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Review Article

Mechanical Strain and Bone Cell Function: A Review

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Introduction: The Strain-Adaptive Response of Bone

Bones are able to withstand functional loads without either breaking or sustaining extensive damage because they have evolved the capacity to adapt their architecture in relation to changes in their habitual loading environment [1–3]. Reduced loading due to long-term bed rest, cast immobilization, or microgravity conditions induces significant bone loss and mineral changes [4–6], which only begin to be recovered following the reintroduction of normal activity. It is assumed, therefore, that the functional input required to stimulate and maintain normal bone architecture is the loading environment encountered during normal activity. Although most fractures occur as a result of the loads engendered during accidents such as falls or collisions, these loads (since they are encountered only at the time of fracture) cannot be used as a controlling input for bone cells to adapt bone strength. It is most likely therefore that bone cells respond directly or indirectly to the local strains engendered in their vicinity by the loads of normal functional activity. These strains are the product of the bones' external loads and their structural properties and so contain all the information necessary to be the controlling input for adaptive bone modeling and remodeling.

Frost [7] likened strain-adaptive remodeling to a domestic thermostat (or 'mechanostat') that is 'off' under circumstances of normal physiologic strain and 'on' in response to strain magnitudes outside normal physiologic thresholds. This is an attractive analogy but inevitably limited in its applicability. Just as the precise input and mechanism by which loading is transduced into cellular control of bone remodeling is unknown, so are bone's objectives (the on/off points) in terms of strain. A number of studies have shown that bone's adaptive (re)modeling behavior is more complex than on/off formation/resorption responses to strain magnitude. For instance, static strains do not engender adaptive responses [8,9] whereas dynamic strains which change at high physiologic rates (as in impact loading) engender greater adaptive responses than those which change more slowly [10–13]. The on/off points therefore relate to a strain-related stimulus rather than a particular strain value [9].

The inference, derived from animal experiments, that bone cells respond preferentially to a subset of their mechanical environment dominated by high strains changing at fast rates and presented in unusual distributions, has been substantiated by exercise studies in humans. Thus high impact activities such as squash, tennis, and badminton, for example, are more osteogenic, than running, cycling, swimming, or ice hockey [14]. These human exercise studies also support the data from animal studies that local loading induces local site specific changes in bone architecture [15–17]. In tennis players and baseball pitchers, for example, humeral hypertropy occurs only in the playing arm in which the stimulatory loading is actually experienced [16,18]. Conversely, protection from strain-related stimuli causes a localized reduction in bone mass. External fixation, for example, causes an increase in the diameter of the medullary cavity and reduced bone mineral content [4,19,20]. Even with generalized decreases in skeletal loading, as occurs during space flight, bone loss occurs in a non-uniform manner, with the distal leg bones experiencing the highest bone loss [21,22]. This has been hypothesized to occur because of the absence of normal high frequency heel strike activity under microgravity conditions [23]. It seems, therefore, that

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although bone cells must use their functional strain environment to 'assess' the structures' suitability to withstand fracture from aberrant loading conditions, they compensate for the inevitable inappropriateness of this measure by responding preferentially to the 'error rich' components of their functional strain environment. It is for this reason that activities such as squash, tennis, triple jumping etc. engender large osteogenic responses whereas 'error poor' activities such as swimming and cycling do not [14,15,17,24].

Experimental Measures of Functional Strain and Adaptation in Bone

The observation that bone responds to its mechanical environment is ancient, but its origin in modern times is generally attributed to Wolff [25]. The concept has received qualitative support from many sources, but was not subjected to systematic experimentation until Hert and co-workers developed a methodology for the controlled experimental loading of individual bones in vivo [8,26–30]. In a classic set of experiments Hert et al. applied loads through Bowden cables to the diaphysis of the rabbit tibiae via trancutaneous pins [31]. Regardless of whether tensile or compressive forces were applied, the bone responded to intermittent, but not static loading. Unfortunately, although Hert and his colleagues could estimate the size of the loads necessary to engender adaptive change they had no means of knowing either the normal locomotor strains in that region or the strains which the loading engendered. This deficiency was remedied ten years later when it became possible to attach strain gauges directly to bone surfaces in vivo [10]. This development allowed the measurement of normal (usually locomotor) strain magnitude, rate of change of strain, and strain distribution. These values could then be used as reference points for the strains engendered under experimental loading. The first study to combine these approaches was that of O'Connor et al. [10] who used a pneumatic actuator to apply bending loads to sheep radii through metaphyseal pins. This study demonstrated a significant correlation between maximum strain rate and the degree of bone hypertrophy. This inference has been confirmed by a number of subsequent studies [11,19,32].

The animals used in O'Connor's experiments were allowed to ambulate normally between experimental loading episodes. This superimposition of experimental loading onto normal activity made it difficult to establish a unique mechanistic correlation between the experimental mechanical loading regimen and the adaptive response. It was also uncertain whether the opposite limb was, indeed, 'normal' for use as a control, since increased load was likely transferred to this limb following intervention in the experimental limb.

The development of the functionally isolated avian ulna model [33] afforded the means to document the adaptive response to strain engendered by artificial loading in the absence of the confounding strains superimposed by normal daily activity (though it was also potentially confounded by the withdrawal of these strains). By using the ulna of the wing of a flightless bird, rather than a weight-bearing bone of a quadruped, the animals' lifestyle was not incommoded and the opposite wing could be used as a control without concerns regarding possible load transfer. In these experiments, the ulnar diaphysis was functionally isolated by proximal and distal transverse metaphyseal osteotomies, and transfixing pins were used to either mechanically load the ulna or protect it from mechanical loading by external fixators. Functional load-bearing producing physiological strain magnitudes, but an altered strain distribution, prevented remodeling which would otherwise have led to disuse osteoporosis. Consistent with the findings of Hert, this strain-adaptive response required dynamic, rather than static loading [12,33] and showed that strains within the normal physiologic range could engender substantial adaptive modeling when presented in a novel distribution. Experiments using this model also led to the important finding that the maximal adaptive response to an osteogenic stimulus could be achieved with as few as 36 cycles of loading per day [33–36]. This finding prompted the idea that bone cells adaptively remodel bone architecture in response to a subset of their total mechanical experience, and that this subset is dominated by the strains encountered during 'error loading' rather than controlled coordinated activity [19].

The search to define the active subset of bone's loading environment has continued with non-invasive rodent loading models of which data have been published from two studies in cortical long bones and one in trabecular bone of the spine. In the long bone models, loads have been applied to the rat tibia by four-point bending [37], or to the ulna by axial compression [38]. Both these models avoid the potentially confounding reactions that may be associated with the use of implants, but the four-point bending model introduces the problem of reaction at the points of pressure on the periosteum [39,40]. In the trabecular bone model, pins inserted into the seventh and ninth caudal vertebrae are used to load the eighth caudal vertebra [41], allowing study of the response to compression. These rodent models reintroduce the feature of a short period of artificial loading embedded in a longer period of normal activity. They confirm the inference from the avian experiments that loading periods need only be very short to stimulate adaptive responses [11,42–45]. Both long bone models have consistently demonstrated that bone formation is threshold-driven and influenced by strain rate, frequency, amplitude, duration of loading, and interpolation of rest periods [11,40,43,44,46–48]. The introduction of transgenic mice overexpressing or underexpressing various genes means that this in vivo approach may be used to dissect out the mechanisms involved in strain-related adaptation. The axial ulna loading model has been successfully adapted for mice [49] but no data are currently available from its use in transgenics.

Both the waveform and distribution of strain influence bone's adaptive response [50–54]. In the turkey ulna, for

example, the remodeling response to strain varies depending on whether it has been loaded in an axial or torsional manner [53]. Osteogenic exercises probably derive their osteogenic character from engendering a strain environment that deviates from that habitually encountered, and to which bone architecture has previously adapted [52,55–57]. Porcine ulnar ostectomy, for example, increases strain levels in the radius, causing an adaptive hypertrophy. After 3 months, the increase in radius cross-sectional area serves to reduce surface bone strains to normal physiologic levels [58]. Similarly, both the avian ulna and rat four-point bending models have demonstrated that prolonged loading regimens stimulate modeling with slowly forming circumferential lamellae despite their initially potent osteogenic effect producing woven bone [59,60]. Cessation of new bone formation presumably equates to a new equilibrium where the stimulus for adaptive change has reduced to zero. Once the loading regimen that engendered the adaptive change ceases the bone will remodel back towards the customary status quo ante for strain. The precise nature of the target strain environment for each location is unknown.

In Vivo Measurements of Bone Strain

Quantification of the 'habitual' bone strain milieu has been an important part of the continuing investigation of the mechanism by which bone adapts to loading. Using strain gauges, peak bone strain and strain rates have been measured during various activities in numerous species, including humans. These studies have been comprehensively reviewed by Fritton and Rubin [61]. For most animals, peak functional strains range from less than -1000 microstrain during walking to between -2000 and -3200 for more vigorous activities, and can almost reach -5000 microstrain in galloping racehorses [61]. These values reflect the deformation that bone normally experiences and have been helpful in defining the parameters within which in vitro experiments should be designed in order to more fully characterize the cellular mechanisms that initiate an appropriate loading-related adaptive response. However, while these are the normal strain parameters, the 'active subset' of the strain environment to which bone cells are actually responsive remains undefined and may be very different from the overall strain data determined from in vivo measurement. Using the isolated avian ulna model, for example, it has been shown that a mechanical load of 500 microstrain has little consequence if applied at 1 Hz, but is highly osteogenic if the frequency is increased to between 10 and 60 Hz [62,63]. Furthermore, the minimum strain magnitude that is osteogenic decreases with increases in frequency. For instance Rubin et al. [64] report that strains as low as two orders of magnitude below physiological levels can stimulate large increases in bone mass if presented at 30Hz. Extremely low-magnitude (<10 microstrain), high frequency biomechanical intervention may also prevent bone loss associated with disuse [65] and improve both the quantity and quality of trabecular bone [66].

In Vitro Strain Experiments

One particularly valuable experiment in vivo demonstrated that normally quiescent, adult periosteum can be directly transformed to active bone formation in response to the bone being exposed to only a single short period of osteogenic loading [67]. Within a few minutes of loading, the osteocytes within the loaded bone show an increase in uptake of tritiated uridine and increased levels of glucose 6-phosphate deyhydrogenase (G6PD) that are in proportion to local strain magnitude [68,69]. Thus the full cascade of cellular events between quiescence and active bone formation can be followed under any circumstances in which a bone, or bone-derived cells, can be kept alive. Long bone explants can thus be studied for approximately 24 hours ex vivo, whereas cultured bone cells may be examined practically indefinitely. Thus while remodeling per se can still only be studied in vivo, the earliest stages of bones' adaptive response can be studied in explants [70,71]. Such studies have demonstrated that if cores of cancellous bone are loaded in organ culture there is increased release of prostacyclin (PGI_2) and prostaglandin E_2 (PGE_2) [72]. The exogenous administration of PGI_2, but not PGE_2, caused early increases in G6PD activity and RNA synthesis in bone cells that were similar to those caused by in vivo mechanical loading [70].

The Cellular Response to Mechanical Stimuli

Explants cannot be used for experiments of longer than 24 hours duration and so many investigators have used bone cells either in primary cultures or cell lines. Early attempts to generate mechanical strain in bone cells in vitro were highly inventive, but their interpretation is somewhat limited because of uncontrolled variables. Glucksmann [73] pioneered studies on mechanotransduction in the 1930s using biologic materials, such as intercostal muscle, to apply load to bone cell cultures. Subsequently, several other systems have been developed in an effort to provide distinct mechanical stimuli for experimental study. More recently, hydrostatic compression [74–76], longitudinal and axisymmetric substrate distension [77–83], substrate bending [82,84–86], fluid shear [87,88], and combinations of these models [83,89] have been developed to mimic the physiologic components of the mechanical stimuli presumed to occur in vivo. These methodologies have been reviewed by Brown [90]. The response of bone cells to the varied mechanical stimuli provided by these diverse devices has provided some insight into the relative importance of each type of stimulus to the ultimate generation of an adaptive response.

Early studies which used tensile stretching of a deformable substrate to strain cells often generated

very large, heterogeneous [79], or static strain stimuli [77] which are difficult to relate to the situation in vivo [78]. Subsequent longitudinal substrate distension models were able to take advantage of advances in culture substrates to achieve greater strain homogeneity [81]. One of the more popular systems used to strain cells has been marketed under the name 'Flexercell', and has undergone several modifications to meet criticisms regarding the lack of uniformity and high magnitude of the strains applied [91,92]. Relatively simple substrate bending devices have been developed which allow homogeneous, physiologic strains to be applied using four-point bending [82,85]. Some more sophisticated substrate bending systems allow waveform, peak strain, and frequency to be altered while input strains are characterized using laser displacement and strain gauge monitoring [86]. Hydrostatic compression models can also readily apply a distinct waveform of a given magnitude to cells in culture, but this type of cellular deformation may not mirror that experienced in vivo, and the high oxygen and CO_2 pressures require alterations in the nutrient medium.

The Effects of Fluid Flow

Comparative histomorphometric studies have demonstrated a consistent appearance of the lacuno-canalicular network, regardless of species, such that osteocytes are always oriented in a manner which maintains gap junction connections with the osteoblasts on the bone surface as well as neighboring osteocytes [93–95]. This arrangement permits the exchange of mechanical and metabolic signals [96–98], and provides a network which may be important for both mechanosensation and mechanotransduction (as reviewed by Burger and Klein-Nulend [99]). Mechanical loading of bone not only deforms the bone tissue, but also engenders movement of extracellular fluid through the bone's lacuno-canalicular system. Such fluid flow may stimulate bone cells via streaming potentials, wall shear stress, or chemo-transport related effects [100–102]. Although there are no definitive studies on the magnitude of periosteocytic strain-induced fluid flow, Biot's porous media theory has been used to relate whole bone stress to canalicular interstitial fluid flow past osteocytic processes [103]. These calculations predict that loading regimens engendering peak physiologic strains will induce fluid induced shear stresses of 0.8–3 Pa. Subsequent experiments have used pulsating fluid flow to generate fluid shear stress of 0.2–6 Pa, and have found that bone cells in vitro readily respond, lending experimental support to the theory that one of the stimuli to which bone cells are mechanosensitive may involve strain-driven fluid flow [104,105].

The dominant determinant of the bone cell's response to fluid flow is hypothesized to be due to fluid shear stresses that stimulate cell wall processes [83,99,103,104], causing cell deformation and subsequent metabolic activity via integrins and the cytoskeleton [106–109]. One experiment that compared the stimulus of hydrostatic pressure with fluid shear stress found that a response to hydrostatic pressure required 6 hours, whereas the response to pulsating fluid flow could be measured after 1 hour [110]. This is perhaps not surprising since cellular phenomena in many biologic systems are influenced by fluid shear [111]. Osteoblast osteopontin expression is also more readily influenced by fluid forces than mechanical stretch, which tempts investigators to believe that extracellular fluid flow is important to the response of bone cells [84]. One recently developed model demonstrates that the tensile forces necessary to maintain the cytoskeleton's attachment to its substrate in the face of fluid forces may be much greater than the fluid shear force applied [112]. If drag forces on the pericellular matrix are transmitted to the intracellular actin cytoskeleton, they would amplify the strain-related stimulus. This could explain why tissue level (physiologic) strains are generally smaller than those typically required to elicit a response in vitro [113,114]. This model has been used to hypothesize that drag forces on the pericellular matrix may be the cell's chief mechanostimulus [112]. However, in most experiments the acute cellular responses engendered by the mechanical stimuli employed are rarely if ever causally related to subsequent adaptive remodeling. It is likely that the cellular responses to mechanical stimuli in many experiments are actually unrelated to the control of adaptive modeling and remodeling.

It is more than probable that bone cells respond to more than one component of their mechanical environment. This is suggested strongly by the ability of mechanical strain as well as fluid flow stimuli to promote bone cell activity [101,103,115–118]. Since in vivo loading is always accompanied by fluid flow, bone cells may respond to changes in their strain environment via an integrated assessment of changes in multiple parameters associated with both fluid flow and physical deformation. Such a response might allow for a more structurally relevant remodeling response in different anatomic locations or in bone with different architectural (cortical vs. cancellous) properties [119,120].

Prostanoids and Nitric Oxide

Since osteocytes are well situated within the bone matrix to sense strain, convey strain-related information, and influence appropriate bone remodeling [99,121,122], osteocytes are likely to be the primary mechanosensors in bone. These cells have been demonstrated to be mechanoresponsive and, within the range of strains they encounter in vivo, to produce significantly higher levels of the signaling molecules PGE_2 and PGI_2 than osteoblasts in response to pulsating fluid flow [106,123,124]. This in vitro osteocytic response is significant since in vivo inhibition of prostaglandins using indomethacin prevents bone adaptation in response to mechanical strain [43,125,126]. If indomethacin is administered in vitro or ex vivo strain-related increases

in G6PD and RNA are eliminated [72,125,127,128]. This suggests that a prostanoid-dependent step precedes these early signaling events. In vivo inhibition with NS-398 demonstrates the even more specific role of COX-2 in mediating the bone formation that occurs in response to loading [129]. Fluid shear stress induction of COX-2 mRNA expression involves the ERK signaling pathway as well as the C/EBP β, cAMP-response element binding protein, and AP-1 regions of the COX-2 promotor gene. [130,131]

In order to understand the cellular mechanism by which strain stimulates prostaglandin production, researchers have used selective blockers of prostaglandin production in order to inhibit specific aspects of this pathway [117]. Pulsating fluid flow-induced PGE_2 release can be decreased using specific inhibitors of Ca^{2+}-activated phospholipase C, protein kinase C, and phospholipase A_2. By blocking calcium channels and intracellular calcium release pulsating fluid flow-induced PGE_2 production is also markedly inhibited. Disruption of the actin cytoskeleton with cytochalasin B, likewise has a similar effect. These findings suggest that pulsating fluid flow transduces mechanical events into cellular signals by raising intracellular Ca^{2+} through ion channels and inositol trisphosphate (the product of phospholipase C)-induced Ca^{2+} release from intracellular stores. Ca^{2+} and protein kinase C could then stimulate phospholipase A_2 activity, arachidonic acid production, and finally PGE_2 release [117]. Furthermore, results from ex vivo loading of rat ulna explants suggest that loading-related release of PGI_2 and PGE_2 utilizes arachidonic acid-derived from the activity of different phospholipase A_2s [132]. In osteocytes and osteoblasts, arachidonic acid for PGI_2 synthesis is liberated by G-protein-dependent secretory phospholipase A_2 alone. In osteoblasts, arachidonic acid for PGE_2 synthesis is released by G-protein-dependent, cytosolic phospholipase A_2-mediated activity, which also requires upstream secretory phospholipase A_2 and protein kinase C activities. These findings support those of earlier researchers who used models of fluid shear [133] and longitudinal substrate distension [78] to demonstrate the involvement of PGE_2 in the remodeling response via activation of G proteins [133] and phospholipase A_2 [134].

The release of PGE_2 has important anabolic actions in bone as demonstrated by its ability to promote recruitment of osteoblast precursor cells, and increase subsequent osteoblast proliferation, alkaline phosphatase (AP) activity, and collagen synthesis [121,135,136]. In response to pulsating fluid flow and organ culture rat ulna loading, PGE_2 is released from calvarial osteocytes and surface lining cells [72,137]. In vivo administration of PGE_2 enhances bone formation in response to four-point bending [138]. Fluid shear stress and mechanical stretching likewise increases cAMP in a manner that is PGE_2 dependent [101,134]. PGE_2 is partly responsible for fluid flow induced increased levels of inositol trisphosphate (IP_3), which may act to further increase intracellular calcium and activate protein kinase C [133].

Mechanical stretching of young osteocytes results in increased influx of extracellular Ca^{2+} [117,139–141]. In explants of rat ulna subjected to physiologic levels of compressive axial loading (-3000 microstrain), blocking stretch sensitive cation channels with gadolinium chloride abolished loading-related increases in the release of PGI_2, NO, and osteocyte G6PD. Gadolinium also reduced the loading-related release of PGE_2 and G6PD in osteoblasts. Blocking of L-type voltage-dependent calcium channels with nifedipine prevented loading-related increases in PGE_2, NO, and osteoblast G6PD activity [142]. These studies suggest that calcium influx precedes the rapid increase in G6PD activity that has been demonstrated to occur in proportion to peak strain magnitude [69]. Further study has shown that the strain-related responses of osteocytes and osteoclasts involve gadolinium-sensitive cation channels, whereas the osteoblast response involves both gadolinium-sensitive and nifedipine-sensitive channels [141–143].

Nitric oxide (NO) has been shown to act as a mediator of mechanically induced bone formation. Both nitric oxide (NO) and prostanoid release are increased following exposure to physiologic levels of mechanical strain induced by axial loading and fluid flow in organ and cell cultures of calvarial and long bone osteocytes and osteoblasts [72,85,137,144–146]. This suggests a prominent role for both of these endogenous mediators in the adaptive response. Mechanically induced formation of NO appears to result from activation of endothelial NOS (eNOS) in bone cells [147,148], suggesting that the mechanism for bone cell upregulation of NO may be similar to the sensitivity of blood vessel endothelial cells to shear stresses associated with blood flow. If true, this similarity may provide yet further support for the involvement of cation channels and increases in intracellular calcium in bone's cellular response to mechanical strain [147,149]. Transients in fluid flow have been shown to stimulate NO release in osteoblasts which, like PGE_2 release, occurs in a manner that is dependent on G-proteins and calcium [146]. The in vivo inhibition of NO or prostaglandin production hinders mechanically induced bone formation in rats, providing a tentative link between the strain-related prostanoid and NO production in osteocytes, and the mechanotransduction of an osteogenic response [125,150–152]. The inhibition of NOS activity prevents release of PGE_2 following a fluid flow stimulus, suggesting that upregulation of NO production precedes PGE_2 upregulation in the cellular response to a strain stimulus [137]. However, it has also been shown that indomethacin (the non-selective COX inhibitor) and 15-hydroperoxyeicosatetraenoic acid (a selective inhibitor of prostacyclin synthase), can prevent loading-induced nitric oxide release [85,153,154], suggesting that strain-related NO production and prostacyclin upregulation may occur concurrently. Since cytochrome P450 activity is responsible for the release of PGI_2, and P450 reductase activity is responsible for the release of NO, it has been suggested that an interaction may occur at this level [155].

Growth Factors

A feature of the early proliferative response to mechanical loading stimuli is the release of insulin-like growth factors (IGFs). There are two forms, with IGF-I more predominantly produced in human osteoblasts and IGF-II more common in murine osteoblasts [156]. In human bone cell cultures, there are skeletal site-dependent differences in the production of IGF system components (with IGF binding proteins estimated to be produced at a higher magnitude than the IGFs), and IGF-II levels are higher than IGF-I [157]. Both IGF-I and -II stimulate proliferation and differentiation of bone cells [158]. In sites of bone resorption, large amounts of stored IGFs are released, suggesting that IGFs could couple bone formation to bone resorption [158]. When primary cultures of rat long bone-derived osteoblast-like cells are subjected to dynamic four-point bending (3400 $\mu\varepsilon$, 600 cycles, 1 Hz), both G6PD activity and the smallest transcript of IGF-II (IGF-II T3) increase, but IGF-I levels are unaffected [159]. Prostacyclin, but not PGE_2, also stimulates the early release of IGF-II in organ culture of adult canine cancellous bone [70]. Similarly, strain-related proliferation of ROS 17/2.8 cells appears to be mediated by IGF-II, rather than either IGF-I or the IGF-I receptor [160]. Exogenous administration of PGE_2 can cause a dramatic increase in IGF-I along with increased G6PD activity and increases in the largest transcript of IGF-II (IGF-II T1), but this transcript may not be actively translated into the IGF-II protein [159]. In contrast, in vivo mechanical loading of the rat tail vertebrae (peak microstrain magnitude = 700 microstrain) increased osteocytic expression of mRNA for c-fos and IGF-I that led to subsequent expression of type-I collagen and osteocalcin on the bone surface [161,162]. Similarly, cells stretched by the Flexercell apparatus (expected peak microstrain = 4000 microstrain, possible range = 0–30,000 microstrain) cause newborn rat calvarial cells to respond with an increase in IGF-1 production when they are in the late osteoblast/early osteocyte stage of differentiation [163].

Circulating parathyroid hormone (PTH) increases with weight-bearing exercise [164]. Using a hypotonicity-induced stretch-loading model, PTH has been shown to stimulate an increase in Ca^{2+} while synergistically elevating IGF-I mRNA levels in rat and chicken osteocytes [141]. Nucleotide activation of P2Y receptors may also sensitize cells to the action of PTH, providing a means for adaptive bone remodeling in response to both local and systemic signals [165]. The pathway by which this occurs is integrated with the production of other factors and likely involves the influx of Ca^{2+} and activation of PKA. This, in turn, activates c-fos and COX-2 transcription resulting in the production of IGF-I and osteocalcin [140,162]. Dissimilarity in the reported importance of IGF-I and IGF-II in response to mechanical loading may result from the diversity of model systems used to induce mechanical strain, the differences in calvarial versus long bone responses to mechanical strain [148,166], or the variable age-related responsiveness of cell cultures to mechanical and biochemical stimulation [167].

Physiologic levels of strain have recently been shown to enhance bone-resorbing activity associated with TRAP and cathepsin K mRNA expression in osteoclasts [143]. As with osteocytes and osteoblasts, Ca^{2+} entry may be involved in osteoclast mechanosensitivity and activity regulation. Osteocytes, osteoblasts, and osteoclasts attach to their substratum via osteopontin (OPN) interaction with integrins (particularly $\alpha_v\beta_3$-integrin) and other adhesive receptors, which may also be involved in the responses to bone strain [168–170]. Integrins interact with extracellular matrix proteins and intracellular actin filaments to create a connection which may promote fluid shear-induced increases in COX-2 and c-fos in MC3T3-E1 osteoblasts [109]. Cyclical pressure results in membrane hyperpolarization and PGE_2 production via integrin-mediated release of IL-1β in human bone cells [171]. Cytoskeletal reorganization associated with mechanical deformation may be involved in osteocyte detection of bone strain and enhancement of osteoclast bone resorbing functions [143,169,172]. Osteocyte OPN mRNA expression increases in response to mechanical loading, and in sites of bone formation and resorption, suggesting that it may have a paracrine effect on the activity of osteoblasts and osteoclasts [173–176].

It is important to realize that mechanical loading results in a number of different physical events, all of which may direct different responses at different levels. Not all of these are the natural stimulus for functional adaptation. For example, osteoblasts subjected to approximately 70,000 microstrain at 0.05 Hz have $\alpha_v\beta_3$-integrin-dependent enhanced extracellular matrix mineralization [168]. Conversely, short-term experiments have shown that cyclic strain at physiologic magnitude (1000 microstrain) causes osteoblast proliferation and collagen synthesis associated with matrix production while simultaneously decreasing alkaline phosphatase (AP) activity and osteocalcin release associated with matrix maturation and mineralization [177]. Pulsatile fluid flow has similarly been shown to cause a reduction in AP mRNA expression [178]. Continuous compressive pressure has been shown to increase PGE_2 while decreasing AP activity, with PGE_2 administration decreasing AP activity in a dose-dependent manner [75]. This decrease in AP activity is followed by mineral deposition in three-dimensional culture with flow [178]. These studies are consistent with the concept that a threshold physiologic strain is necessary to increase matrix production in a manner necessary for functional adaptation [35,177,179].

Failure of the Adaptive Response to Loading: Osteoporosis and Estrogen

Although the mechanisms of adaptive bone (re)modeling are among the most reliable in the body there are instances where their failure reminds us of their

fundamental importance. One of the most widespread of these is osteoporosis. This condition, which affects 28 million Americans, 80% of whom are women [180], is characterized by a failure to maintain bone architecture sufficiently robust to withstand the loading of everyday life without substantial risk of fracture. Although the condition has traditionally been approached as being the result of endocrine disorder, dietary deficiency, or genetic predisposition, we consider it may be a primary failure in the mechanically adaptive mechanism normally responsible for ensuring a safe relationship between bone loading and bone structure [120].

Although exercise is important to bone maintenance throughout life, bone mass changes less in response to exercise in adults than in growing animals [181–184]. Experimental evidence suggests that this may be due to changes in cellular response to mechanical stimuli with ongoing bone formation, age [184–186], estrogen loss [187,188], and the presence of osteoporosis [189]. Normally, bone cells respond to mechanical loading by increasing their metabolism, activating genes, producing growth factors, and synthesizing bone matrix [99]. Reductions in estrogen may impair the capacity of bone cells to respond to mechanical stress, resulting in osteoporosis [187,190–193]. Prevailing strain, however, likely directs the localized remodeling effects that occur in conjunction with more ubiquitous changes in systemic hormonal status [13,192].

The normal adaptive response of bone cells which accurately matches bone structure to prevailing weight-bearing changes dramatically as a consequence of estrogen loss at menopause [191,194]. The reason for this is uncertain, although it has been suggested that the presence of estrogen facilitates the recognition of a strain threshold which is necessary for an appropriate adaptive response [195,196]. Estrogen deficiency leads to preferential bone loss in anatomical sites of low mechanical strain [192]. The PGE_2-related response to mechanical strain is enhanced in the presence of estrogen and reduced in isolated bone cells from osteoporotic patients [188,189]. Both estrogen and mechanical strain have been shown to influence the expression of other paracrine factors, such as TGF-β and IGF-1, in several in vitro and in vivo models of bone metabolism [197–200]. In contrast to osteoblasts from normal donors, osteoblasts isolated from human osteoporotic bone fail to increase proliferation and TGFβ release in response to 1% (supraphysiologic) cyclic strain [201]. Both mechanical loading and estrogen reduce osteocyte apoptosis, effectively sustaining osteocyte populations [202–204]. Estrogen also promotes osteoclast apoptosis [205,206], reducing their capacity for resorption. In contrast, both loading and estrogen increase periosteal cell division and matrix synthesis in bones of male and female rats [207].

Such compelling associations between estrogen and mechanical strain have led several authors to suggest that strain and estrogen likely share a common mechanistic pathway [187,191,192,201]. Recent studies suggest that important components of this pathway include the estrogen receptor (ER) and ERK [208–212]. Functional ER is required for estrogen's ability to block ovariectomy-induced bone loss in rats [213], and it has been suggested that estrogen responses in bone may become defective as a result of impaired ERα expression in men and women [209]. The early responses of osteoblasts to strain and estrogen have been demonstrated to share a common pathway of ERK-mediated phosphorylation of ser^{122} within the amino terminus of ERα and to stimulate estrogen response element (ERE) activity [210,214]. ERK activation in response to strain and estrogen is dependent on changes in calcium levels [212,215], and may also be induced via cytoskeletal components, including FAK and integrins [216,217]. This supports the notion that ERα is involved in the early cascade of events which occur following mechanical strain [210]. Furthermore, the osteoblast proliferation stimulated by mechanical strain and estrogen can be inhibited by selective estrogen receptor modulators (SERMs) [160,211,218].

It may be that changes in ERα level provide a mechanism by which strain-related information and circulating estrogen can be integrated to engender appropriate adaptive remodeling. Bone cells have a relatively low level of ERα [219,220] which may confine, or create a threshold for, the extent of possible adaptive activity which can occur in response to estrogen and strain. By increasing osteoblast ERα number through stable transfection, for example, the proliferative responses to strain and estrogen can be enhanced [210,214]. In vivo osteocyte ERα levels decrease with estrogen withdrawal and idiopathic osteoporosis [221–223], suggesting that estrogen levels play an important feedback role in the control of ERα levels. Mechanical strain may also be an important determinant of osteocyte ERα expression, affording a potential means for localized direction and refinement of estrogen's influence on bone remodeling. In vivo studies of adult male rats have demonstrated that exposure to short daily periods of artificial loading (superimposed on normal loading) diminishes the number of osteocytes expressing ERα by 46% [224,225].

Osteoporotic bone has a reduced mass and a structurally inadequate architecture and thus probably experiences relative increases in functional strain during 'normal' daily activity. These relative increases in strain could downregulate ERα levels, compounding the reduction of ERα expression associated with estrogen deficiency [221], and further impair the capacity for appropriate adaptive remodeling in postmenopausal women. The ability of strain to effect ERα expression provides a potential means by which estrogen's influence on bone can be anatomically targeted to ensure a mechanically competent skeleton. The combination of strain and estrogen-related reductions in ERα expression could explain the reduced ability of bones to adaptively remodel following the menopause [120]. Alternatively, the level of ERα expression in bone may alter the ability of bone cells to respond to mechanical strain.

Summary

1. The fracture resistance of bones is established and maintained as the result of an adaptive mechanism in which bone cells' modeling and remodeling activity is directly or indirectly influenced by their strain environment.
2. The mechano-responsive cells are most likely osteocytes and osteoblasts. Many studies show acute mechanically related changes in behavior of these cells in culture but many of these have not been related to the long term control of remodeling on which adaptive control of bone architecture depends. Not all bone cells' responses to mechanical stimuli are part of the cascade of responses involved in adaptive (re)modeling. The specific mechanical stimuli to which osteocytes and osteoblasts respond in vivo probably include changes in strain itself and strain generated changes in their fluid environment. Physiologic levels of bone strain have been demonstrated to be directly and indirectly involved in increasing the release of signaling molecules and anabolic growth factors, such as PGE_2, PGI_2, NO, and IGF-I and IGF-II, that stimulate bone cell proliferation and matrix formation.
3. The osteoregulatory nature of a natural strain regimen appears to be determined primarily by the peak strains achieved, the rate of strain change, and the extent to which the strain distribution is different from the normal strains to which the bone has adapted. Loading regimens producing high strains, high strain rates and unusual strain distributions appear to have a high osteoregulatory potential stimulating osteogenic responses and maintaining high bone mass. Static strains, strains which change slowly, and 'error-free' strains may have little or no osteoregulatory potential and may thus permit (if not engender) bone loss and low bone mass.
4. The positive osteoregulatory influence of a strain regimen is most effective during growth. Appropriate activity at this time can lead to a larger and more robust skeleton that can maintain its strength despite loss of bone tissue later in life.
5. The reduced ability to maintain bone strength in postmenopausal women is a failure of the normally adaptive response to mechanical strain under the conditions of the postmenopausal state. Evidence that early strain-related responses of bone cells involve the estrogen receptor could explain the decreased effectiveness of this pathway when estrogen receptor levels are low postmenopausally. The high strains assumed to be associated with low bone mass may downregulate ERα expression thus further diminishing the responsiveness of bones to loading.

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