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Bone Homeostasis and Repair: Forced Into Shape

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Abstract Mechanical loading is a potent anabolic regulator of bone mass, and the first line of defense for bone loss is weight-bearing exercise. Likewise, protected weight bearing is the first prescribed physical therapy following orthopedic reconstructive surgery. In both cases, enhancement of new bone formation is the goal. Our understanding of the physical cues, mechanisms of force sensation, and the subsequent cellular response will help identify novel physical and therapeutic treatments for age- and disuse-related bone loss, delayed- and nonunion fractures, and significant bony defects. This review highlights important new insights into the principles and mechanisms governing mechanical adaptation of the skeleton during homeostasis and repair and ends with a summary of clinical implications stemming from our current understanding of how bone adapts to biophysical force.

Keywords Bone remodeling · Mechanical adaptation · Mechanobiology · Osteocyte · Osteoblast · Stem cell

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Introduction

The skeleton is a multifunctional system involved in locomotion, protection of internal organs, hematopoiesis, immunity, endocrine status, and mineral homeostasis. Bone, though seemingly an inert tissue, has the ability to change in size and shape in response to mechanical cues [1, 2, 3•]. In fact, mechanical signals are primary regulators of bone mass and architecture, a phenomenon better known as *Wolff's Law* [4]. Mechanical signals likewise influence bone regeneration and repair by activating pathways involved in cellular recruitment, proliferation, osteogenic differentiation, and angiogenesis [5–8]. Thus, homeostasis and repair can be considered adaptive processes in which mechanobiochemical signals are integrated to generate mechanically optimized bone [9, 10].

Bone tissue is composed of hydroxyapatite (hard matrix), type I collagen, bone cells, blood vessels, nerves, and extracellular fluid, which permeates the hard matrix and lacuno-canalicular space [11]. It is a living viscoelastic material with organizational units at the macro-, micro-, and nano-length scales. In adults, its organization, shape, and size are governed by global and local stimuli to meet changing physical and biochemical demands. These changes in size and shape are accomplished by groups of cells located throughout the mineralized matrix and on bone surfaces.

Osteocytes, which are dispersed throughout the bony matrix and connected by the lacuno-canalicular network, communicate with one another and with cells on the bone surface by way of gap junctions and paracrine signaling [12]. The periosteum, a sheath of fibrous tissue covering bone, contains bipotent progenitors that can form both bone and cartilage [13, 14]. Likewise, the bone marrow (BM) is a rich source of self-renewing, multipotent skeletal progenitors for bone, cartilage, fat, and stroma [15]. Bone, the home of hematopoiesis, is a highly vascularized tissue. The complex vessel network in

bone and marrow is a conduit through which paracrine and endocrine signals, as well as circulating cells, can access the bone microenvironment [16, 17]. Osteoblasts, derived from the mesenchymal lineage, and osteoclasts, derived from hematopoietic stem cells, are the workhorses of bone formation and bone resorption, respectively.

How these "effector" cells and other "supporter" cells sense and respond to mechanical cues driving new bone formation has been comprehensively reviewed in several previous papers [7, 8, 18–20]. With the advent of genetically engineered mice and the use of advanced computational methods, our understanding of the complex processes involved in mechanical adaptation of bone has advanced significantly. This review highlights some of these important new insights into the principles and mechanisms governing mechanical adaptation of the skeleton during homeostasis and repair.

Bone Adapts to Mechanical Cues as Part of Its Homeostatic Program

Principles of Mechanical Adaptation of Bone

Load-induced mechanical signals in bone, or the absence of those signals (e.g., disuse), result in either new bone formation or bone loss, respectively, with consequent changes in bone mechanical properties. Principles that govern this adaptive response have been formulated using data from in vitro and in vivo experimentation and are reviewed in detail elsewhere [18, 21, 22]. These principles are as follows: (1) Bone responds to dynamic, rather than static, loading; (2) bone responds only when strain magnitude or strain rate reaches distinct thresholds or mechanical set points—a paradigm first coined by Frost as the *mechanostat* [23]; (3) the amount of new bone formation correlates with strain magnitude and rate—and thus in a site- (i.e., site along longitudinal axis of bone) and envelope-specific manner (i.e., periosteal, endosteal, trabecular); (4) bone responds to short loading periods, whereas longer loading periods have diminished returns; (5) bone grows accustomed to routine mechanical signals; and (6) bone is highly responsive to loading during the growth and development phase, with effects maintained well into adulthood and old age [3•, 24, 25]. These principles guide development of weight-bearing exercise protocols to minimize bone loss in osteoporotic patients [26] and to enhance repair in patients with fracture and large bone deficits [27–29].

Mechanical Signals Generated During Daily Activities

Deformation or strain at the tissue level on bone surfaces during walking varies between 500 microstrain (με; 0.05 %) and 2000 με (0.2 %) [30]. During vigorous activity, strains can reach up to 10,000 με (1 %) [31]. Bone strain mediates fluid flow within the hard matrix, lacuna-canalicular space [32], and marrow [33], and is proposed to result in amplified strain at the level of the cell due to fluid drag through glycocalyx proteoglycans and integrin attachments located along the osteocyte processes [34, 35]. Using computational models, the resulting flow-induced shear stress in the canalicular space in bone has been estimated to be 0.5 to 2 Pa [34, 36]. Similarly, estimated shear stress in the marrow near individual trabeculae is 0.5 to 2 Pa [33, 37]. In some cases, shear stress may reach up to 5 Pa when the marrow is modeled as a highly viscous material [38]. Using bone explants, Verbruggen et al. [39, 40••] recently showed that application of uniaxial compressive strains of up to 3000 με resulted in membrane strains in osteocytes and osteoblasts of up to 30,000 and 25,000 με, respectively, a 10× amplification. These estimated load-induced strains at the tissue and cellular levels exceed the experimentally determined anabolic strain threshold at the tissue level for initiating new bone formation (>1050 με) [41] and activating mechanoresponsive signaling pathways in bone cells (>10,000 με) [42].

Anabolic Strain Threshold: Refinement of the Mechanostat

The mechanostat theory predicts that bone formation and resorption are each limited to specific strain ranges and that there is a "lazy zone" in which bone mass remains constant [23, 43]. This theory further proposes that an age-related decline in bone mechanoresponsiveness is a result of a shift in the anabolic mechanical set point, that is, there is an increase in the amount of strain required to elicit new bone formation [43]. Seminal studies that demonstrate an anabolic strain threshold [41, 44–46] compare bone formation rates to tissue level strain (measured with uniaxial strain gauges) in young adult rodent bones subjected to noninvasive mechanical loading. Inherent limitations of these early studies are the averaging of strain across a finite area (e.g., 0.38×0.51 mm gauge area) and the variability in the strain measurement due to operator-dependent gauge placement [47].

More recent in vivo studies incorporate finite element (FE) models to more precisely estimate strain on complex bone surface geometry and compare these data to bone formation parameters. Sugiyama et al. [48] reported that cortical bone formation rates in response to mechanical strain follow a continuous, linear response curve without an apparent lazy zone. Using high-resolution microCT imaging and image registration, Schulte et al. [49] showed that regions of high local mechanical strain coincide with trabecular bone formation and regions of low local mechanical strain coincide with trabecular bone resorption with no apparent lazy zone. Importantly, mechanical stimulation enhances the mineralizing surface (% of bone surface undergoing active bone formation) in

cortical bone rather than increasing the rate at which new matrix is deposited at a given location independent of age [50]. The same group also showed that mechanical adaptation occurred through short-term modeling events in which formation and resorption were decoupled [51•].

In a series of studies by Checa, Willie, and colleagues [52, 53••, 54], the effects of aging on tissue-level mechanical adaptation in the mouse tibia were examined. Using novel image registration techniques and FE models, Razi et al. [52] showed that load-induced strain magnitudes at the cortical mid-diaphysis were significantly reduced with aging (20 % lower in adult versus young mice, 15 % lower in aged versus adult mice) due to alterations of whole bone morphology. This shift was also observed in trabecular bone. Next, they showed that young, adult, and aged mice all exhibited new bone formation and reduced resorption in response to loading [53••], which corroborates previously published work showing that aged bones can, in fact, respond to physical force [55–58]. However, adult mice exhibited clear strain ranges in which formation and resorption occurred, whereas the strain threshold in aged mice was blurred rather than shifted, as proposed by the mechanostat theory. That is, mechanical signals lost their specificity to increase bone formation and reduce bone resorption, with formation and resorption occurring in similar strain ranges. This preferentially puts aged bone at a disadvantage in that increased amounts of high-load exercise (and strain) may not significantly improve bone mass in the elderly long-term [54].

Osteocytes as the Master Regulator of Load-Induced Bone (Re)Modeling

Osteocytes, the fully differentiated bone cells dispersed throughout the bony matrix, play a critical role in bone mechanoadaptation [12]. Osteocytes subjected to mechanical stimulation respond rapidly by mobilizing a series of second messengers, including calcium [59, 60], nitric oxide [61, 62], and prostaglandins [63]; by activating kinase signaling cascades, including the MAP kinase and PKC pathways [64]; and by exhibiting alterations in gene expression [65]. In addition, osteocytes respond to biophysical cues by releasing soluble factors important for cellular proliferation and differentiation and for recruitment of osteoblasts and osteoclasts [66–70].

Leucht et al. showed that *Cxcl12*, the gene encoding the chemotactic molecule stromal cell-derived factor-1 (SDF-1), is upregulated in osteocytes and periosteal cells using in vivo and in vitro mechanical loading models. Systemic inhibition of CXCR4 (SDF-1 receptor) signaling attenuated in vivo load-induced bone formation, suggesting that CXCL12 is an important paracrine regulator of osteoblast function. Using in vitro techniques, Govey et al. [67] reported the altered expression of a variety of genes in osteocytes exposed to fluid flow shear stress. The greatest increases were observed in three chemokine genes (*Cxcl1*, *2*, and *5*) involved in chemotaxis and inflammation [71], suggesting that these factors may also serve as important paracrine signals. Osteocytes also negatively regulate osteoblast function through the basal expression of sclerostin, a known inhibitor of the pro-osteogenic Wnt signaling pathway [72]. Robling et al. [68] and others [73] have shown that in vivo mechanical loading suppresses the expression of sclerostin in osteocytes, thereby removing its inhibitory effect and permitting new bone formation to proceed. More recent in vivo studies have been able to delineate the osteocyte-specific factors that are important in mechanical adaptation by utilizing Cre-LoxP technology to knock out genes in an osteocyte-specific manner. These factors include IGF-I [74], Wnt/β-catenin [75], and nuclear factor erythroid 2-related factor (Nrf2) [76].

Mechanisms linking osteocyte damage and osteoclastic activity have also been described. Kennedy et al. [69] showed that in vivo fatigue-induced osteocyte apoptosis leads to increased RANKL/OPG ratios in osteocytes adjacent to apoptotic cells. Dolan et al. [70] reported that thermally damaged osteocytes in culture exhibit increased expression of pro-osteogenic genes (decrease in RANKL/OPG, increase in Cox2). Treatment of mesenchymal stem cells (MSCs) with conditioned media from damaged cells leads to increased osteogenic differentiation. This link between osteocyte-expressed paracrine factors in response to mechanical loading and MSC recruitment and differentiation is the focus of new and interesting ongoing work by various groups.

Load-Driven Differentiation of Multipotent Mesenchymal Stem Cells

Applied mechanical stimuli and intracellular forces regulate the differentiation of multipotent MSCs (reviewed in [19]). Physical properties of the extracellular matrix also control cell lineage specification [77] by regulating changes in cell shape, density, and cell-cell contact [78]. Several signaling molecules involved in load-driven differentiation include integrins [79], cadherins [80], RhoA and ROCK [81], Wnt/β-catenin, and Yes-associated protein (YAP)/transcriptional co-activator with PDZ-binding motif (TAZ) [82], all of which regulate cytoskeletal dynamics. YAP/TAZ signaling localizes to the cell nucleus with increased matrix stiffness [82], and this translocation is dependent on Rho activity and stress fibers, but not F-actin polymerization, suggesting that force generation is required to activate YAP/TAZ. Furthermore, the inhibition of YAP/TAZ activity attenuates osteogenic differentiation and enhances adipogenic differentiation on a stiff substrate. Recent studies have shown that YAP/TAZ mediates pro-osteogenic Wnt signaling [83, 84]. Wnt signaling results in accumulation of β-catenin, YAP, and TAZ in the nucleus [83], thereby upregulating β-catenin and YAP/TAZ target genes [85].

The emerging paradigm is that osteocytes, the master regulators of re(modeling), regulate cellular recruitment and osteoblast function on bone surfaces and in the marrow. In response to loading, osteocyte-osteocyte and paracrine signaling [66, 67], via the release of soluble factors from osteocytes that travel through the canalicular space to bone surfaces, are likely key mechanisms in bone adaptation.

Bone Adapts to Mechanical Cues as Part of Its Regenerative Program

Bone repair is acutely sensitive to the prevailing mechanical environment [9, 10]. Compressive strain, tensile strain, hydrostatic pressure, shear strain, and fluid flow have all been implicated as important mechanical stimuli regulating regeneration and repair (reviewed in [86–88]), and there is a growing body of quantitative work describing relationships between mechanical factors, tissue formation, and tissue-specific differentiation in bone healing.

In an early study, Bostrom and colleagues [89] used a mouse tibial osteotomy model to investigate the load magnitude and the time post-fracture before initiation of loading required for optimal healing. Compressive axial loading (100 cycles/day, 1 Hz, 5 days/week for 2 weeks at 0.5, 1, or 2 N) was applied across the flexed knee and ankle using an external loading device directly after fracture (0 day) or after a 4-day delay. They found that, of all combinations of load magnitude and timing of load application, only a 0.5-N load applied at 4 days post-fracture resulted in a stronger callus relative to the nonloaded controls. Loading immediately after fracture (0 day) inhibited callus formation regardless of load magnitude; this may have been due to the disruption of early vascularization, particularly if the tissue failure strain threshold was exceeded. Callus strength and stiffness were reduced in these groups compared to the nonloaded controls. Higher loads applied after a 4-day delay also inhibited callus formation, which the authors also attributed to exceeding the tissue strain threshold.

In a separate study, Morgan and colleagues [90] used a rat femoral osteotomy model with external fixation to investigate how mechanical stimuli may direct differentiation to the cartilage phenotype rather than to bone. They used a loading protocol (cyclic bending, +35°/−25° at 1 Hz, 15 min/day for 5 consecutive days/week) beginning at 10 days post-surgery and continuing for 1, 2, or 4 weeks. Cyclic bending enhanced the cartilage formation and the expression of cartilage-related genes, COL2A1 and COL10A1, and downregulated bone morphogenetic genes (BMP)-4, BMP-6, and BMP-7.

Guldberg and colleagues [91] investigated the role of functional loading across a 6.0-mm segmental defect in rat femora that was fixed with either stiff or compliant plates. Compliant plates allowed transfer of ambulatory loads to the defect at 4 weeks post-fracture. Compliant fixation resulted in increased regenerate volume, which contains both bone and cartilage, a greater relative amount of cartilage within the regenerate, and decreased remodeling to lamellar bone compared to stiff fixation. Torsional stiffness was almost 60 %

Table 1 Regulation of selected genes by mechanical loading in uninjured and injured bone. Bone-specific genes (BSP, COL1a1, BGLAP, SPP1) are upregulated in response to loading in both uninjured and injured bone

	Response to loading		Reference
	Uninjured	Injured	
α5β3 integrin	Increase	Unknown	[5]
Bone morphogenetic protein-3 (BMP-3)	No change	Increase	[90]
Bone morphogenetic protein-4 (BMP-4)	Increase	No change	[6]
Bone morphogenetic protein-10 (BMP-10)	Increase	No change	[6]
Bone sialoprotein (BSP)	Increase	Increase	[6, 90]
Collagen type I (COL1a1)	Increase	Increase	[7, 90]
Collagen type II (COL2a1)	No change	Increase	[5, 90]
Collagen type X (COL10a1)	No change	Increase	[5, 90]
CXCR4	Increase	Unknown	[66, 6]
Epidermal growth factor (EGF)	Increase	Unknown	[5]
Fibronectin (FN)	Increase	Unknown	[5]
Hif-1α	Increase	Unknown	[5]
Osteocalcin (BGLAP)	Increase	Increase	[6, 90]
Osteopontin (SPP1)	Increase	Increase	[6, 90]
Sclerostin (SOST)	Decrease	Unknown	[68, 6]
Tissue inhibitor of metalloproteinases (Timp1, Timp2)	Increase	Unknown	[6]

higher in the compliant-plate group, although failure torque was not different between groups. The authors concluded that the greater micromotion increased the amount and distribution of bone formed. In addition, they found that functional loading early in the healing process significantly inhibited vascular invasion into the defect by 66 % and reduced bone formation by 75 % compared to stiff-plate controls [92]. In contrast, delaying the onset of loading by 4 weeks significantly enhanced bone formation by 20 % and stimulated vascular remodeling, that is, loading resulted in larger vessels.

Unsurprisingly, some of the same bone-specific genes are upregulated in uninjured and injured bone in response to mechanical loading (Table 1). The effects of loading on other mechanosensitive genes in injured bone are currently under investigation. Ongoing studies are beginning to elucidate the effects of mechanical stimulation on different phases of repair, and there are current efforts to combine models of mechanically driven repair with genetically altered mice to delineate the roles of specific genes in the complex repair environment.

Clinical Implications

Family medicine doctors and orthopedic surgeons alike utilize the concept of mechanoadaptation in their daily clinical practice. While the family practitioner will prescribe an exercise program to prevent osteopenia or osteoporosis, the orthopedic surgeon will advise the post-operative patient to perform weight-bearing activity on the involved extremity in an effort to enhance bone healing. Both scenarios employ activities that result in high bone strains, while maintaining them below the damage threshold. For the osteoporotic patient, these exercises will focus on weight training, jumping, and plyometrics, with a special emphasis on the skeletal elements that are most severely affected by osteoporotic fragility fractures, such as the wrist, the proximal humerus, and the hip [26]. On the other hand, exercises serve a different purpose for the post-operative fracture patients. In this case, exercises to maintain bone mass are only of secondary importance, while the pro-osteogenic effect of mechanical loading is the primary reason for early weight bearing [28, 93]. Recent implant designs have taken this stimulatory effect into account and now allow for immediate weight bearing after surgical fracture fixation. This development resulted in changes in post-operative care for many fractures, resulting in decreased complications associated with immobility. Ultimately, however, clinical experience and patient-specific factors, such as comorbidities and compliance issues, will determine how much weight and how early this weight is applied to the injured extremity.

The recent understanding and knowledge of the effects of mechanical stress on bone homeostasis has resulted in a change in orthopedic implant material [94, 95]. While stiff, load-bearing implants are known to result in significant stress shielding, which is most commonly seen in the bone around a total hip replacement and which can result in prosthetic loosening and fracture [95], newer implant designs and metal alloys have resulted in reduced incidence of this inadvertent bone loss.

Conclusions

Mechanical loading in the form of functional weight bearing and exercise is the first line of defense for mitigating age-related bone loss and enhancing fracture repair. Seminal studies in the field provided the basis for the principles of bone adaptation. Early in vivo studies used mouse bone loading models and periosteal strain gauging to establish a relationship between strain and new bone formation. More recent studies have utilized FE models and high-resolution image registration techniques to more accurately predict load-induced strain and to quantify new bone formation in a site-specific manner. These studies are helping to refine the mechanostat theory and the concept of the anabolic strain threshold, particularly in the context of aging in which the responses to loading become increasingly variable. In addition, signaling pathways critical for bone mechanoadaptation in the context of homeostasis and repair continue to be clarified using in vivo systems.

Compliance with Ethics Guidelines

Conflict of Interest The authors declare that they have no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by the authors.

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