Mechanical Loading Increases Osteocyte Uptake and Retention of Fluorescent Nanoparticles *In Vivo*

Melia D. Matthews[1], Nada Naguib[1], Ulrich B. Wiesner[1], Karl J. Lewis[1]
[1]Cornell University, Ithaca, NY
mdm345@cornell.edu

Disclosures: Melia D. Matthews (N), Nada Naguib (N), Ulrich B. Wiesner (N), Karl J. Lewis (N)

INTRODUCTION: Endocytosis and membrane trafficking are fundamental components of cellular mechanotransduction[1]. Recycling of mechanosensitive transmembrane proteins like integrins provides spatiotemporal regulation of cell adhesion and sensitivity to mechanical stimulation[2]. Osteocytes, the resident mechanosensing cells of bone, utilize integrins and other membrane-bound proteins to sense and respond to fluid flow within the lacuno-canalicular system[3-4]. Their responses to mechanical stimulation include calcium signaling, cytoskeletal reorganization, and fluctuation in their secretory phenotype[5-6]; together, these downstream signaling reactions suggest reciprocal cross-talk between mechanical stimulation and membrane trafficking pathways. However, whether mechanical stimulation itself impacts osteocyte endocytic dynamics has not been studied. In previous work, we established the use of non-targeted and integrin-targeted fluorescent nanoparticles as tools to visualize and study endocytic dynamics in osteocytes in a living mouse long bone[7]. Here, we interrogate how physiological loading modulates osteocyte internalization and clearance of fluorescent nanoparticles within metatarsal cortical bone *in vivo*.

METHODS: Skeletally mature (16-18 wk) male and female wild-type mice on a C57BL/6 background were used for the experiments (n=3-5/group). Untargeted PEG-C'Dots or integrin-targeted RGD-C'Dots (15uL, 10uM) were injected subcutaneously above the third metatarsal (MT3) and incubated for 45 min. Mice were allowed free cage activity during incubation. The MT3 was then surgically isolated, stabilized in a 3-point bending configuration, and submerged in DPBS for imaging. C'Dot Cy5 fluorescent signal was excited at 1090nm using 2-photon intravital microscopy. In loaded groups, the MT3 was subjected to a physiological strain of 1000με at a frequency of 1Hz for 60 seconds every 15 min, starting before the first imaging session. Z-stacks were collected from the same 35μm volume of cortical bone every 15 min over 2.5 hrs, between loading bouts. Osteocyte uptake and clearance of nanoparticles was quantified in ImageJ (NIH). Two-way ANOVAs with multiple comparisons (Fig. 1 A-D) were used to compare non-loaded and loaded groups (GraphPad Prism, **** = $p<0.0001$, Error = SEM). Group-wide statistical comparisons were plotted. Representative z-projections at the 60 min timepoint were taken (Fig. 2 A-H, scale bars =50μm). All experiments were approved by the Cornell IACUC.

RESULTS: Mechanical loading increased osteocyte nanoparticle uptake and retention in all groups compared to non-loaded controls (Fig. 1 A-D). Male mice showed a larger increase in retention between loaded and non-loaded groups (Fig. 1 A,C). However, loaded female mice still had higher overall uptake and retention compared to loaded males (Fig. 1 B,D), matching relative clearance patterns of the non-loaded data. Representative images an hour into the experiment highlight the substantial change in nanoparticle retention between loaded and non-loaded groups (Fig. 2 E-H).

DISCUSSION: Repeated mechanical loading of the MT3 directly increases nanoparticle uptake in osteocytes. This effect is consistent across both PEG- and RGD-functionalized nanoparticles and in both sexes, demonstrating a conserved response to physiological levels of mechanical strain. Prior studies have shown that catabolic conditions with decreased mechanosensitivity *in vivo* (i.e., OVX) reduce membrane protein availability along osteocyte dendrites[8]. Our findings extend this work by revealing mechanical regulation of uptake and clearance pathways, supporting a model where loading and membrane trafficking are linked in a feedback loop. This interaction between loading and membrane dynamics may be a plausible redistribution mechanism for mechanosome proteins under disruptive conditions, particularly given that other canonical mechanisms like internal cytoskeletal trafficking are unlikely due to space constraints in the osteocyte dendrites[9]. This new understanding could be leveraged to improve osteocyte-targeted drug delivery or modulate cellular sensitivity to mechanical stimulation.

SIGNIFICANCE: Our findings establish mechanical stimulation as an active regulator of osteocyte internalization dynamics, linking mechanotransduction to membrane activity within bone. Recognizing this connection increases our understanding of osteocyte signaling and offers loading as a potential strategy to improve the efficacy of bone targeted therapies.

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IMAGES AND TABLES:

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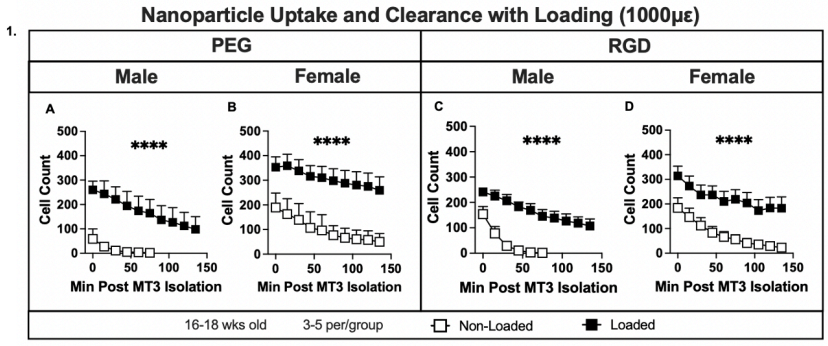

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