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Perspective Article

Hylonome

Biological underpinnings of Frost's mechanostat thresholds: The important role of osteocytes

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Abstract

Harold Frost first proposed the existence of several mechanical thresholds in bone, two of which determine whether bone is added to, or lost from, the skeleton. Recent evidence from bone biology helps elucidate the role of osteocytes in determining these mechanical thresholds. Specifically, when mechanical stimuli fall below the resorption threshold, osteocyte apoptosis occurs, followed by bone resorption. Conversely, mechanical loading maintains osteocytes viability, and consequently, no bone is lost. With a greater than customary mechanical stimulus, osteocytes perturbation from pulsatile fluid flow results in release of anabolic factors and subsequent bone formation. Osteocytes also play a pivotal role in bone remodeling in response to alterations in the mechanical environment. In particular, osteocyte apoptosis results in bone turnover in disuse as well as in response to greater than customary mechanical stimuli due to microdamage accumulation. Given the important role of osteocytes in bone modeling and remodeling, these cells provide an ideal target for both drug therapies and exercise to prevent bone fragility.

Keywords: Mechanostat, Osteocytes, Modeling, Remodeling, Mechanical Loading

Introduction

Harold Frost first introduced the mechanostat theory in which he outlined how postnatal human load-bearing bones adapt to changes in their mechanical environment[1]. Specifically, Frost proposed the existence of a homeostatic regulatory mechanism in bone responsible for forming or resorbing bone in response to deviations in customary mechanical loading. While the cells responsible for this bone formation and resorption (osteoblasts and osteoclasts, respectively) have been appreciated for some time, the sensory role in bone has only recently been hypothesized to be fulfilled by a third cell type—osteocytes. In this article, we review recent evidence from bone biology that osteocytes are indeed the primary mechanosensory cells in bone, and therefore, are critical for bone functional adaptation. We first introduce Frost's mechanostat theory and then review evidence for the role of osteocytes in determining the mechanostat's thresholds for bone formation and resorption. We conclude with some practical thoughts regarding the importance of targeting osteocytes for the prevention of bone fragility in later life.

Frost's Mechanostat

Although Frost was not the first to recognize that bones are responsive to mechanical loading, he was the first to provide a detailed theory regarding how load-bearing bones adapt to maintain mechanical competence in response to alterations in the mechanical environment. Frost suggested the existence of a homeostatic regulatory mechanism in bone responsible for sensing changes in the mechanical demands placed on bone and subsequently altering the mass and conformation of bone to better meet these new mechanical demands. Specifically, Frost postulated that several mechanical thresholds control whether bone is added or taken away from the skeleton. He theorized that below a certain threshold of mechanical use, bone is resorbed, and is therefore rid of excess mass. Above another threshold, in which bone is exposed to greater than typical peak mechanical loads, bone formation occurs on the existing structure to increase bone strength[1]. Thus, bone tissue has an intrinsic "mechanostat" which regulates bone functional adaptation. As with any homeostatic control system, bone's mechanostat

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must have several independent components, including a stimulus, a sensory mechanism that is capable of detecting the stimulus, and an effector mechanism that is able to bring the system back to homeostasis. We review these components below.

The mechanostat's stimulus

Frost originally proposed that the stimulus for bone functional adaptation is strain magnitude. Strain refers to the relative change in length of bone, or deformation of bone tissue, that occurs with loading. Evidence that bones appear to regulate the magnitude of strain comes from several animal studies that demonstrated that peak strains are kept within a close range across many different species[2]. However, since the mechanostat theory was first proposed, there have been a number of other strain-related characteristics that have been shown to play a role in the functional adaptation of bone including strain rate, the frequency of loading cycles, the amount of rest between loading cycles and bouts of loading, and the distribution of strain within the bone structure[3]. Skerry coined a new term for the stimulus of bone functional adaptation that incorporates these various strain characteristics into a unified concept—the customary strain stimulus (CSS, Figure 1). Importantly, Skerry acknowledged that the CSS is both sex and site specific and that it is genetically, biochemically, and pharmacologically modified[3].

The mechanostat's effector mechanisms

The roles of osteoblasts and osteoclasts in forming and resorbing bone, respectively, have been appreciated for some time. However, it was Frost who characterized two distinct and dynamic effector processes carried out by these cell types. Frost proposed that the process of modeling involves the independent action of osteoclasts and osteoblasts on the surfaces of bone whereby new bone is added along some surfaces and removed from others. Thus, modeling affects the size and shape of bones and is therefore a critical process for reshaping long bones during growth[4]. Given that modeling can refer to either the independent actions of bone formation or resorption, Frost coined the terms, "formation modeling" and "resorption modeling" to distinguish between these two processes. Bone's other major effector process, remodeling, is a localized process that involves the coupled action of osteocalsts and osteoblasts in which osteoclasts first resorb a small trench of bone, and osteoblasts are subsequently recruited to the site to form and mineralize new bone. Frost was the first to identify this coupled action of osteoblasts and osteoclasts[5]. Except in disuse, the amount of bone formed is generally equivalent to the amount of bone resorbed in each remodeling unit[6]. Nonetheless, this processes of bone resorption followed by formation can take several weeks to months to complete, and consequently, there is a temporary increase in porosity caused by remodeling[7] that can transiently alter whole bone strength. While the two distinct processes of modeling and remodeling are responsible for altering bone's material properties, structure, and strength in response to changes in the mechanical environment, there still remains confusion as to the different roles of these effector mechanisms in various mechanical states such as in disuse or overload. Understanding the biology of a third cell type of bone, osteocytes, in bone functional adaptation helps clarify both the stimuli for, and effects of, these two distinct processes of bone adaptation.

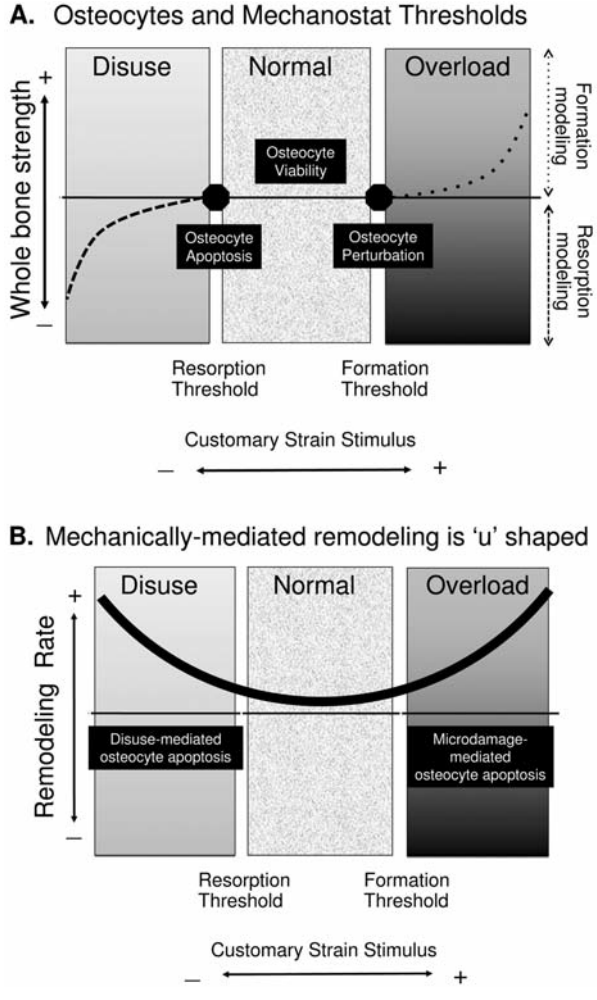

Figure 1. Schematics showing the role of osteocytes in determining mechanostat thresholds for bone modeling (A) and remodeling (B). **(A)** Whole bone strength is altered by formation modeling (dotted line) and resorption modeling (dashed line). Above the CSS, osteocytes are perturbed and formation modeling occurs to increase whole bone strength. A lower than customary CSS causes osteocyte apoptosis followed by resorption modeling primarily on the trabecular and endocortical surfaces, resulting in decreased whole bone strength. In the normal loading range, osteocytes remain viable and no bone is lost. **(B)** Mechanically mediated remodeling also occurs in response to mechanical loading but in a U-shaped manner such that the rate of remodeling increases with both increased loading as well as unloading. With an increase in customary loading, microdamage accumulates, resulting in osteocyte apoptosis and subsequent targeted bone remodeling to repair damage. In disuse, osteocytes apoptosis also occurs, possibly due to nutrient insufficiency, and the rate of remodeling is increased with each remodeling cycle resulting in a negative bone balance.

The mechanostat's sensory mechanism

As mentioned, the mechanostat's effector cells have been well characterized. However, the sensory cells of bone have only recently received more attention. This role is fulfilled by members of the mesenchymal stromal cell lineage—osteoblasts, osteocytes, and bone lining cells. Of these cells, osteocytes are highly connected by dendritic processes, are linked to the dendrites of neighboring osteocytes by gap junctions, and are abundantly distributed throughout the bone matrix allowing them to provide local indications of changes in the mechanical environment[8]. As discussed below, recent evidence reveals a crucial role for osteocytes in resorbing, forming, and maintaining bone mass in response to alterations in the mechanical environment.

Osteocytes perturbation with a higher than customary strain stimulus

Given that osteocytes are surrounded by a network of interstitial fluid-filled lacunae and canaliculi, it has been postulated that when bone tissue is deformed by mechanical loading, fluid pressure gradients are generated in which interstitial fluid will move from areas of compression toward areas of tension[9]. This fluid flow results in the perturbation of both osteocytes in their lacunae and dendrites in their canaliculi[8]. The osteocyte's integrins, G-proteins, cytoskeleton, ion channels, and cilia, all appear to play a role in sensing the mechanical signal and the transduction of this mechanical signal into a biochemical signal[8,10].

Within minutes of fluid shear stress on cultured osteoblasts and osteocytes, mobilization of intracellular calcium and release of several biochemical signals such as nitric oxide (NO), prostaglandins (PGE_2 and PGI_2), and adenosine triphosphate (ATP) occurs[8,10]. These signaling pathways are only now emerging and are not well characterized. However, the necessity of these factors in initiating an anabolic response to mechanical stimuli has been shown by an observed suppression of bone formation in response to an increase in mechanical loading with the use of the nitric oxide synthase inhibitor, L-NAME[11,12], and nonsteroidal anti-inflammatory drugs (prostaglandin synthesis blockers)[13-15]. Moreover, calcium channel blockers have been shown to prevent mechanical loading-induced release of prostaglandins[16], and mice with a null mutation in $P2X_7$ receptor—an ATP receptor that plays an important role in PGE_2 release[17]—show suppressed bone formation with mechanical loading. Following mechanical loading, the release of NO and PGE_2 from osteoblasts and osteocytes has been demonstrated to lead to the recruitment of osteoblasts from the marrow stroma[12,18]. *In vitro* studies of cultured bone cells have demonstrated that in response to mechanical stimuli, osteoblast proliferation as well as synthesis and mineralization of the extracellular matrix occurs[10]. This bone formation, in response to osteocyte perturbation with a higher than customary strain stimulus, occurs primarily on existing trabeculae as well as on the periosteal surface of long bones[19,20]. This type of bone formation (without prior bone formation), consequent to surpassing the formation threshold, is an example of the process of formation modeling.

Formation modeling is also dependent on sclerostin, a product of the Sost gene[21]. Sclerostin is secreted from osteocytes and negatively regulates canonical Wnt signaling—an important signaling pathway for osteoblast differentiation and function[22,23]. A recent study demonstrated that sclerostin was inhibited from secretion by mechanical loading, and moreover, regions of bone that experienced the highest strain stimulus had a greater reduction in the proportion of sclerostin-positive osteocytes[24]. By suppressing the release of sclerostin, mechanical loading results in enhanced Wnt/β-catenin signaling[25], and consequently, greater bone formation.

Osteocyte apoptosis with a lower than customary strain stimulus

Osteocytes have been implicated as the mechanosensors on the other end of the strain spectrum as well. In the case of a lower than customary strain stimulus, however, osteocyte apoptosis is the stimulus that results in bone functional adaptation to alterations in mechanical loading. Abundant evidence from both animal and human literature shows that, consistent with the mechanostat theory, bone is lost when strains in bone are lower than typical, such as in immobilization, bed rest, and spaceflight[26,27]. While the mechanisms for disuse-mediated bone loss are not well known, recent evidence suggests that osteocytes are an important regulator of bone loss[28,29]. Aguirre et al.[28] showed that within 3 days of tail-suspension in mice, osteocyte apoptosis incidence increased in both trabecular and cortical bone, followed by osteoclastogenesis and bone resorption two weeks later. Of note, in cortical bone, osteocyte apoptosis was concentrated on the endocortical surface which was subsequently resorbed—effectively reducing cortical thickness and whole bone strength[28]. In a supportive study, when approximately 70% of osteocytes were ablated *in vivo* in a rat model, the animals were resistant to subsequent disuse-mediated bone loss from hindlimb unloading—unlike control animals (with intact osteocytes) who experienced significant bone loss as expected[29]. These findings indicate that osteocyte apoptosis is necessary for bone resorption to be initiated when in a state of disuse. Below this "resorption" threshold, bone mass is lost, and according to animal studies, this loss occurs primarily on the endocortical surface of long bones in mature animals, as well as along trabecular surfaces[30,31]. This resorption of bone, independent of bone formation, is an example of resorption modeling.

How disuse leads to death of osteocytes is not well understood. However, a possible reason for osteocyte apoptosis with disuse is inhibition of nutrient supply to the osteocyte and inadequate removal of waste—both critical for metabolism in any living tissue. Knothe Tate et al[32], demonstrated in immature and mature rats that diffusion alone is insufficient for osteocyte supply of large molecules (e.g. proteins). The authors concluded that convective transport by means of a mechanism such as load-induced fluid flow is needed to supply osteocytes with important larger molecules[32]. Thus, in a state of disuse, lack of mechanical strain may lead to nutrient deficiencies in osteocytes and subsequent apoptosis and resorption of bone.

As in the case of a higher than customary strain stimulus, disuse-mediated bone loss may also be dependent on sclerostin. Sclerostin, as previously mentioned, is an inhibitor of Wnt/β-catenin signaling, and therefore, bone formation. A recent study[21] in which Sost-deficient mice were immune to bone loss from hindlimb unloading, highlights that sclerostin is necessary for bone loss to occur in disuse. Given that sclerostin decreases the viability of osteoblasts and osteocytes[21], it follows that an increase in sclerostin with unloading in Sost-replete animals likely plays a role in osteocyte apoptosis and subsequent bone resorption. However, this theory remains to be empirically tested.

Recent *in vitro* experimental studies have investigated the means by which osteocytes may be able to recruit osteoclasts for bone resorption, and it has been demonstrated that osteocytes secrete both receptor activator of NF-κB ligand (RANKL) from their dendritic processes and macrophage colony-stimulating factor (MFC)[8,33]. Both are essential cytokines for the stimulation of osteoclast differentiation. Furthermore, osteocytes are in direct contact with osteoblasts and bone lining cells (which also secrete RANKL) as well as the bone marrow (through their dendritic processes), which may allow for direct contact with osteoclast precursors[8,34].

Maintenance of osteocyte viability with a customary strain stimulus

While a lack of customary loading results in osteocyte apoptosis, conversely, several studies have demonstrated that mechanical stimulation actively prevents osteocyte apoptosis[35,36]. Noble et al.[37], demonstrated that short periods of mechanical loading of the ulnae of rats resulted in a 40% relative reduction in osteocyte apoptosis *in vivo* three days following loading compared to the same site on the contralateral limb. Similar findings were observed in an *in vitro* study which showed that fluid shear stress prevented serum starvation-induced osteocyte apoptosis and promoted osteocyte survival through increased expression of the anti-apoptotic marker, Bcl-2[35]. It has recently been demonstrated that in response to loading, NO plays a role in the expression of Bcl-2, and by extension, loading-induced osteocyte apoptosis[36]. The findings from these *in vitro* studies suggest that mechanical stimuli not only prevent osteocyte apoptosis but also promote osteocyte survival. It therefore follows that, in congruence with the mechanostat theory, a threshold of strain stimuli must be met to maintain osteocyte viability, and consequently, maintain bone mass (Figure 1A).

Summary of osteocytes and the mechanostat thresholds

Although bone mechanotransduction pathways are just beginning to be identified, it does appear that osteocytes provide a pivotal function in bone adaptation to mechanical demands (Figure 1). If a large enough strain stimulus is generated from customary loading, osteocytes will remain viable and no bone will be lost. Conversely, if strain stimuli are lower than normal, osteocyte apoptosis and subsequent bone loss will ensue. Should the strain stimulus be great enough to surpass a threshold of osteocyte perturbation, sufficient anabolic factors will be released from osteocytes to result in bone formation. In summary, osteocytes represent a primary step in bone modeling to alter whole bone strength in response to mechanical loading and unloading. Modeling, however, is not the only cellular process in bone that responds to mechanical stimuli. The process of remodeling is also regulated, often indirectly, by changes in the mechanical environment of bone.

Remodeling and microdamage repair

Like modeling, the rate of remodeling can increase with various alterations in the mechanical environment, and again, osteocytes play a critical role in this process. Like any structure bearing repetitive loads, bone accrues microdamage that can compromise its mechanical competence. However, unlike inert materials, biologically active bone is able to sense accrual of microdamage and replace it. It is estimated that human load bearing bones such as the tibia would fracture in only three years of normal loading[38] without such a mechanism of material repair. Similar to bone loss in disuse, bone resorption in response to microdamage is preceded by dying osteocytes. Evidence for this process comes from several animal studies, one in which fatigue loading in rat ulnae was shown to result in accumulation of microdamage, resulting in osteocyte apoptosis and subsequent intracortical remodeling of damaged bone[39]. These findings are particularly interesting given that cortical bone of rats do not typically experience remodeling. Similar results were found by Bentolila et al.[40], who reported intracoritcal remodeling in 14 of 16 rats that underwent 10 days of fatigue loading using the isolated ulna loading model. Further support for the role of microdamage in stimulating turnover comes from the two animals in this study that did not accrue bone microdamage—they did not experience intracortical remodeling[40].

How osteocyte apoptosis results in the bone resorption phase of remodeling is not fully evident, but osteocytes directly at the site of microcracks have been shown to express the apoptotic biomarker Bax, while adjacent osteocytes are shown to express the anti-apoptotic marker Bcl-2[41]. This suggests that dying osteocytes send out signals to be turned over while adjacent healthy bone cells send out protective signals -effectively providing an area code for bone resorption. The biochemical signaling between apoptotic osteocytes and osteoclasts remains to be determined. Yet, as previously mentioned, osteocytes are able to secrete pro-osteoclast factors such as MCF and RANKL, and there is evidence suggesting that damage to the osteocyte processes causes up-regulation of these factors[42]. Osteoclasts, in turn, are capable of recruiting osteoblasts to fill resorption cavities. With the observations that osteons (the remnants of bone remodeling in cortical bone) and trabeculae are aligned with the dominant loading direction, it has been postulated that this coupling of osteoclasts and osteoblast in remodeling is mechanically regulated. Several pathways for this cellular communication have been postulated including bidirectional signaling between osteoclasts and osteoblasts through the transmembrane ligand ephrinB2 expressed by osteoclasts and its receptor EphB4 expressed by osteoblasts[43].

In summary, it appears that bone is remodeled in response to a disruption in the osteocyte synctium from microdamage. This type of remodeling is often referred to as "targeted re-

	CUSTOMARY STRAIN STIMULUS		
	LOW	**NORMAL**	**HIGH**
MODELING			
Osteocytes	Apoptosis	Maintenance of viability	Perturbation
Bone mass	Decreased	Maintained	Increased
Primary surfaces of action	Endocortical, Trabecular	N/A	Periosteal, Trabecular
Whole bone strength	Decreased	Maintained	Increased
REMODELING			
Remodeling rate	Increased	Baseline	Increased
Osteocytes	Apoptosis	Maintenance of viability	Apoptosis
Stimulus for osteocyte action	↓ Nutrients/ Waste removal	Pulsatile fluid flow	Microdamage
Result	Transient loss of bone with a negative bone balance	No Change	Maintenance of material quality

Table 1. Summary table showing the role of osteocytes in the different processes of mechanically-mediated bone modeling and remodeling in response to changing customary strain stimulus (CSS).

modeling"[44], and it prevents microdamage from accumulating in bone tissue. Targeted remodeling, as with all types of remodeling, results in newly-formed bone that is less mineralized than adjacent, older bone. This can have a positive effect on bone material properties, and in a sense, keeps bone tissue young. Thus, the greater levels of turnover observed with a higher than customary strain stimulus can be explained by targeted remodeling. As loading increases, microdamage accumulates, and this damaged bone is then remodeled[44].

Remodeling and disuse

In disuse, like resorption modeling, remodeling also occurs following osteocyte apoptosis. Though much of the bone is lost only transiently, bone formation in each remodeling unit does not quite equal the amount of bone that was resorbed[6]. This is referred to as a "negative bone balance" and results in increased porosity. These observations can be explained by evidence that osteoblast differentiation, lifespan, and activity are under regulation of mechanical loading[45-49]. Consequently, in a state of disuse, osteoblasts may not be able to finish the job due to fewer osteoblasts being recruited to a site or premature apoptosis in the absence of adequate strain. Disuse-mediated remodeling helps clarify why the remodeling rate in response to mechanical demands is 'U' shaped (Figure 1B)[20]. Although osteocyte apoptosis is the primary step in bone remodeling on either end of the strain spectrum, targeted remodeling is responsible for increased remodeling seen with a higher than customary strain stimulus, and disuse-mediated remodeling is responsible for higher remodeling rates with a lower than customary strain stimulus (Figure 1B).

Remodeling and bone mineral demands

Bone remodels for nonmechanical reasons as well, and this turnover is often under control of hormones such as parathyroid hormone (PTH) which is secreted in response to a systemic demand for calcium. The effects of PTH has traditionally been attributed to its direct effects on osteoblasts. However, transgenic mice expressing a constitutively active PTH receptor exclusively in osteocytes demonstrated increased remodeling[50], pointing to a role of osteocytes in PTH-regulated remodeling. An example of this type of remodeling is seen with increased intracortical remodeling of the ribs of deer when a large amount of mineral is needed for seasonal antler formation. This type of remodeling likely also occurs as a support system for formation modeling. As reviewed by Bilzikian et al.[51], because a cubic centimeter of bone contains as much calcium as does the entire blood volume, bone formation consequently generates a hypocalcemic environment. For this reason, when bone formation modeling occurs during growth, the process of remodeling may provide needed bone mineral.

Summary of the roles of modeling and remodeling in bone adaptation

Like modeling, remodeling modifies whole bone strength, but often only does so transiently. Therefore, bone remodeling is a process of a materialstat—performing a part in maintaining bone material quality and either transiently ridding bone of material as in disuse or providing bone material when needed for formation modeling or metabolic demands. Modeling, on the other hand, is the process of the mechanostat that efficiently rids bone of excess mass or adds bone to the existing structure in order to alter whole bone strength to the prevailing strain environment.

Osteocytes and prevention of bone loss

Given that osteocyte viability must be maintained for bone to be preserved, a means of prevention of bone loss is by targeting osteocytes with drug therapies. Several drugs in use are known to have anti-apoptotic effects on osteocytes, including

bisphosphonates, sex steroids, and PTH[51]. A decrease in osteocyte apoptosis may partially explain why bone loss is suppressed with such therapies in conjunction with direct inhibition of oseoclast function. However, as reviewed above, osteocytes are critical for suppressing accumulation of microdamage, and several animal studies[52,53] have demonstrated increased accumulation of microdamage with bisphosphonate therapy at dosages congruent with human therapy. As sclerostin also augments osteocyte apoptosis, it too provides a potential target for prevention of bone loss.

Nonpharmacological therapies should also be considered for prevention of skeletal fragility, including exercise prescription. As reviewed above, mechanical loading can prevent osteocyte apoptosis, and therefore, exercise interventions to prevent bone loss should theoretically generate a high enough strain stimulus to prevent osteocyte apoptosis. The strain stimulus may be composed of various strain characteristics beyond just strain magnitude[3], and therefore, the mechanostat threshold for prevention of bone loss may be reached by altering strain rate[54], strain distribution[55], and frequency (i.e. vibration)[56], as well as adding rest-insertion between loading cycles and bouts[57]. Animal studies focusing on identifying effective loading doses and modalities with osteocyte apoptosis as an outcome may help identify optimal physical activities for the prevention of bone loss.

Summary

Osteocytes are an important part of the cellular machinery of bone functional adaptation. In response to a strain stimulus that is below the mechanostat's resorption threshold, osteocytes undergo apoptosis, primarily in trabecular and endocortical bone, which is followed by osteoclastic resorption modeling and consequently, lower whole bone strength. When there is a normal strain stimulus, osteocytes are protected from apoptosis, and bone mass is preserved. When the strain stimulus surpasses the mechanostat's formation threshold, tissue level strains lead to fluid flow-mediated osteocyte and dendrite perturbation and release of anabolic factors. In turn, osteoblasts are recruited and bone is subsequently formed primarily on trabecular and periosteal surfaces—effectively increasing whole bone strength. This resorption independent of formation and formation independent of resorption are the result of the cellular process of modeling. The rate of remodeling in bone is also influenced by changes in the mechanical environment of bone. It is increased when there is a higher than customary strain stimulus due to osteocyte apoptosis in response to generation of microdamage and is also increased in unloading in response to disuse-mediated osteocyte apoptosis. Remodeling transiently alters whole bone strength while providing mineral for metabolic demands, aids in ridding bone of excess mass in disuse, and protects bones from accruing excessive microdamage. Given that osteocytes represent the initial cellular sensing mechanism in bone, and therefore, a primary step in bone modeling and remodeling, they are an important cell type for further study as targets for prevention of bone loss.

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