Institute Inc., Cary, NC).

Results

Changes in microstructure could be observed qualitatively upon inspection of pre- and post-deformation images (Fig. 1). Mixed model ANOVA models were significant for DA ($p < 0.0001$), Tb.Th.Av ($p < 0.0001$), Tb.N.Av ($p < 0.05$), Tb.Th.SD ($p < 0.03$), Tb.N.SD ($p < 0.0003$), Tb.N.CV ($p < 0.004$), and Tb.Sp.CV ($p < 0.05$), whereas other parameters were not ($p > 0.07$ – $p > 0.71$).

Degree of anisotropy was less at failure than at baseline ($p < 0.03$) and less at postfailure than at failure ($p < 0.0001$) (Fig. 2). A significant portion of DA was recovered from postfailure levels upon unloading ($p < 0.02$), but the final DA was still less than at failure ($p < 0.0001$) and baseline ($p < 0.0001$). Up to 12.8% change from baseline was noted for DA. The smallest difference detected was 2% (from baseline to failure).

Tb.Th.Av increased with displacements at postfailure ($p < 0.0001$) and did not recover when unloaded ($p > 0.96$; Fig. 3). Up to 5.1% change from baseline was noted for Tb.Th.Av. The smallest statistically detectable difference was 3.6% (from failure to postfailure). Moreover, Tb.N.Av did not change with increasing strain levels ($p > 0.17$) but decreased (5%) when unloaded ($p < 0.006$).

Tb.Th.SD increased ($p < 0.005$) and Tb.N.SD decreased ($p < 0.003$) with displacements at postfailure and these changes

were not recovered after unloading ($p > 0.33$ and $p > 0.44$, respectively) (Figs. 4–5). Up to 16.7% and 22.8% change from baseline was noted and the smallest difference detected was 11.1% and 18.3% (from failure to postfailure) for Tb.Th.SD and Tb.N.SD, respectively.

Tb.N.CV, as for Av and SD, decreased at postfailure displacements ($p < 0.02$) and was not recovered upon unloading ($p > 0.72$) (data not shown). Tb.Sp.CV at unload was also less than that at baseline ($p < 0.03$) (data not shown). Up to 17.3% change from baseline was noted and the smallest difference detected was 15.3% (from baseline to postfailure) for Tb.N.CV. Up to 15.7% change from baseline was noted and the smallest difference detected was 15.7% (from failure to unload) for Tb.Sp.CV.

Discussion

The demonstration that mechanical load changes the organization of the trabecular architecture is perhaps obvious. What is important, however, is the demonstration that such changes can be detected using specific micromorphometric parameters that quantify the texture of the cancellous bone. The current results show that trabecular anisotropy, trabecular thickness, number and microstructural heterogeneity reflected in the variability of Tb.Th, Tb.N and Tb.Sp changed significantly under strain. Tb.Th.SD and Tb.N.SD had larger changes than average values, suggesting that heterogeneity of the microstructure is especially sensitive to deformation and these could be good parameters to use to estimate strain history in the tissue.

Interestingly, average BV/TV was not different between deformation stages indicating that changes in total volume due to the lateral expansion of the samples was sufficient to compensate for the loss of volume from compression. This would likely be different if the cancellous bone was tested in situ with constraint from the cortical bone. Nevertheless, the results collectively indicate that measures of bone texture can pick up deformation-related changes in cancellous bone that bone mass cannot.

It must be noted that the changes in the microstructure due to loading are quantified as perceived by the methods of stereology. While some of these changes such as the number density of trabeculae per plane are likely real due to displacement of trabecular struts from one plane to another, some other changes such as those in trabecular thickness may be perceived as such due to changes in total volume and compaction from compression. Nevertheless, quantifiable changes in these parameters would be useful in developing indices of strain history. While significant changes were demonstrable for failure and postfailure levels with the current data, it is possible that the relationships between

Fig. 1 Central slice from images of a typical specimen before (left, zero strain) and post-failure (right, unloaded). Encircled areas show the nature of deformation that led to the reported microstructural changes.

Fig. 2 (a) Changes in the degree of anisotropy with different levels of strain representing baseline, elastic, postyield, postfailure, and unloaded states. Changes in the asterisks indicate significant changes in DA as strain levels change (from left to right). The levels of strain connected with a bar (–) are not significantly different. This graph indicates that there is no change in DA from baseline at the elastic level (the bar); however, there is a change at the failure (F) level (* versus **), further changes at the post-failure (PF) level (** versus ***) and further changes at the unload (U) level (*** versus ****) but that changes in DA are not fully recovered upon unloading (**** versus *). The error bars represent within-subject standard deviations which take into account the repeated nature of the measurements [18]. (b) Replot with error bars representing standard deviations without taking into account the repeated nature of the measurements (no test of significance is performed).

Fig. 3 (a) Changes in average trabecular thickness (Tb.Th.Av) with different levels of strain representing baseline, elastic, postyield, postfailure, and unloaded states. Changes in the asterisks indicate significant changes in Tb.Th.Av as strain levels change (from left to right). The levels of strain connected with a bar (–) are not significantly different. This graph indicates that there is no change in Tb.Th.Av from baseline at the elastic and failure levels (the bar), but there is a change at the postfailure (PF) level (* versus **) after which no further changes are observed (the second bar). The error bars represent within-subject standard deviations, which take into account the repeated nature of the measurements [18]. (b) Replot with error bars representing standard deviations without taking into account the repeated nature of the measurements (no test of significance is performed).

microstructural changes and strain levels exist on a continuous scale and may be demonstrable with a larger sample size.

The finding that parameters of trabecular microstructure can change due to mechanical deformation indicates that measurement of bone microstructure without consideration of pre-existing deformation could introduce inaccuracy in determining bone quality based on trabecular microstructure. For example, higher levels of Tb.N.SD have been associated with increased levels of trabecular stress variability within cancellous bone [6]. Large variability of trabecular stress distributions would indicate the presence of stress risers in the structure. The current results indicate that the trabecular structure becomes more uniform in Tb.N after damaging levels of apparent strains. Without consideration to this history, a microstructural analysis would suggest stiffer and stronger cancellous bone based on Tb.N distributions. On the other hand, high variability of trabecular thickness values within a bone has been associated with lower stiffness of the cancellous structure

[6,19]. Although the increase in the SD of Tb.Th after damaging levels of deformation is consistent with an expected reduction in bone stiffness due to damage, it is unlikely that there is a practically meaningful change in the true trabecular thickness due to loading alone. However, changes in plane-to-plane variation of Tb.Th may be observed in correlation with buckling and displacement of trabeculae from one plane to another [8] (also Fig. 1) and provide a useful index of strain in cancellous bone.

Despite the large intersample (Figs. 2–5) variability, deformation-caused changes in the cancellous microstructure within a sample could be detected. This observation suggests that methods measuring microstructural heterogeneity to monitor progression of cancellous bone damage can be conceived. Due to progress in imaging, it is possible to quantify cancellous bone texture using morphometric and/or statistical properties of the image. For instance, fractal measurements from radiographs or tomosynthesis images correlate with intra-individual heterogeneity

Fig. 4 (a) Changes in the standard deviation of trabecular thickness (Tb.Th.SD) with different levels of strain representing baseline, elastic, postyield, postfailure, and unloaded states. Changes in the asterisks indicate significant changes in Tb.Th.SD as strain levels change (from left to right). The levels of strain connected with a bar (–) are not significantly different. This graph indicates that there is no change in Tb.Th.SD from baseline at the elastic and failure levels (the bar), but there is a change at the postfailure (PF) level (* versus **) after which no further changes are observed (the second bar). The error bars represent within-subject standard deviations which take into account the repeated nature of the measurements [18]. **(b)** Replot with error bars representing standard deviations without taking into account the repeated nature of the measurements (no test of significance is performed).

Fig. 5 (a) Changes in the standard deviation of trabecular number (Tb.N.SD) with different levels of strain representing baseline, elastic, postyield, postfailure, and unloaded states. Changes in the asterisks indicate significant changes in Tb.N.SD as strain levels change (from left to right). The levels of strain connected with a bar (–) are not significantly different. This graph indicates that there is no change in Tb.N.SD from baseline at the elastic and failure levels (the bar) but there is a change at the post-failure (PF) level (* versus **) after which no further changes are observed (the second bar). The error bars represent within-subject standard deviations which take into account the repeated nature of the measurements [18]. **(b)** Replot with error bars representing standard deviations without taking into account the repeated nature of the measurements (no test of significance is performed).

of cancellous bone [20]. The sensitivity of such measurements under physiological and clinical conditions remains to be determined.

A significant limitation is that the extent to which remodeling, independent from or in relationship with mechanical loading, would affect estimation of strain history from changes in bone microstructure is currently unknown. While short term assessment of mechanical loading (such as pre, during, and postmechanical activity) may be possible from microstructural changes, it may be more challenging to extend this concept over to time periods where remodeling can be significant. Future work should address the time window in which useful information on mechanical loading can be obtained from longitudinal analyses of bone microstructure.

Another limitation is that a mixed tibial and femoral group of specimens without demographic data was used. It is not uncommon to use specimens from various anatomic sites or from different species to represent a range of cancellous bone structures in studies such as this one [21]. The mean and variance of the baseline BV/TV values in the current group of specimens are consistent

with published values from the proximal tibia and the distal femur for a wide range of donor age, various anatomic sites within the respective bones, and males and females [22–27]. Therefore it appears that the current sample is sufficiently representative of the human tibial and femoral cancellous bone population, although the effect of anatomic site, age, or gender cannot be inferred from the current results.

In conclusion, we identified potentially important microstructural parameters sensitive to mechanical deformation. Consideration of these parameters in new imaging approaches and in interpretation of bone quality may enhance our understanding of the damage mechanisms in cancellous bone and our ability to predict fracture risk.

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