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Original Article

Hylonome

Skeletal adaptation to mechanical stimuli in the absence of formation or resorption of bone

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Abstract

Too often, unique loading environments fail to alter bone mass and morphology, calling to question the validity of Wolff's Law; the skeleton's plasticity to mechanical signals[1]. We propose that bone can accommodate new loading environments *without* the need to form or resorb tissue, and that a critical aspect of bone tissue's ability to adapt to mechanical stimuli is first achieved via the plasticity of the osteocyte. We suggest that the osteocyte is capable of "normalizing" its local mechanical environment by modulating its cytoskeletal architecture, attachment to the matrix, configuration of the periosteocytic space, and communication channels to surrounding cells. We believe that through this local adaptive mechanism the osteocyte can accommodate the majority of changes in the mechanical milieu *without* altering the tissue architecture. It is only when bone tissue is subject to more severe (albeit rare) increases or decreases in the functional environment, the osteocyte participates in the formation and/or resorption of bone by coordinating site-specific recruitment of osteoblasts and/or osteoclasts. *In vivo* models of bone adaptation, combined with *in situ* reverse transcriptase-PCR, semi-quantitative RT-PCR, Northern analysis, immuno-cytochemistry and histomorphometry, can demonstrate how distinct mechanical stimuli influence the osteocyte's cytoskeletal and lacunar architecture, coupling (and uncoupling) of the osteocyte to the matrix and neighboring cells, and the osteocyte's participation in the recruitment and differentiation of osteoblasts and osteoclasts. Thus, the osteocyte controls three strategies to modulate its local and global environment in response to three distinct functional stimuli: 1) exogenous mechanical stimuli which are distinct from normal but sufficient to maintain bone mass, 2) mechanical stimuli which are osteogenic, and 3) disuse. If it is true that the resident cell population is capable of accommodating subtle changes in the functional milieu before modification of tissue morphology is deemed necessary, a novel strategy for the development of prophylaxes for osteopenia, osseointegration and fracture healing may become apparent.

Keywords: Osteocyte, Bone, Skeleton, Remodeling, Anabolic, Catabolic, Osteoblast, Osteoclast, Adaptation

It is only when bone tissue is subject to more severe (*albeit* rare) increases or decreases in the functional environment, the osteocyte participates in the formation and/or resorption of bone by coordinating site-specific recruitment of osteoblasts and/or osteoclasts by as of yet unidentified factor(s).

The hypothesis that bone mass and morphology are orchestrated by the activity of the osteocyte is indirectly supported by the attenuation of Wolff's Law by age[2], indicating that some responsive element of the cells is suppressed by this systemic distress and thus the tissue's ability to respond to adaptive stimuli is compromised. With the goal of identifying aspects of cellular change which may parallel the aging process and thus contribute to this dysfunction, we modified reverse transcriptase polymerase chain reaction (RT-PCR) such that it can be used *in situ* in cortical bone[3]. Beginning with one-year-old, skeletally mature turkeys, IS-RT-PCR has demonstrated that the number of osteocytes involved in mRNA expression of many matrix proteins (e.g., type I collagen, osteopontin), cell adhesion molecules (e.g., integrin β3), and gap junction proteins (e.g., Cx43) are upregulated by mechanical stimuli within three days, far preceding evidence of new bone formation[4,5].

By the time bone is laid down (i.e., tissue level adaptation), the percentage of osteocytes involved in mRNA expression for these proteins has lowered, suggesting the osteocytes have "normalized" to the new adaptive stimulus (Fig. 1). Importantly, loading caused no change in the number of osteocytes expressing matrix metalloproteinase-1, demonstrating that

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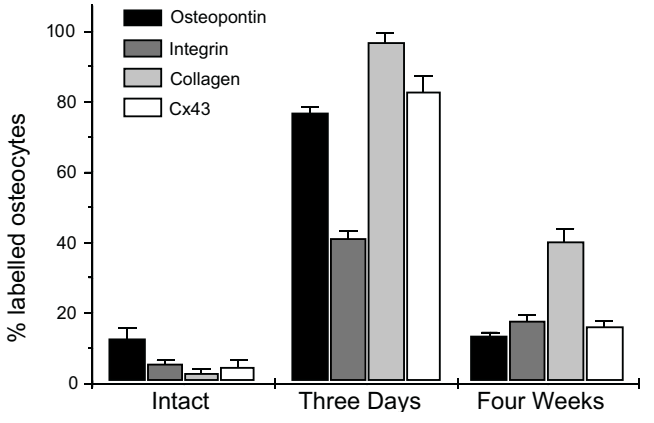

Figure 1. In the intact, adult ulna, relatively few osteocytes are involved in the expression of osteopontin, integrin β3, collagen type I or connexin 43. The percent of active, producing osteocytes rises markedly within three days of subjecting the ulna to a strongly osteogenic mechanical stimulus. By four weeks of loading, a time period in which new bone has been formed on the periosteal and endosteal surfaces, mRNA activity falls back towards the levels seen in the intact bone. It is proposed that this reflects osteocyte plasticity, in an effort to regulate the mechanical stimulus to which the cell is exposed.

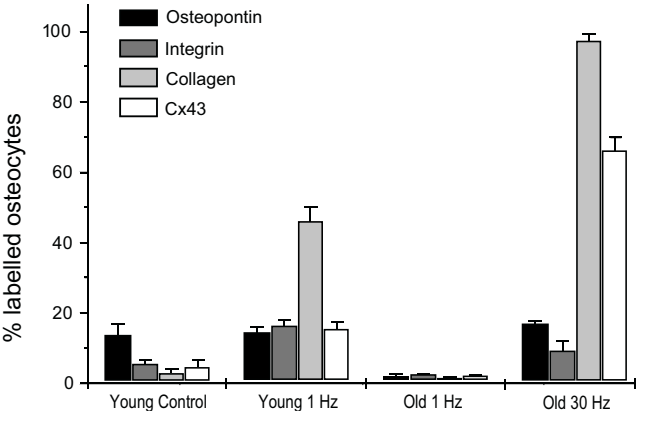

Figure 2. Effect of age and loading regimen on osteopontin, integrin β3, collagen type I and connexin 43 mRNA expression. Shown are percent of total cells (815±41 osteocytes/mm^2) labeled for mRNA expression. N=3 for each load group, n=6 for the intact controls. Note the sharp decrease in the number of osteocytes involved in mRNA expression in the loaded ulnae of the old animals as compared to the young. While the high magnitude mechanical stimulus fails to alter gene expression in osteocytes in the old bone, increasing the frequency of the stimulus succeeds in stimulating bone formation and gene expression.

upregulation of gene expression is selective in response to the loading environment. From these data, it appears that cell/matrix interactions are intimately involved in the osteocyte's ability to perceive any mechanically mediated regulatory information. Functional load causes matrix strain, and a well-coupled osteocyte will perceive this deformation and thus participate in an appropriate response. We believe these data are indicative of the osteocyte "normalizing" its perception of the mechanical environment, perhaps towards an "optimal strain environment". While we do not know what the optimal environment may be, it is clear that changes in the cytoskeletal, coupling to the matrix, and periosteocytic boundaries will ultimately conspire to alter the mechanical signal to which the cell is exposed.

Could this cell attachment hypothesis partly explain old bone's inability to respond? While a mechanical regimen of 100 cpd @ 1Hz to 3000με was potently osteogenic in the young animals, four weeks of this stimulus failed to stimulate new bone formation in the older tissue and did not alter the relatively low number of osteocytes expressing mRNA for any of these four proteins (Fig. 2). Interestingly, reducing the mechanical signal to 500με, but increasing the frequency to 30 Hz, stimulated substantial new bone formation in these older animals (+14% over the animals' intact control). This signal also increased the number of osteocytes expressing integrin ß3 mRNA from 0.8% in intact ulnae to 7.4% in the loaded bone, to the high Hz ulnae osteocytes expressing osteopontin mRNA increased from 2.1% in intact ulnae to 13.9% in loaded bone, and osteocytes expressing collagen type I expressing osteocytes increased from 1.2% in the intact and 1 Hz bone to 92.4%, a 77-fold increase. Finally, osteocytes involved in Connexin 43 mRNA expression rose from 1% in the intact ulnae to 53% in the 30 Hz condition. Further, these data suggest that some aspect of the mechanical domain other than strain magnitude (500με at 30 Hz was osteogenic, 3000με at 1 Hz was not) is what makes the signal osteogenic. A reduction in the amount of these proteins will diminish the efficiency of transmitting the regulatory signal to the cell.

As described above, *in situ* RT-PCR shows that mechanical stimuli which are osteogenic will upregulate gene expression

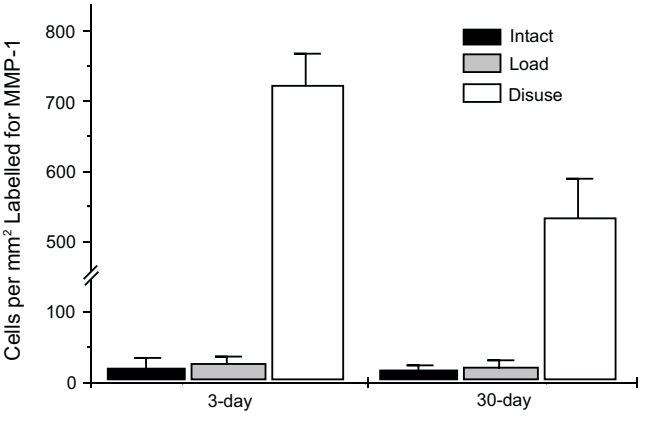

Figure 3. Loading effects on osteocytes expressing MMP-1 mRNA. The number of positive cells for MMP-1 mRNA were converted to percentage of total cells/mm^2. Note that the number of osteocytes involved in MMP-1 transcription do not increase with mechanical load (in contrast to proteins involved in cell attachment or coupling; see above), yet disuse causes significant increase in MMP-1 expression by three day (in contrast to osteopontin, which did not change). The percent of cells involved in MMP expression remains elevated at 30 days, but is significantly lower ($p<0.05$).

for several proteins involved in the formation of bone. Importantly, mechanical signals which *fail* to stimulate new bone formation also fail to upregulate gene expression for these proteins. These reports emphasize that the osteocytes are selectively sensitive to anabolic stimuli, and are capable of responding via a spectrum of pathways which correlate with the formation of bone, even though bone is actually formed remotely, on the periosteal/endosteal surfaces. Either this transcriptional activity occurs as a remnant activity inherited from the cell's pre-differentiated life as an osteoblast, or the activity is directly involved in the adaptive process, even though it is occurring within the cortex. Our governing hypothesis is based on the latter, proposing that the plasticity (e.g., increasing or decreasing connections) between the osteocyte and the matrix represents a first tier response of the cell to deal with subtle changes in mechanical stimuli. The cell is "normalizing" its perceived strain environment by altering the means by which mechanical signals are transmitted. If normalizing the physical environment is truly a goal of the osteocyte, than a *reduction* in the mechanical stimuli (e.g., weightlessness, cast immobilization, bedrest) should stimulate the osteocyte to secret factors responsible for the deterioration of its physical environment. To test the osteolytic side of the osteocyte plasticity hypothesis, young (ly) skeletally mature turkeys were separated into groups of 3 or 30-day stimulus periods. In each animal, the left ulna was functionally isolated and subsequently subject to either 600cpd @ 1Hz to 3000με or disuse[6]. The right ulna remained intact and served as an intra-animal control. Using IS-RT-PCR, less than 2% of the osteocytes examined from the intact control ulnae expressed MMP-1 mRNA (Fig. 3). The low percentage of MMP-1 mRNA, is similar to that percentage observed in ulnae subject to osteogenic mechanical stimuli, even though a host of other genes are turned on during this period. When the ulnae were subject to either 3 or 30 days of disuse, MMP-1 mRNA was strongly evident in a high percentage of osteocytes (89 ± 5% & 73 ± 8%, respectively). In contrast, osteopontin expression was not altered by disuse, again demonstrating the upregulation of gene activity to be specific to the load regimen (data not shown). These data suggest that a first line (within 3 days) strategy of bone to accommodate disuse is to upregulate MMP-1 mRNA levels in osteocytes[6].

While synthesis of collagen is a major step in forming bone, removal of collagen is a principal step in bone resorption. A role of the osteocyte in the degradation of matrix, however, has not been shown. Indeed, the concept of osteocytic osteolysis has essentially been abandoned, as it is

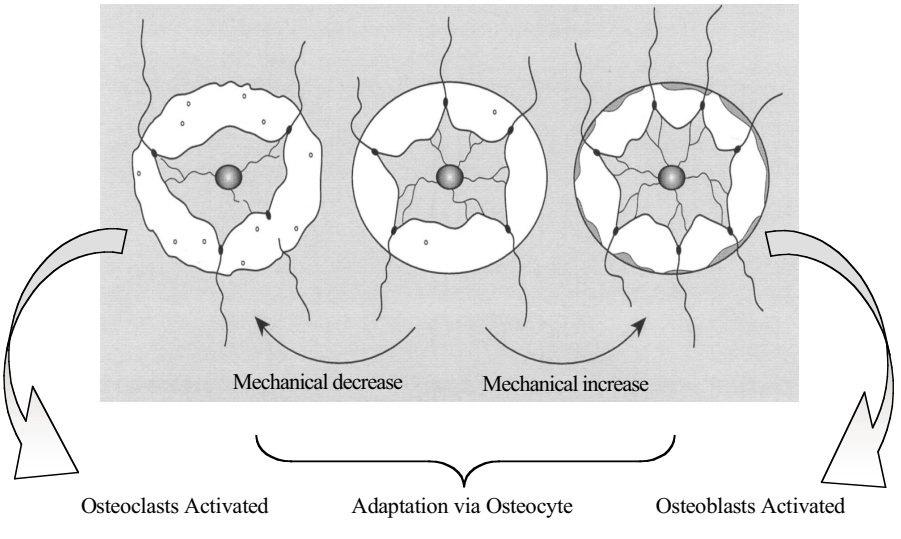

Figure 4. Cartoon to show how the osteocyte, within its lacunae, is coupled to the by connections between collagen and integrin. Intracellularly, cytoskeletal architecture is represented by actin. Deformation of the matrix (functional loading) will cause distortion of the lacunae as well as fluid flow through the bone, thus mechanically stimulating the osteocyte. The premise of the osteocyte plasticity hypothesis is that the cell will actively alter its cytoskeletal architecture, coupling to the matrix, and modulation of the periosteocytic space to achieve an "optimal strain environment". The osteocyte in the center has "adjusted" its attachments such that the mechanical signal is now filtered to the desired level. When there is a subtle increase in load (osteocyte on the right), the cell will rapidly enhance its cytoskeletal infrastructure, attachment to the matrix via the upregulation of actin, osteopontin, and integrins. Within the lacunae, the osteocyte has modified its periosteocytic boundaries by depositing collagen. Higher levels of mechanical stimuli will cause the osteocyte to secrete factors such as BMP-2, and thus participate in the recruitment and proliferation of osteoblasts. As the newly recruited osteoblasts deposit bone, the strain perceived by the cell would fall towards the optimal strain environment. When there is a decrease in mechanical signal, the osteocyte adapts to this change by degrading its attachment to the matrix via MMP-1 (cell on left). MMP-1 is shown as small circles within the periosteocytic space, which have destroyed connections between the cell and matrix and expanded the lacunar space (osteocytic osteolysis). In this scenario, osteocytes "adapt" to subtle decreases in the mechanical environment without necessitating the recruitment of osteoclasts. At even lower levels of stimuli, the osteocyte will secrete factors such as MCSF and ODF, and thus participate in the recruitment and differentiation of osteoclasts. Osteoclast resorption would increase the strain back towards the optimal strain environment.

believed these cells do not have the machinery to digest the matrix[7], leaving essentially all responsibility for the resorption of bone matrix to the osteoclast. However, alternative means to adapt to disuse, independent of osteoclasts, is via enzymes which could degrade collagen, such as matrix metalloproteinases (MMPs). If the osteocyte could focally produce MMPs, it would establish a means to modulate the cell's attachment to the mineralized matrix, and modify the confines of the periosteocytic space. Though this form of strain regulation may not result in a change in bone mineral, *per se*, it certainly would alter the cell's perception of the physical environment, whether fluid or deformation induced. The upregulation of coupling which follows increased mechanical demand, and the active process to uncouple the osteocytes from the matrix when subject to disuse, would be an integral part of a set of feedback loops in which the osteocyte can mediate the degree to which it is exposed to mechanical signals (Fig. 4). Thus, an osteocyte would alter its own microenvironment to achieve a genetically mandated mechanical signal generic to all osteocytes in all bone locations, in all vertebrates, regardless of whether the cell is in the humerus of a goose, the cranium of a chimp, or the tibia of a human. This scenario would allow a cell in a high strain region to be attached differently than one subject to low strain, yet each would normalize their local environment to "achieve" the same stimulus. **The "optimal strain environment" achieved without the need to form or resorb bone.**

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