A Brief Review of Bone Adaptation to Unloading

Ping Zhang, Kazunori Hamamura, and Hiroki Yokota*

Department of Biomedical Engineering/Department of Anatomy and Cell Biology, Indiana University–Purdue University Indianapolis, Indianapolis, IN 46202, USA.

Weight-bearing bone is constantly adapting its structure and function to mechanical environments. Loading through routine exercises stimulates bone formation and prevents bone loss, but unloading through bed rest and cast immobilization as well as exposure to weightlessness during spaceflight reduces its mass and strength. In order to elucidate the mechanism underlying unloading-driven bone adaptation, ground-based *in vitro* and *in vivo* analyses have been conducted using rotating cell culturing and hindlimb suspension. Focusing on gene expression studies in osteoblasts and hindlimb suspension studies, this minireview introduces our recent understanding on bone homeostasis under weightlessness in space. Most of the existing data indicate that unloading has the opposite effects to loading through common signaling pathways. However, a question remains as to whether any pathway unique to unloading (and not to loading) may exist.

Key words: weightlessness, unloading, osteocytes

Introduction

Spaceflight challenges molecular and cellular machineries that are at a homeostatic equilibrium under Earth's normal gravity. Unloading-driven physiological alterations during spaceflight often result in a short-term and long-term impaired function in many organs including the cardiovascular system, the immune system, the nervous system, the urinary system, and the musculoskeletal system (*1*, *2*). Bone is constantly remodeled under normal gravity on ground, and this remodeling process (bone forming activities by osteoblasts and bone degradation by osteoclasts) is sensitive to alterations in mechanical environments (*3–5*). Unloading disturbs the delicate balance of homeostasis of weight-bearing bones that is fine tuned under normal gravity (*6–14*). In fact, examinations of pre- and post-flight bone mass of astronauts have revealed significant reduction in bone mass with the highest rate of bone loss in the femur (*15*).

In order to evaluate unloading effects at a molecular level, this minireview first focuses on *in vitro* microarray studies using cultured osteoblasts. Since spaceflight opportunities for basic life sciences are limited, ground-based pseudo simulations of weightlessness have been exploited. Two frequently used simulators are a rotating wall vessel bioreactor and a random positioning machine. Cells in the rotating bioreactor are maintained in a nearly free-fall state (*16*), while the random positioning machine constantly changes orientation of the cells at a variable speed. Either device does not achieve weightlessness in spaceflight, but through rotation the cells are cultured not to receive loads in a fixed direction. In the second part of this minireview, *in vivo* wild-type and transgenic mouse studies using hindlimb suspension are highlighted, where animals are suspended by their tails without touching their hindlimbs on ground. The hindlimbs do receive gravitational force because of their mass, but suspension can remove a major portion of loading because of no reaction force from the cage floor.

In Vitro Studies

Unloading-driven mRNA expression in osteoblasts

Homeostasis of bone remodeling involves three types of bone cells: osteoblasts, osteoclasts, and osteocytes (Figure 1). Osteoblasts are bone-forming cells derived from mesenchymal stem cells, while osteoclasts are multi-nucleated bone-degrading cells differentiated from hematopoietic progenitor cells. Osteocytes are terminally differentiated from osteoblasts and exist

***Corresponding author.**
E-mail: hyokota@iupui.edu

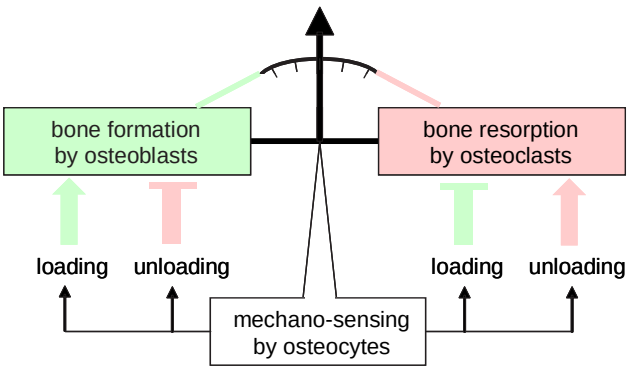

Fig. 1 Interactions of osteoblasts, osteoclasts, and osteocytes in response to unloading and loading.

embeddedly in caves called lacunae (*17*). To date, *in vitro* microarray data under unloading are available only for preosteoblast cells (*16*, *18*).

Pardo *et al* (*18*) showed that the mRNA expression of 140 genes in 2T3 preosteoblasts was significantly altered during 3-day weightlessness simulated by the random positioning machine. For instance, the mRNA level of alkaline phosphatase (marker for bone formation), runt-related transcription factor 2 (Runx2), and parathyroid hormone receptor 1 (PTHR1) was down-regulated by 5, 2, and 5 fold, respectively. Loading-driven up-regulation of alkaline phosphatase and Runx2 has been reported in cultured osteoblasts (*16*, *19*). The parathyroid hormone receptor is considered as one of the key molecular targets in mechanotransduction (*20*), and constitutively active parathyroid hormone receptor signaling in osteoblastic lineage cells has been shown to suppress mechanical unloading-induced bone resorption (*21*). Taken together, the microarray results suggest molecular interactions between osteoblasts and osteoclasts at least in part through regulation of PTHR1 (Figure 1).

Comparison between unloading and loading

Patel *et al* (*16*) conducted a pair of microarray experiments using the same 2T3 cells with the rotating wall vessel bioreactor as well as the random positioning machine. Those results were compared with microarray data derived from mechanically loaded mouse tibiae (*22*). Three genes, which were down-regulated in the two *in vitro* unloading experiments and up-regulated in the *in vivo* loading experiment, were osteoglycin, procollagen C-proteinase enhancer protein, and platelet-derived growth factor receptor-like protein. Signaling pathways for regulating those three genes as well as their specific roles in unloading and loading are yet to be identified. Computational tools such as Gene Set Enrichment Analysis (GSEA) (*23*) and Ingenuity Pathways Analysis (IPA) (*24*) might be useful to predict molecular networks responsible for unloading/loading-linked responses.

In Vivo Studies

Role of a sympathetic nervous system

An increasing number of studies suggest that nerve-derived signals play an important role in the regulation of bone remodeling (*25*), and mouse studies indicate involvement of a sympathetic nervous system in the responses to unloading (*10*). Neuropeptides and receptors/transporters of adrenergic, glutaminergic, serotoninergic, dopaminergic, and sensory nature have been described in osteoblasts *in vitro* (*25*). Particularly, an inhibitory role of leptin in bone formation has been well documented (*26*, *27*). Leptin is a small polypeptide hormone primarily secreted by the adipocytes and it binds to a specific receptor located in the hypothalamus (*28*). Leptin's antiosteogenic function is mediated by the sympathetic nervous system through β2-adrenergic receptor (Adrb2), which is the only adrenergic receptor known to be expressed in osteoblasts (*29*).

Using C57BL/6J mice, Kondo *et al* (*10*) employed hindlimb suspension and evaluated the role of the sympathetic nervous system in unloading with propranolol (blocker of β-adrenergic receptor) and isoproterenol (stimulator of β-adrenergic receptor as an agonist). First, administration of propranolol suppressed the unloading-induced reduction in bone mass. Second, isoproterenol reduced bone mass in mice under normal activities but unloading did not significantly

alter bone mass. Those observations support the notion that the sympathetic nervous system mediates unloading-induced bone loss, although its role in loading-driven bone formation is yet to be investigated.

Osteopontin and *cas*-interacting zinc finger protein

Two transgenic mouse studies support that osteopontin (OPN) and *cas*-interacting zinc finger protein (CIZ) mediate unloading-driven bone resorption. First, unloading of $OPN^{-/-}$ mice does not increase the number of osteoclasts, which are elevated by unloading in wild-type mice (*13*). Furthermore, no reduction in osteoblastic bone formation is evident in $OPN^{-/-}$ mice. OPN is a noncollagenous protein abundant in the bone matrix. It is believed to facilitate osteoclast attachment to the mineralized extracellular matrix, but its function is not clearly understood (*30*). Second, Hino *et al* (*31*) have reported that CIZ-deficient mice suppress unloading-driven reduction of bone mass. CIZ is localized at adhesion plaque with other adhesion-related molecules in the cells, and in response to mechanical stimulation it is translocated into nuclei for regulation of various genes including matrix metalloproteinases (*32*, *33*). It is not clear whether either OPN or CIZ is involved in loading-driven acceleration of bone formation or suppression of bone resorption.

Osteocytes as an unloading sensor

In order to examine the role of osteocytes in unloading, Tatsumi *et al* (*34*) developed elegant transgenic mice in which osteocytes were specifically designed to express a diphtheria toxin receptor. After injection of diphtheria toxin followed by ablation of 70%–80% osteocytes, hindlimb suspension was conducted. Note that unloading has been known to induce osteocyte apoptosis and recruitment of osteoclasts (*8*). The osteocyte ablation experiment has shown that osteocyte-deficient mice are resistant to unloading-induced bone loss. In reloading experiments, however, those osteocyte-deficient mice gained an amount of bone similar to control mice. Those results indicate that osteocytes are indispensable for unloading-induced bone loss, while bone recovery by reloading may not depend on osteocytes. Namely, the mechanisms responsible for maintaining bone mass with normal loading might not be the same as those for recovering bone mass after unloading. It will be important to determine whether the remaining 20%–30% osteocytes are involved in or responsible for the observed recovery in bone mass (*35*).

Conclusion

Bone can be exposed to a variety of stresses including hypoxia, ischemia, infection, trauma, loading, and unloading. Many lines of evidence support that the effects of unloading are the opposite to those of loading, but recent *in vivo* experiments request re-examination of this simplistic view. Bone adaptation to unloading is regulated by coordinated molecular machineries of osteoblasts, osteoclasts, and osteocytes in the presence of various signals (*in situ* mechanical, neural, and hormonal). Comparison of unloading effects to loading effects in wild-type and transgenic mice using microarray data would be useful to identify molecular pathways common and unique to unloading and/or loading. Since unloading is a cause of disuse osteoporosis in bedridden patients, studies on bone adaptation in spaceflight should contribute to the development of diagnostics and therapeutics for patients with bone diseases as well as astronauts.

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