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Exercise and the Prevention of Osteoporosis

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Osteopenia, a condition of diminished bone mass, becomes osteoporosis when mechanical demands exceed the ability of the skeletal structure to support them. Consequences of bone loss are exacerbated by an age-related decrease in muscle strength [1] and postural stability [2], markedly increasing the risk of falls, fracture and—ultimately—mortality [3]. Mechanical signals generated by exercise can mitigate bone loss as well as help preserve the musculoskeletal "system." The physical and/or biologic basis of how mechanical signals are transformed into anabolic agents for bone and other tissues is called mechanotransduction and may represent the foundation for a nondrug approach to treat osteoporosis [4].

Bone quantity and quality is enhanced by exercise and compromised by inactivity, age, and disuse [5]. Cross-sectional studies illustrate the sensitivity of bone morphology to physical extremes. Astronauts subject to microgravity lose up to 2% of hip bone density each month [6], while professional tennis players possess 35% more bone in the dominant arm as compared to the arm that simply throws the ball into the air [7]. A broader benefit can be seen in professional athletes involved in a variety of demanding activities, including soccer players, weight lifters, speed skaters, and gymnasts [8, 9].

Several prospectively designed trials emphasize that functional loading result in increased bone mass. Intense exercise in young army recruits [10] stimulated large increases in bone mineral density (BMD), while a 10-month, high-impact strength building regimen in children significantly increased femoral neck BMD [11]. A number of longitudinal exercise studies, however, have reported only modest increases in bone mass [12]. For example, a 1-year high-resistance strength training study in young women significantly increased muscle strength but failed to influence bone mass [13]. And even if the goal of exercise is to slow the loss of bone in the elderly, there is increasing evidence that bone tissue is less responsive to mechanical stimuli as we age [14]. Before exercise in general, and mechanical signals in particular, can be effectively used to prevent the degradation of the musculoskeletal system with age, we must improve our understanding of both the mechanical milieu generated by exercise and the complex cellular machinery that perceive and respond to these key regulatory signals [15].

MECHANICAL FACTORS REGULATING BONE CELL RESPONSE

Skeletal loads and bending moments resolve into strain in the bone tissue, reaching up to 0.3% (3,000 microstrain) during strenuous activity, a level of matrix deformation common across a range of species [16]. The strain levels actually "experienced" by bone cells *in vivo* is unclear, but may be as much as 10 times that experienced by the matrix [17]. Bone cells also experience interstitial fluid flow dynamic pressure changes during mechanical loading [18]. Furthermore, functional loading also induces pressure in the intramedullary cavity [19], shear forces through canaliculi [20], and dynamic electric fields as interstitial fluid flows past charged bone crystals [21]. As

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such, the complex loading environment of the skeleton generates a diverse range of mechanical signals that are ultimately inseparable [22], but each of these biophysical factors may differentially target tissue, cell, and molecular activity.

Animal models demonstrate that bone remodeling is sensitive to changes in strain magnitude [23], the number of loading cycles [24], the distribution of the loading [25], and the rate of strain [26]. Importantly, the load signal must be dynamic (time varying), as static loads are ignored by the skeleton [27], while the anabolic potential increases when rest periods are inserted between the mechanical events [28]. Even extremely low-magnitude bone strains, three orders of magnitude below peak strains generated during strenuous activity, when induced at high frequencies similar to the spectral content of muscle contractibility [29], are anabolic to bone tissue [30], enhancing not only bone mass but bone quality and strength (Fig. 46.1). Together, these findings emphasize that the "key ingredient" to an osteogenic exercise regimen cannot simply be distilled to "bigger is better."

Physiologic levels of strain reduce osteocyte apoptosis, suggesting matrix deformation is critical to the survival of these cells [31, 32]. Too much strain, though, can cause matrix microdamage and exacerbate death of adjacent cells [33]. Factors other than matrix strain also cause an adaptive cellular response; e.g., acceleration, achieved independently of direct loading, can be anabolic to bone [34], suggesting that cells may act as "accelerometers" that respond to dynamic changes in force [35], emphasizing that *by-products* of the strain signal, such as shear stress or strain-generated potentials, may be critical to regulating the biology of the adaptive response. But *how* do the cells sense these mechanical signals?

MECHANICALLY RESPONSIVE BONE CELLS

The sensitivity of bone cells to mechanical signals, including stromal cells, osteoblasts, and osteocytes has been well documented [36], but it is difficult to designate a critically responsive cell. While the osteoblast is critical for the adaptive response, the osteocyte, representing 95% of adult skeletal cells, may prove key to bone tissue plasticity [37]. The antenna-like three-dimensional morphology of this osteocyte syncytium, interconnected by regulated gap-junctional connexins [38], are ideally configured to perceive and even amplify biophysical stimuli [20]. In particular, the removal of load is tied to osteocyte activity; loading regulates the release of sclerostin from osteocytes, an osteocyte product involved in Wnt signaling [39]. The osteoclastic bone resorption accompanying hind limb unloading in mice is ablated when osteocytes are absent [40], as the unloaded osteocytes release receptor activator of nuclear factor-κB ligand (RANKL) [41].

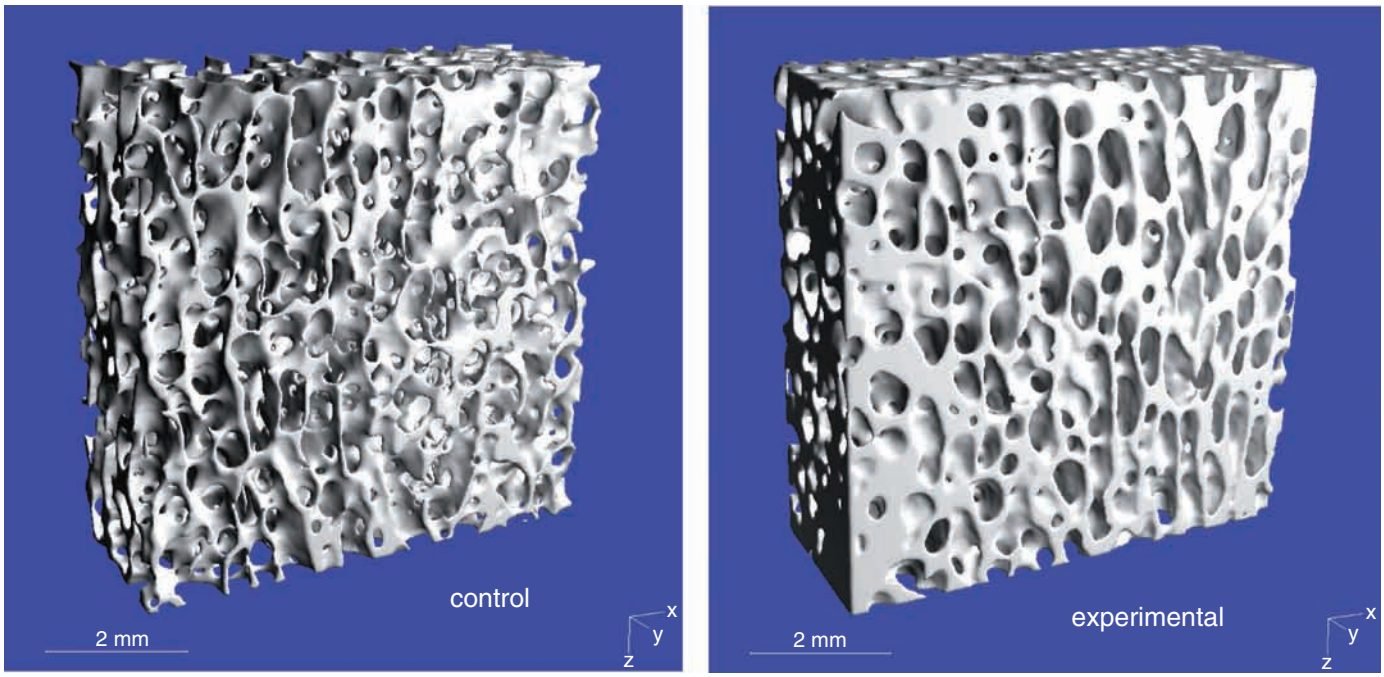

Fig. 46.1. Microcomputed tomography of the distal femur of adult (8-year-old) sheep, comparing a control animal (left) to an animal subject to 20 min/day of 30 Hz (cycles per second) of a low-level (0.3 g) mechanical vibration for 1 year (Ref. 76). The large increase in trabecular bone density results in enhanced bone strength, achieved with tissue strains three orders of magnitude below those that cause damage to the tissue. These data suggest that specific mechanical parameters may represent a nonpharmacologic basis for the treatment of osteoporosis (Ref. 71).

The connectivity of the osteocyte network deteriorates markedly with age and may contribute to the progressive loss of sensitivity of bone to chemical and physical signals [14].

Marrow stromal cells change proliferation and gene expression in response to mechanical stimulation [42] and through mechanical regulation of RANKL expression [43] also affect osteoclast number and function. The osteoclast also responds directly to mechanical signals limiting bone resorption [44]. Other cells present in bone, such as shear sensitive endothelial cells in the penetrating vasculature, likely contribute to the adaptive response by producing nitric oxide [45], which has pleiomorphic effects on the skeleton [46].

The role of mesenchymal stem cells (MSCs) in regulating the adaptive response to mechanical signals is also being investigated, with the demonstration that exercise can bias MSCs toward osteoblastogenesis [47], while disuse increases adipogenesis within the bone marrow [48]. Even low magnitude mechanical signals have been shown to influence the fate of MSCs, driving them toward a musculoskeletal lineage [49] while suppressing adipogenesis [50]. Considering the interdependence of fat and bone tissue [51], it may be that exercise-based prevention of obesity and osteoporosis could be achieved through regulation of MSC lineage rather than necessarily the resident cell population in fat (adipocytes) or bone (osteocytes) [52].

TRANSDUCING MECHANICAL SIGNALS INTO CELLULAR RESPONSE

When bone strains of 3,000 microstrain, realized during strenuous activity [16] are resolved to the level of the cell, these deformations are on the order of Angstroms, requiring an exquisitely sensitive receptor system. Further, cell mechanoreceptors must either be in contact with the outside, through the cell membrane and its attachment to substrate, or the mechanoreceptor must be able to sense by-products of load, such as fluid shear on the apical membrane. While in sensory organs there are examples of channels that are regulated by movement of mechanosensory bristles [53], or by tension waves [54], a unified model of proximal events inducing intracellular signal transduction in non-sensory tissues does not yet exist. There are, however, several components of the cell that could act as the mechanoreceptor, transducing a physical challenge into a cellular response.

Ion channel activity in osteoblasts stimulated by stretch/strain of the membrane [55] or by parathyroid hormone (PTH) [56] have been associated with bone cell activation. Patch-clamp techniques demonstrate at least three classes of mechanosensitive ion channels [57]. In limb bone cultures, gadolinium chloride, which blocks some stretch/shear-sensitive cation channels, blocked load-related increases in PGI2 and nitric oxide [58].

Membrane deformation and shear across the membrane, as well as pressure transients, are transmitted to the cytoskeleton and ultimately to the cell-matrix adhesion proteins that anchor the cell in place [59]. Membrane-spanning integrins, which couple the cell to its extracellular environment, and a large number of adhesion-associated linker proteins are potential molecular mechanotransducers. The architecture of the cytoskeleton with its microfilamentous and microtubular network linking adhesion receptors to the cell nucleus plays a role in perceiving small deformations and directly informing the nucleus [60]. Application of strain to MSCs induces focal adhesion assembly, which amplifies force generated signaling [61].

Cellular cytoskeletal adaptation to force is even more subtly reflected in a compartmentalization of signals within the several phases of liquid-ordered and liquid-disordered lipid making up the plasma membrane [62]. Organized lipid rafts are thought to act as mechanical sensors: in endothelial cells, shear stress causes signaling molecules to translocate to caveolar lipid rafts, and if the caveolae are disassembled, both proximal and downstream mechanical signals, including MAPK activation, are abrogated [63]. In bone cells, many of the outside-in signaling systems that respond to force are sequestered in lipid rafts.

With the multiplicity of mechanical signals presented to the cell, it is likely that no single mechanosensor or receptor mechanism is responsible for perceiving and responding to the mechanical environment (Fig. 46.2). At the very least, multiple mechanosensors are likely to interact to integrate both mechanical and chemical information from the microenvironment.

Since the distal responses to mechanical factors are similar to those elicited by ligand-receptor pairing and result in changes in gene expression, mechanotransduction must eventually end up utilizing similar intracellular signaling cascades. Mechanical forces have been shown to activate every type of signal transduction cascade, including β-catenin [64], mTORC2 [65], cAMP [66], IP3 and intracellular calcium [42, 67], guanine regulatory proteins [68], and MAPK [69], among others [70]. Given the pivotal role of mechanical stimuli in regulating anabolic and catabolic cell activity, perhaps it should not be so surprising that so many signaling pathways are involved.

TRANSLATING MECHANICAL SIGNALS TO THE CLINIC

There is growing clinical evidence that mechanical signals, even those that are extremely low magnitude, can be anabolic to bone [30], particularly in the young skeleton. In the first study, the ability of these low level signals to improve bone mass, delivered using low intensity vibration, was examined in children with disabling conditions [71]. Children were randomized to stand on an actively vibrating (0.3 g, 90 Hz) or placebo device for 10 min/day. Over a 6-month trial, proximal tibial volumetric trabecular BMD (vTBMD) in children on active devices increased by 6.3% while vTBMD decreased by 11.9% on placebo devices ($p < 0.01$). In the second study,

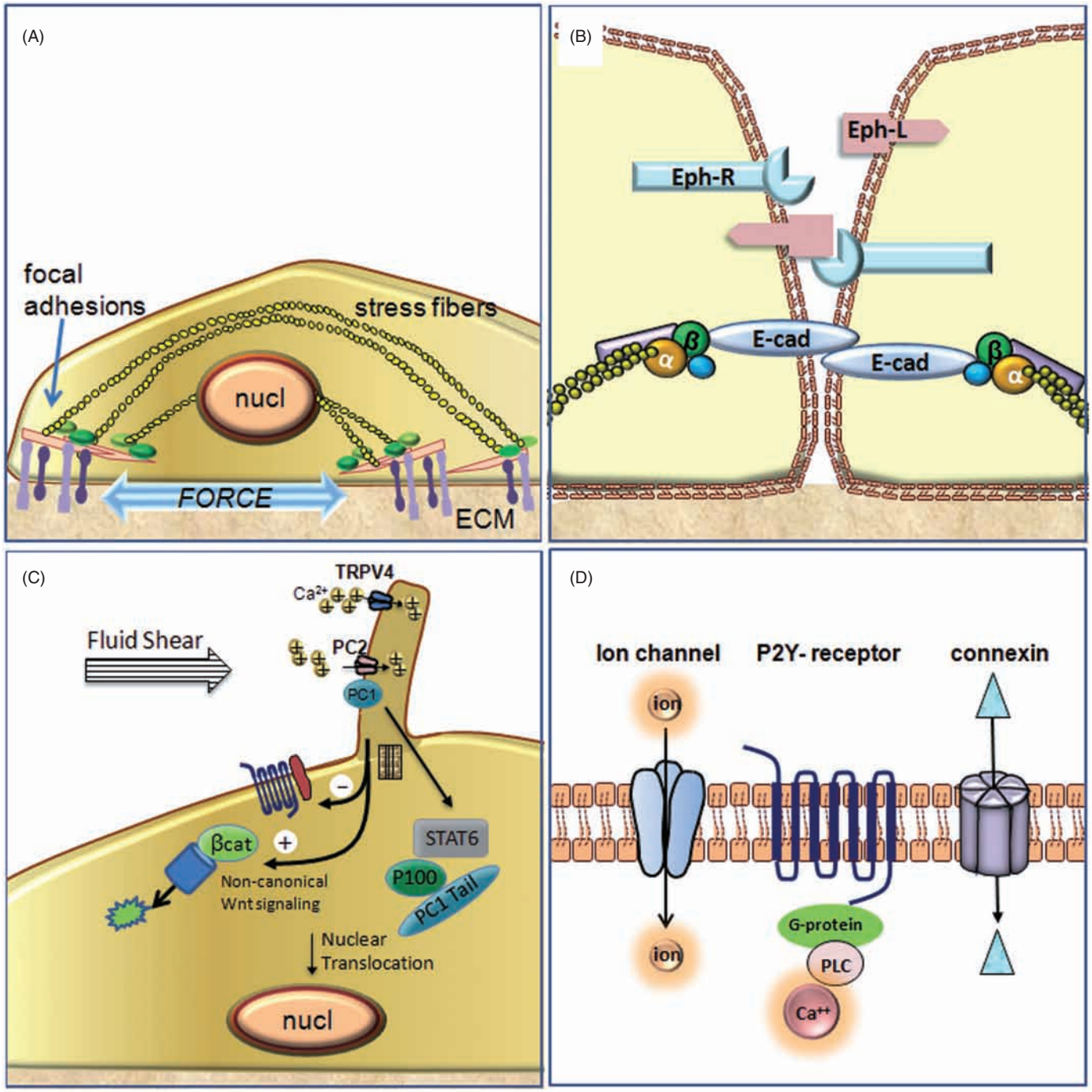

Fig. 46.2. Skeletal loading generates deformation of the bone matrix and acceleration/deceleration of the tissue system, resulting in strain across the cell and pressure within the marrow cavity and mineralized cortices. Functional load bearing also results in shear forces through the canaliculi, with the bulk fluid movement causing drag over cells and streaming potentials from charged interactions with the bone crystals. Some aggregate of these physical signals distorts the morphology of the cell. Some composite of these physical signals interacts with the morphology of the cell through interactions such as potentiated by the membrane–matrix or membrane–nucleus structures, or distortion of the membrane itself, to regulate transcriptional activity of the cell. Several candidate mechanotransducer systems are illustrated. (A) Cell cytoskeleton senses loading at the membrane through integrins that transmit force through focal adhesions and F-actin stress fibers. (B) Cadherins, which connect to the cytoskeleton, are examples of outside-in signaling modifiers. Ephrins exemplify an intercellular signaling system regulated by movement of components within the plasma membrane. (C) Primary cilia may sense flow, pressure, and strain, activating ion flux through PC1 and TRPV4, which can activate Stat signals. Cilia also modulate Wnt signaling via noncanonical antagonism that leads to β-cat degradation. (D) Membrane spanning proteins such as ion channels, purinergic receptors and connexins can be regulated through shear and strain. Modified from Thompson WR, Rubin CT, Rubin J. 2012. Mechanical regulation of signaling pathways in bone. *Gene* 503(2): 179–93.

a 12-month trial was conducted in 48 young women in the lowest quartile of bone density and at least one skeletal fracture [72]. Subjects were randomly assigned either into a daily, low-magnitude whole body vibration group (10 min/day, 30 Hz, 0.3 g) or control. Intention-to-treat data indicated that cancellous bone in the lumbar vertebrae and cortical bone in the femoral midshaft of the experimental group increased by 2.0% ($p = 0.06$) and 2.3% ($p = 0.04$), as compared to controls. Importantly, the cross-sectional area of paraspinous musculature was 4.9% greater ($p = 0.002$) in the experimental group versus controls. Per protocol analysis indicates that subjects who used the device at least two minutes each day realized a much greater musculoskeletal benefit from the mechanical signals. These low intensity mechanical signals have also been shown to help protect postural stability during chronic bedrest [73] and increase bone mass and muscle strength in the upper limbs of disabled children, thereby improving their autonomy [74].

SUMMARY

As bone geometry and material properties vary between individuals, there are also genome-specific sensitivities to mechanical loading which may help explain the variability in exercise based trials.[75] However, evidence in the animal and human indicates that exercise in general, and mechanical signals in particular, are both anabolic and anti-catabolic to the musculoskeletal system, and benefit both bone quantity and quality. The challenge remains to identify those parameters within the complex mechanical milieu induced by exercise that are critical to driving anabolism in bone, and that ultimately may represent the basis of a nondrug strategy to control musculoskeletal health. While bone-targeted pharmaceutical interventions represent effective means of curbing osteoporosis, exercise, in its many varied forms, is self-targeting, endogenous to the musculoskeletal system, and auto-regulated, causing site-specific positive adaptation in bone mass and structure. And as effective as drug treatments for osteoporosis may be, physical signals have had a 525 million year head start in terms of optimizing skeletal structure to withstand the loads placed upon them.

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