MECHANICAL LOADING IS REQUIRED TO MAINTAIN OSTEOCYTE HOMEOSTASIS

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INTRODUCTION

Bone rapidly transduces the loss of mechanical loading (i.e., disuse) into a cellular response resulting in diminished bone mass. While cytokine pathways responsible for controlling osteoblastic and osteoclastic functions are increasingly well defined (1,2), the pathways by which physical stimuli (or their absence) are transduced into biochemical signals within the tissue remain unknown. In bone, the osteocyte is an ideal candidate to initiate cellular responses leading to tissue adaptation. Osteocytes are ubiquitous throughout the tissue (3), form a network capable of cell to cell communication with adjacent osteocytes and lining cells (4), and demonstrate rapid biochemical responses to altered physical stimuli (5). At the level of the osteocyte, mechanical loading serves to locally enhance nutrient exchange and diffusion (6,7). This process may be particularly important to osteocyte function as these cells typically reside at locations far distant from blood vessels. As osteocytic metabolic function appears to rely upon diffusion, we hypothesized that a loss of bone loading would rapidly induce osteocyte hypoxia. To address this hypothesis, we developed the ability to detect osteocyte hypoxia in an *in vivo* model of bone adaptation.

METHODS

Seven adult male turkeys were included in the study. The left ulna diaphysis of four turkeys was unloaded for 1 d (n=3) or 3 d (n=1) via the isolated avian ulna procedure (8). Each animal was injected 6 hr prior to sacrifice with 40 mg/kg of pimonidazole. This compound is reduced via the nitroreductase pathway by any cell that is hypoxic. Hypoxic cells are then detected using a monoclonal antibody raised against the reduced form of the pimonidazole (9). The remaining three turkeys served as negative controls (no surgery, no pimonidazole). Upon sacrifice, 5 mm thick mid-diaphysis cross-sections were removed from the left and right ulnae of each turkey. The sections were fixed in 10% buffered formalin, decalcified in EDTA, paraffin embedded, sectioned at 6 μm, and mounted on charged slides. To detect osteocyte hypoxia, slides were deparaffinized, pronase digested, blocked with 10% horse serum, and incubated with a mouse anti-pimonidazole antibody followed by an anti-mouse FITC secondary antibody. Imaging was performed using a scanning confocal microscope. Osteocyte hypoxia was examined at four repeatable locations on each cross-section corresponding to the sites of peak tension and compression and the neutral axis induced by wing flap. At each site, a 60x objective was used to obtain 4 adjacent fluorescent and corresponding bright field images (each containing approximately 40 osteocytes). The images were overlaid and the total number of osteocyte lacunae and FITC positive osteocyte lacunae were counted. Identical laser intensity and gains were used for all imaging. Imaging and counting were performed with the operator blinded to the identity of the slides. Statistical comparison between the unloaded bones and intact control bones was performed with a paired t-test.

RESULTS

A mean (± S.E.) 0.9 ± 1.4% of osteocytes demonstrated FITC positive staining in the negative control bones. Intact contralateral control bones from the animals undergoing disuse demonstrated a similarly low level of FITC positive osteocytes (1.4 ± 0.5%). Brief unloading of the bone precipitated a significant increase in osteocyte hypoxia (8.1 ± 2.1%; data pooled; p=0.02; *Fig 1.*). Each animal demonstrated elevated hypoxia in the unloaded ulna compared to the intact contralateral ulna, with a 14.1 (± 4.0) fold increase in FITC positive osteocytes precipitated by disuse. Hypoxia was equally evident at locations normally accustomed to high magnitude strains (7.8 ± 2.2%) and low magnitude (8.4 ± 2.4%) strains.

DISCUSSION

From these data we conclude that unloading the skeleton, even briefly, rapidly induces osteocyte hypoxia. The extremely rapid onset of hypoxia when bone is unloaded emphasizes the critical role that mechanical stimuli play in maintaining osteocyte homeostasis. The avian ulna model is particularly advantageous in this experiment as the bone is completely deprived of mechanical loading via its articulating surfaces, but the model requires an invasive surgery that holds the potential to confound interpretation of these data. However, the brief duration of this experiment and the observation that mid-diaphyseal bone blood flow (and thus passive oxygen exchange) is not altered by the surgical procedure combine to minimize this risk (10). Interestingly, osteocyte hypoxia was induced equivalently in portions of the bone normally exposed to high magnitude tension and compression as compared to locations normally exposed to low magnitude strains (i.e., the neutral axis of bending). These data suggest that osteocytes acclimatize to a range of local nutrient and oxygen conditions, but that diminishment of these levels, regardless of their initial state, evokes a rapid cellular response. In other cells, hypoxia is known to elicit a variety of responses such as calcium fluctuation, cytoskeletal reorganization, and apoptosis. We therefore hypothesize that this physiologic event plays a vital role in initiating bone cell activity responsible for disuse induced osteopenia.

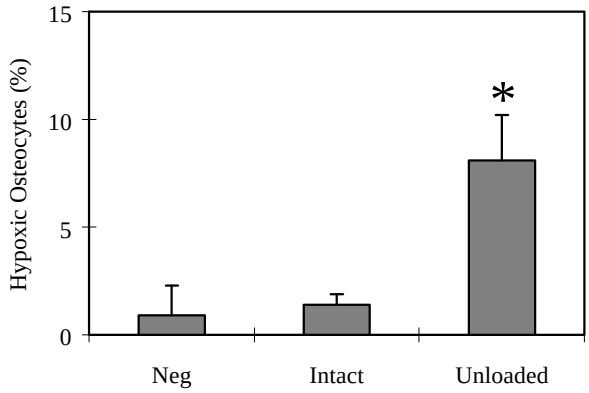

Fig 1. Mean (+S.E.) percentage of osteocytes staining positive for hypoxia in response to brief skeletal unloading. Negative control bones (intact bones in animals not receiving the hypoxia marker pimonidazole; Neg) and intact contralateral control bones (intact contralateral bones in animals exposed to disuse and receiving pimonidazole; Intact) both demonstrated minimal positive staining (@ 1%). Bones exposed to disuse (Unloaded) demonstrated significantly increased osteocyte hypoxia (n=4; asterisk; p=0.02).

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ACKNOWLEDGMENTS

Supported, in part, by the University of Cincinnati Research Council and the University of Cincinnati Orthopaedic Research and Education Foundation.

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