Bone Strain Distribution on (Re)modeling Transcriptome-Level Responses to Two Different Loading Modes

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INTRODUCTION: Bone (re)modeling is a cellular activity to reconstruct bone microstructure in response to mechanical loading, resulting in the combination of old bone resorption by osteoclasts (Ocs) and new bone formation by osteoblasts (Obs). Osteocytes (Ots) sense and transduce mechanical signals through several signaling pathways, to mediate Ocs and Obs activities. The complete picture of these biological pathways is not well discovered and can be accumulated from transcriptomic information. Previous studies showed that load-induced strain distribution influences the osteogenic response of the skeleton. Among various *in vivo* loading models, bone appears to be more sensitive to non-physiological strain distribution compared to strains elicited during normal locomotion.[1,2] However, given the same site-specific strain magnitudes, the effect of tissue-level strain distributions on the transcriptomic-level activities of Ots remains unclear. This study aims to reveal the effects of differences in strain distribution on the gene expression-level of the (re)modeling response. We hypothesize that Ots experienced by non-physiological strain distribution will express stronger level of osteogenic genes than physiological strain distribution.
METHODS: Four dead female 16wk old C57Bl/6J mice with strain gauges attached to the antero-medial surface of tibiae were subjected to the tibial axial compressive[4] (AC, physiological) and medial-lateral cantilever-type[1] (ML, non-physiological) loading models (fig. 1). Finite element analysis (FEA) was performed and calibrated with experimental strain to evaluate the strain at the posterior-lateral (PL) region, 37% of the tibial length from the proximal end, where Ots are exposed to opposite strain directions in AC (compressive strain) and ML (tensile strain) models. The peak load levels for AC and ML models are determined from the FEA to induce ±1800 με at the PL region to activate Ot gene expression[3]. Our next step is to acquire female 16wk old C57Bl/6J mice subjected to either AC or ML loading for three days (n=4/group) before euthanasia. Non-loaded contralateral limbs will be used as controls. The tibial cross-sectional cortical bone will be collected, decalcified, and subjected to spatial transcriptomics analysis (GeoMX, Nanostring) to reveal the strain distribution effects on different Ot gene expressions and potential causes for bone (re)modeling. This study is approved by PACUC 0523002393.
RESULTS: Based on the FEA, the AC model showed physiological strain distribution throughout the bone with peak compressive and tensile strains at the PL site and antero-medial surface, respectively (fig. 2A). In contrast, the ML model confirmed non-physiological strain distribution with peak compressive and tensile strains at the posterior-medial and PL regions, respectively (fig. 2B). The gauge sites displayed strong correlation with the experimental strains in both AC and ML models with R^2 of 0.87 and 0.97, respectively. The AC model requires a relatively higher load (-4.27 N) than the ML model (-0.72 N) to generate the absolute strain magnitude of 1800 με in the PL region (Fig 3).
DISCUSSION: The FEA can successfully confirm distinct strain distributions and calculate the effective peak load levels for both AC and ML models. Since tibia has been modeled to take mechanical load axially during physiological locomotion, it has higher stiffness against loads along the bone axis in the AC model than the ML direction (Fig. 3). The lower peak load required in the ML model non-physiological model can reduce tissue damage and may be more effective to induce osteogenesis in clinical application. The *in vivo* experiment to obtain transcriptomic information is currently in progress.
SIGNIFICANCE/CLINICAL RELEVANCE: The outcome of this study is essential for understanding mechanosensation and mechanotransduction at the osteocytes, which could contribute to better osteoporosis clinical intervention through mechanical stimulation or drug development.

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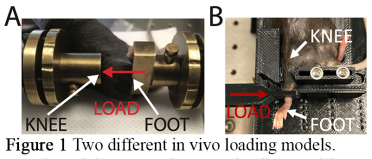

Figure 1 Two different in vivo loading models.
(A) The axial compressive (AC) loading model.
(B) The medial-lateral (ML) loading model.

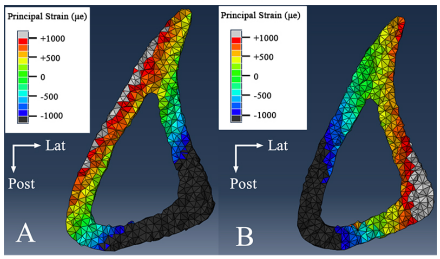

Figure 2 Strain distribution in cross-sectional region at 37% from the proximal end from (A) AC model and (B) ML model.

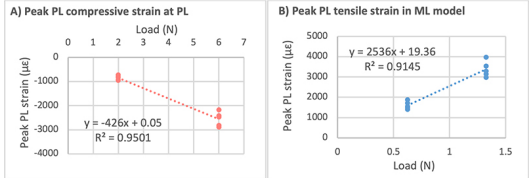

Figure 3 Relationship between load (N) and peak strain at PL region (με) in AC model (A) and ML model (B).