Revisiting Harold Frost's Mechanostat Theory of Bone Functional Adaptation: New Interpretations Based on New Evidence

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Minneapolis, Minnesota, December 2010

Julie Hughes

Dedication

This dissertation is dedicated to Jana and Patrick Hughes. Five hundred thousand dollars later and all you get is this lousy dedication. I love you.

TABLE OF CONTENTS

LIST OF FIGURES

1

INTRODUCTION, BACKGROUND, AND SPECIFIC AIMS

INTRODUCTION

Writing a theoretical dissertation

The main purpose of this dissertation is to explore, update, and add to the existing theory of bone functional adaptation through critically reviewing and interpreting the literature in the bone field. As explained by John Currey in his book, *Bones*, a good theory is not a guess or hunch but rather, "…it tries to explain a whole set of disparate observations with a few basic postulates..." Such theories are important because they determine the questions that are asked about things in the field. The better determined the theory is, the better determined the questions that are asked, and the more efficient the subsequent research will be. For example, for several decades in the bone field, one of the prevailing questions asked in regards to osteoporosis has been, "how can we prevent bone loss by inhibiting *osteoclasts* (bone resorbing cells) from resorbing bone?" In turn, a great amount of effort has been spent trying to target cells that resorb bone. After reading the review and discussion of recent bone biology in this thesis, I hope that a skeletal research scientist (assuming a skeletal research scientist would choose to read this!) would ask, "how do we prevent bone loss by keeping *osteocytes* viable?" Such new questions have all sorts of implications for disuse- and age-related bone loss.

The structure of the dissertation

In this dissertation, three main questions are asked regarding bone functional adaptation and then answered (based on existing literature) in three papers. These papers are not meant to be solely review articles but rather attempts to interpret and integrate new and existing literature with the aim of providing evidence for new ways to think about bone functional adaptation. In the first chapter, background on osteoporosis is provided to highlight the importance of research in the bone field, and relevant concepts for the three

main papers are introduced. Chapters two, three, and four comprise the body of the thesis and each deal with one of the three specific aims (See: Specific Aims section at the end of the Background section). Finally, the fifth chapter summarizes the main conclusions of the dissertation and outlines important implications for both future bone research and for prevention of osteoporosis and related fractures.

BACKGROUND

Epidemiology of Osteoporosis

Osteoporosis is a skeletal disorder characterized by compromised bone strength that results in an increased susceptibility to fracture [1, 2]. It is estimated that over 200 million people worldwide currently have osteoporosis [3], and the prevalence is expected to rise with the increasing lifespan and ageing population [4]. In the US alone, an estimated 44 million individuals (55 percent of the population over age 50) have low bone mass or osteoporosis. These numbers are predicted to increase to 61.4 million by the year 2020 [5]. As osteoporosis is seen mainly as a disease that affects women, men often go undiagnosed and untreated, yet men are increasingly at risk for osteoporotic related fractures.

The clinical relevance of osteoporosis is the dramatic increase in risk of fracture. More than 1.5 million fractures are associated with osteoporosis each year. Osteoporotic fractures are low trauma fractures that occur with forces generated by a fall from a standing height or lower, and are most common at the spine, hip and wrist. Regardless of fracture site, adults who fracture are at much greater risk of fracturing again at any location [6]. It has been estimated that one in two women and one in four men over 50 years of age will suffer from an osteoporotic-related fracture in their lifetime. To put this

in perspective, a woman's risk of hip fracture is equal to her combined risk of breast, uterine, and ovarian cancers [5], and men have a greater risk of developing osteoporosis than prostate cancer. Hip fractures are considered to be the most devastating consequences of osteoporosis as they are associated with severe disability and increased mortality [7]. Furthermore, the economic burden of hip fractures is substantial, with an estimated worldwide annual cost of $131.5 billion [8]. While the combination of all osteoporotic fractures cost the US health care system approximately $17 billion per year, these annual costs are projected to reach $50 billion by the year 2040 [9]. Importantly, osteoporosis is just one of several risk factors for fracture. Fractures are a function of both the strength of the bone and the load on a bone at any given time - whereby the load must exceed bone strength for a fracture to occur. A majority of hip and wrist fractures occur as a consequence of falling. Thus, factors influencing both bone strength *and* risk of falling are important for fracture prevention.

Healthy Bone Physiology and Pathophysiology of Osteoporosis

Basic Bone Physiology

In order to fully understand the pathophysiology of osteoporosis, it is important to first understand the functions and physiology of the skeleton. Bones are dynamic organs that are comprised of different types of bone tissue that are vascularized and innervated. Bones serve many vital functions including serving as a mineral reservoir for calcium and phosphorous, protection of vital organs, and as a site for muscular attachments to aid in locomotion. The primary function of skeletal long bones is to bear loads, which requires contradictory properties. Long bones must be stiff and massive so as not to deform or break easily during normal loading but also light for efficiency of movement and flexible to absorb energy during impact [10, 11]. To fufill these functions, the

appropriate material composition and structure of bones have been selected over the course of evolution and are subject to adaptation during an individual's lifetime.

Bone is a bisphasic material with crystals of hydroxyapatite (calcium-phosphate mineral) incorporated in a collagen matrix. While the collagen gives bone flexible properties, the mineral adds stiffness. This material is fashioned into two types of bone. Cortical bone (also referred to as compact bone) is dense and stiff and comprises the shaft of long bones as well as provides a shell of protection around trabecular bone. Trabecular bone (also referred to as cancellous or spongy bone) is more porous and flexible and is found in flat bones, the ends of long bones, and in cuboidal bones (e.g., vertebrae). In trabecular bone, the bone material is in the form of plates or struts called trabeculae.

The characteristics of bone that determine its strength include the *quantity* of bone material present (the "mass" component), the *quality* of the material (i.e. mineralization, fatigue damage, etc.), and the distribution of the material in space (*structure or geometry*). These factors are determined by the dynamic cellular activities known as bone modeling and remodeling which are regulated by bone's hormonal and mechanical environments. Modeling is the *independent* action of osteoclasts (bone resorbing cells) and osteoblasts (bone forming cells) on the surfaces of bone, whereby new bone is added along some surfaces and removed from others. Modeling affects the size and shape of bones and is especially important for reshaping long bones as they grow in length during adolescence or in response to changing mechanical load throughout life. Remodeling is a localized process that involves the *coupled* action of osteocalsts and osteoblasts, whereby osteoclasts first resorb a pit of older bone, and osteoblasts are subsequently recruited to the site to form and mineralize new bone. This process

happens throughout the lifespan and occurs diffusely within the skeleton. Like any material subjected to repetitive loading, fatigue damage is incurred. However, unlike inert materials, bone is able to replace old damaged bone with new bone through the process of remodeling [12].

Pathophysiology of Osteoporosis

While osteoporosis denotes skeletal fragility, osteoporotic fractures are the result of *both* reduced bone strength and increased rate of falls.

Skeletal Fragility

Many skeletal characteristics contribute to bone strength, and consequently, bone fragility, including the quantity of bone material present, the quality of the material, and the distribution of the material in space.

Bone Quantity and Skeletal Fragility

Bone 'quantity' is typically measured as the amount of mineralized material (bone mineral content, BMC g – also termed 'bone mass') or the areal bone mineral density (aBMD, g/cm^2) by dual energy x-ray absorptiometry (DXA). The actual pattern of bone change is more dynamic both during growth and in later life. During childhood and adolescence for example, modeling dominates to add new bone and alter the size and shape of bone in response to loads from increased muscle force and body mass. Approximately 26% of total adult bone mass is accrued in a two year period during adolescence [13]. This is approximately equivalent to the amount lost in later life [14]. Overall, global bone formation continues at a faster pace than bone resorption until peak bone mineral accretion is attained sometime in the 2nd or 3rd decade (depending on site,

region and gender). In later life, the process of bone formed in each remodeling site no longer equals the bone that was resporbed, and thus, a small amount of bone is lost with each new remodeling cycle. This is refered to as a negative bone balance.

In later life, gonadal hormones (testosterone, estrogen) decrease in both men and women. Estrogen has been demonstrated to suppress activation of new remodeling cycles, and thus, low estrogen levels result in an increased rate of remodeling [15]. As resorption precedes formation in the process of remodeling, and formation and mineralization are time-intensive processes that may take up to several months to complete, an increase in the rate of remodeling results in temporary decreases in bone mass. While temporary losses in bone mass lead to a transient increase in bone fragility, increased rates of remodeling with a negative bone balance lead to true bone losses of approximately 9% to 13% [16] during the first 5 years post-menopause. Bone turnover eventually adapts and slows to a rate similar to pre-menopausal years. Men also experience age-related bone loss but without the rapid period of loss [17]. Differences in bone size (discussed below) may also partially explain differences in fracture rates between men and women [18].

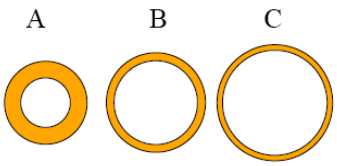
Bone Material Quality and Skeletal Fragility

While the amount of bone in the human skeleton decreases with menopause and advancing age, there is evidence that properties of the remaining bone material may change with age in a way that increases susceptibility to fracture. Bone, like all structural materials, is subject to fatigue damage. This damage occurs in the form of microcracks that increase in number and length with advancing age [19]. Microdamage accumulation is associated with reduced bone strength [20]. This damage is likely a result of increased

mineralization of existing bone that is associated with increasing age [11, 21]. Increased mineralization makes bone more brittle, and thus, less able to absorb energy during impact. While changing material properties of bone with age may contribute to skeletal fragility, it is important to acknowledge that these properties are not captured in any of our current non-invasive techniques of assessing bone [22].

Bone Structure and Skeletal Fragility

An important component of bone strength is the structure and geometry of bone—that is, how the material is distributed within the bone cross-section. To highlight the importance of cortical bone geometry, the schematic in Figure 1 illustrates the cross-section of 3 bones all of which have the same bending strength (represented by the engineering term section modulus).



Areal BMD (DXA)	1.00	0.53	0.36
Volumetric BMD (QCT)	1.00	0.46	0.27
Section Modulus	1.00	1.00	1.00
Bone CSA or BMC	1.00	0.66	0.53

Figure 1. Schematic representation of 3 bone cross-sections with expanding periosteal diameter (from A-C) and constant section modulus. (Figure Courtesy of Tom Beck).

Despite a reduced bone mineral density (areal or volumetric), the bone on the right has equivalent bending strength (section modulus) because the mass is distributed further from the neutral axis. This example highlights the importance of considering the structure rather than solely the mass or density of bone when estimating its strength. Recent advances in technology such as quantitative computed tomography (QCT), peripheral QCT, magnetic resonance imaging (MRI) [23] and software such as Hip Structure Analysis (HSA) [24, 25] allow for measurement of bone geometry and estimates of strength. These techniques, however, are not yet fully developed for use in clinical settings for diagnosis of fracture prediction. Nonetheless, the geometric changes in bone throughout life provide insight into the development of skeletal fragility and bone adaptation to mechanical loading.

Cortical bone gain and loss are not uniform throughout the skeleton or within any single bone and differ in males and females. During growth, boys have greater gains in periosteal (outer) diameter while girls have a narrowing of the endocortical surface during early puberty, resulting in a greater overall bone size in boys that remains throughout life [26 27]. In later life, bone is lost primarily from the endocortical (inner surface of long bones, lining the marrow cavity and intracortical (surfaces within the cortex) surfaces. Thus, the cortex becomes more porous and the cortices become thinner and more fragile. To offset these losses, bone may be added to the periosteum (outside surface of bone), thereby increasing the diameter of bone and maintaining the strength of the structure in bending [10, 28, 29]. However, as more bone is resorbed from the endocortical surface than is formed on the periosteal surface, the cortices continue to thin. The process of adding bone to the periosteal surface appears to be more efficient in men than women, with women showing similar increases in endocortical diameter with

age but less expansion in periosteal diameter. These structural differences may partially explain some of the differences in fracture rates between men and women.

Microarchitecture of trabecular bone is also an important contributor to skeletal fragility but is not possible to measure by traditional densitometric techniques such as DXA or quantitative computed tomography (QCT), which do not have adequate resolution to assess microarchitecture. Newer technology and software for MRI and micro CT allow for measurement of trabecular connectivity, thickness and number [23] – all important components of skeletal integrity. For example, if the resorption phase of remodeling is too aggressive, as is seen at menopause and thereafter, trabeculae may be penetrated and entire trabecular elements lost as well as connections between trabeculae eliminated permanently. In these cases, the loss in structural strength is exaggerated far out of proportion to the amount of bone lost [30]. Furthermore, trabeculae that remain intact may be thinned by excessive remodeling, creating a weakness in the ability to bear loads.

Falls

While skeletal fragility increases susceptibility to fracture, it would be of little concern if damaging loads such as those incurred in a fall, were prevented. A majority of hip fractures occur after a sideways fall on the hip [31, 32]. Therefore, osteoporotic fracture is a function of both increased skeletal fragility *and* an increased rate of falls. The incidence of falls increases with age because several sensory systems that control posture (vestibular, visual, and somasensory) become comprimised with advancing age. Furthermore, muscle mass and strength, which prevent instability and correct imbalance, decline 30 – 50% between the ages of 30 and 80.

Prevention of Osteoporosis

There are sevearl preventative and therapuetic options for decreasing the risk of osteoporosis and related fractures with advancing age. These tools can be characterized as either pharmacological agents or lifestyle modifictions.

Pharmacological Therapy

Several pharmacological agents have been approved by the FDA for the treatment of osteoporosis. These agents can be categorized by whether they act on remodeling (antiremodeling drugs) or directly on formation (anabolic drugs). Antiremodeling agents include bisphosphonates (alendronate, risedronate, etidronate, ibandronate), salmon calcitonin, hormone replacement therapy (HRT), and selective estrogen receptor modulators (SERMs, raloxifene). These drugs act by suppressing the resorption phase of the remodeling cycle, and thus, allow for existing cavities to fill typically resulting in an increase in BMD. Also, by suppressing resorption, these agents can reduce loss of connectivity and trabecular thinning associated with menopause and ageing.

Lifestyle Modifications

All postmenopausal women and older men, regardless of fracture risk, should be encouraged to engage in behavior modifications, including adequate calcium (1000 - 1500 mg/d) and vitamin D (400 - 800 IU/d) intake, regular exercise, smoking cessation, avoidance of excessive alcohol intake, and visual correction to decrease fall risk. *Of all these lifestyle modifications, exercise is the only lifestyle modification that can simultaneously ameliorate low BMD, augment muscle mass, promote strength gain, and improve dynamic balance—all of which are independent risk factors for fracture* [33, 34].

Exercise Prescription for Bone Health

The reason that exercise is an effective tool for the prevention of osteoporosis is that bones are able to adapt to changes in their mechanical environment [35]. The purpose of this functional adaptation of bone is to prevent fractures from typical peak voluntary mechanical loads throughout life [36]. That increased bone strength with loading will aid in prevention of bone fragility provides the theoretical basis for exercise prescription to prevent fragility fractures

Bone Functional Adaptation

When bones are loaded in compression, tension, or torsion, bone tissue is deformed. Deformation of tissue, or the relative change in length of bone tissue, is referred to as strain. Bone tissue strain causes fluid within the bone to move past the cell membrane of osteocytes—the bone cells that are embedded throughout bone tissue and are connected with one another, to other bone cells, and with the bone marrow through slender dendritic processes. The current prevailing theory in the bone field is that this fluid flow along the osteocyte causes a release of molecular signals that lead to osteoclast and osteoblast recruitment to (re)model bone to better suit its new mechanical environment. This process of turning a mechanical signal into a biochemical signal is called mechanotransduction.

It has been suggested by Harold Frost that the response of bone to its mechanical environment is controlled by a “mechanostat” that aims to keep bone tissue strain at an optimal level by homeostatically altering bone structure [36].

Harold 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 [37]. Thus, bone tissue has an intrinsic "mechanostat" which regulates bone functional adaptation. As with any homeostatic control system, bone's mechanostat 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. Each of these components is described in detail in the first paper of this dissertation.

Further Evaluating Mechanostat Theory

Since Frost last updated his mechanostat theory in detail in 2003, there have been numerous advances in the field of bone biology—particularly in regards to osteocyte biology. These advances providing supporting evidence for the mechanostat theory as well as new ways of interpreting the theory of how our bones respond to mechanical load. This new evidence also allows us to interpret 'older' literature differently. For

example, Frost characterized osteocytes in bone several decades ago and reported on their decline in numbers with age. However, given our new insight into the importance of these cells for bone mechanotransduction, these old data now have new implications for the pathophysiology of osteoporosis.

Therefore, this thesis is concerned with updating the mechanostat theory based on recent additions to the bone biology literature as well as interpreting past literature in light of new concepts in the field of bone biology. In particular, three specific questions are asked regarding the mechanostat theory and answered through a review of the literature in three separate papers. Each paper concludes with implications of the reviewed literature for future research as well as implications for prevention of fragility fractures. The three questions asked in this thesis are:

1. **What is the underlying biology of how bones sense and respond to mechanical stimuli?**
2. **Does bone functional adaptation become less effective with increasing age?**
3. **Do the benefits of bone functional adaptation transfer to protection from osteoporotic fractures?**

SPECIFIC AIMS

Specific Aim for Paper One:

To characterize the underlying biology of how bone sense and respond to mechanical stimuli

Secondary Aims:

- To detail the role of osteocytes in bone functional adaptation.
- To characterize the biology that underpins Frost's mechanical thresholds for bone formation and resorption.
- To describe and distinguish between the processes of bone modeling and remodeling in bone functional adaptation.
- To describe and distinguish between the stimuli and processes of (re)modeling in states of disuse and overload.
- To highlight the importance of targeting osteocytes for prevention of osteoporosis and related fractures.

Specific Aim for Paper Two:

To review the evidence, biological plausibility, and practical implications of the age-related decline in bone mechanosensitivity

Secondary Aims:

- To suggest practical implications for the timing of exercise prescription for bone health based on conclusions of the literature review.

Specific Aim for Paper Three:

To describe how the structural benefits gained from mechanical loading transfers to the prevention of bone fragility fractures.

Secondary Aims:

- To defend the proper functioning of the mechanostat in light of the high prevalence of fractures.
- To distinguish between mechanical competence of bone in regards to customary loading and in regards to preventing fractures from abnormal loading.
- To highlight the importance of distinguishing between these goals to properly understand the role of exercise in the prevention of fragility fractures.

2

THE BIOLOGICAL UNDERPINNINGS OF FROST'S MECHANOSTAT THRESHOLDS: THE IMPORTANT ROLE OF OSTEOCYTES

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 [37]. 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. Due to their abundance throughout the bone matrix, high degree of connectivity, and sensitivity to mechanical signals, osteocytes have been implicated as the main sensory cells in bone. In this article, I review recent evidence from bone biology that osteocytes are indeed the primary mechanosensory cells in bone, and therefore, are critical for bone functional adaptation. I 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. I 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 [37]. Thus, bone tissue has an intrinsic "mechanostat" which regulates bone functional adaptation. As with any homeostatic control system, bone's mechanostat 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. I 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 [38]. 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 [39]. 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). Importantly, Skerry acknowledged that the CSS is both sex and site specific and that it is genetically, biochemically, and pharmacologically modified [39].

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 as they grow in length during adolescence [40]. 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 [41]. Except in disuse, the amount of bone formed is generally equivalent to the amount of bone resorbed in each remodeling unit[36]. 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 [42] 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.

The mechanostat's sensory mechanism

As mentioned, the mechanostat's effector cells have been appreciated for some time. However, the sensory cells of bone have only been recently identified. 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 [43]. 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 [44]. This fluid flow results in the perturbation of both osteocytes in their lacunae and dendrites in their canaliculi [43]. 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 [43, 45].

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 [43, 45]. 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 [46, 47], and nonsteroidal anti-inflammatory drugs (prostaglandin synthesis blockers) [48-50]. Moreover, calcium channel blockers have been shown to prevent mechanical loading-induced release of prostaglandins [51], and mice with a null mutation in $P2X_7$ receptor—an ATP receptor that plays an important role in PGE2 release [52]—show suppressed bone formation with mechanical loading. Following mechanical loading, the release of NO and PGE2 from osteoblasts and osteocytes has been demonstrated to lead to the recruitment of osteoblasts from the marrow stroma [47, 53]. *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 [45]. 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 [54, 55]. 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 [56]. Sclerostin is secreted from osteocytes and negatively regulates canonical Wnt signaling—an important signaling pathway for osteoblast differentiation and function [57,

[58]. 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 [59]. By suppressing the release of sclerostin, mechanical loading results in enhanced Wnt/β-catenin signaling [60], and consequently, greater bone formation.

Osteocytes 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 [61, 62]. While the mechanisms for *disuse-mediated* bone loss are not well known, recent evidence suggests that osteocytes are an important regulator of bone loss [63, 64]. Aguirre et al. [63] 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 [63]. 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 [64]. 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 [65, 66]. 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 [67], 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 [67]. 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 [56] 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 [56], 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.

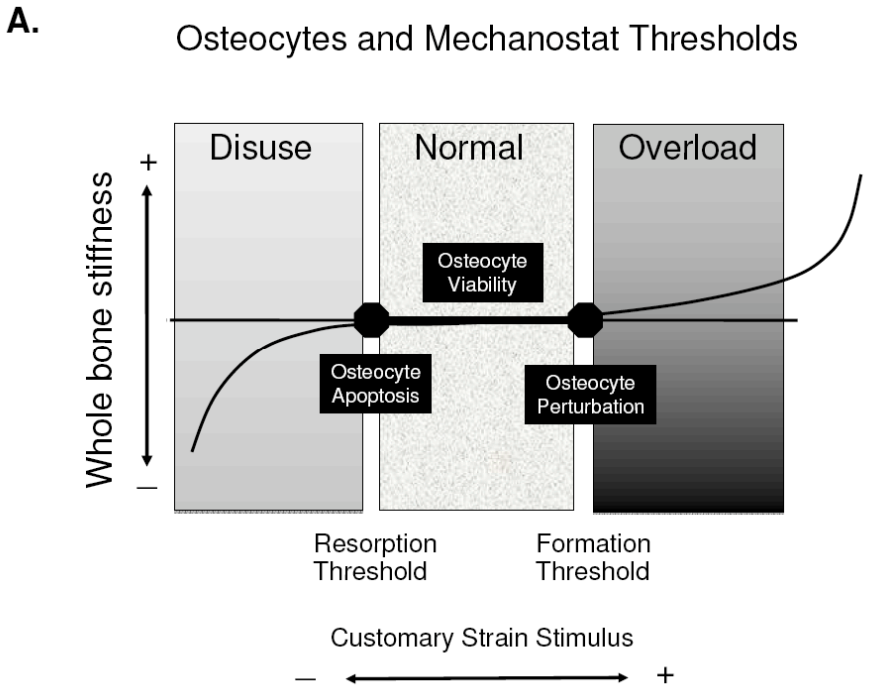
Recent in vitro experimental studies have investigated the means by which osteocytes may be able to recruit osteoclasts for bone resorption, and it has been demonstrated that osteocytes secrete both receptor activator of NF-κB ligand (RANKL) from their dendritic processes and macrophage colony-stimulating factor (MFC) [43, 68]. 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 [43, 69].

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 [70, 71]. Noble et al., [72] 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 [70]. 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 Tan, 2008 #3547}. 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 2A).

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 2). 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 (un)loading. 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.



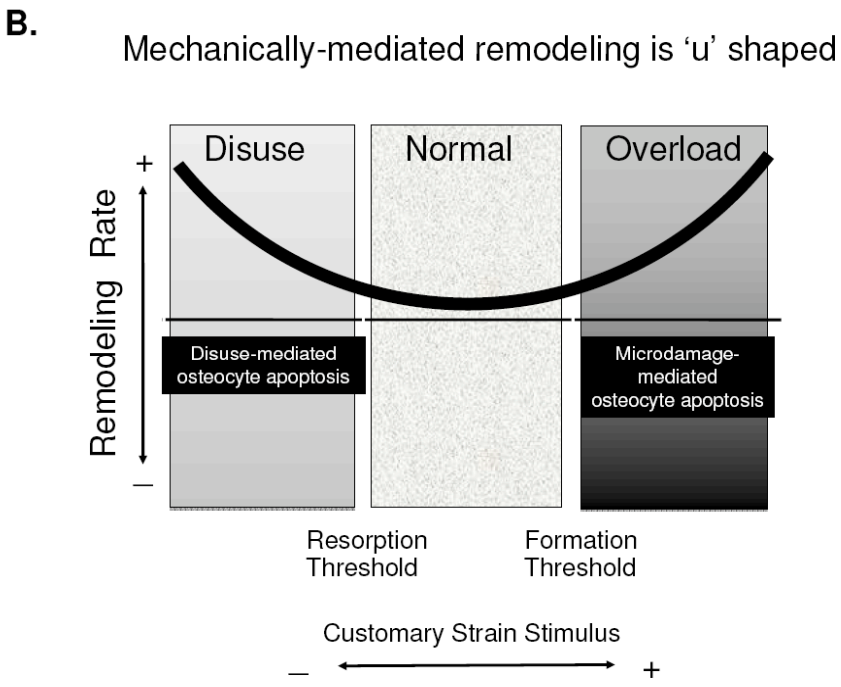


Figure 2: Schematics showing the role of osteocytes in determining mechanostat thresholds for resorption and formation (A) and remodeling rates (B). **A)** Modeling: Above the CSS, osteocytes are perturbed and formation modeling occurs to increase whole bone strength. A lower than customary CSS causes 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)** Remodeling: 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.

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 [73]

without such a mechanism of material repair. Similar to bone loss in disuse, bone resorption 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 [74]. These findings are particularly interesting given that cortical bone of rats do not typically experience remodeling. Similar results were found by Bentolila et al., [75] 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 [75].

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 [76]. This suggests that dying osteocytes send out signals to be turned over while adjacent healthy bone cells send out protective signals [43]—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 [77]. Osteoclasts, in turn, are capable of recruiting osteoblasts to fill resorption cavities. With the observations that trabeculae and osteons (the remnants of bone remodeling in cortical bone) 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 [78].

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 remodeling," [12] 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. 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 [12].

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 [36]. 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 [79-83]. 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 2B) [55]. 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 2B).

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 [84], 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., [85] 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 and/or in response to increased mechanical stimuli, the process of remodeling conveniently provides 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 [85]. 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 [86, 87] 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 [39], and therefore, the mechanostat threshold for prevention of bone loss may be reached by altering strain rate[88], strain distribution[89], and frequency (i.e.

vibration)[90], as well as adding rest-insertion between loading cycles and bouts[91]. 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.

3

IS EXERCISE LESS OSTEOGENIC WITH AGE?

Introduction

It has long been recognized that bones are able to adapt to changes in their mechanical environment [35]. The purpose of this functional adaptation of bone is to prevent fractures from typical peak voluntary mechanical loads throughout life [36]. That increased bone strength with loading will aid in prevention of bone fragility provides the theoretical basis for exercise prescription to prevent fragility fractures. Nonetheless, as highlighted in an eloquent review by Forwood and Burr over a decade ago, 'Physical activity and bone mass: Exercise in futility," exercise has more of an anabolic effect on the young skeleton than on the mature skeleton as well as on the ageing skeleton when osteoporosis is most prevalent[92]. In recent years, not only have there been additions to this literature, but importantly, the intricacies of bone cell mechanotransduction (conversion of a mechanical signal into a biochemical signal) are beginning to be elucidated [45]. Alterations in the differentiation and proliferation, lifespan, and function of the cellular machinery of bone mechanotransduction and functional adaptation with advancing age provide biological plausibility for declines in bone mechanosensitivity with age. These observations have important implications for the timing and type of lifestyle interventions for the prevention of bone fragility.

Is exercise less effective for bone health in maturity than in old age?

Retrospective studies of physical activity in humans indicate that bone responds more favorably to mechanical loading during youth than in adulthood [93, 94]. For example, a retrospective study of female racquet-sport players demonstrated a two-four times greater benefit in side-side differences in bone mineral content between the dominant and nondominant arm bones if the players began playing their sport at or before menarche rather than after it [95]. Furthermore, exercise has generally been shown to be

less effective in the postmenopausal and senescent skeleton than in adulthood and youth[92]. The highest level of evidence available regarding the effectiveness of exercise on skeletal health throughout the lifespan comes from the animal literature. Specifically, several studies of mechanical loading in animals of different age groups provide evidence of the effects of age on bone mechanosensitivity. Results from these studies are not entirely consistent, and therefore, I have stratified these studies below by outcomes in relation to bone mechanosensitivity and age.

Studies that suggest bone becomes less mechanosensitive with age

Steinberg and Trueta et al [96]., found that treadmill running had more of an anabolic effect on the bones of infant rats than mature rats, with the observation of greater bone mass, length, and diameter as well as increased bone x-ray density, cortical thickness, and circumferential ring formation (tetracycline labling) in young exercising rats compared to controls. No such differences were seen in the mature exercising rats compared to age-matched controls. Similar results were found in a study by Rubin et al[97]., using the loadable functionally isolated ulna preparation in young (1 year-old) and old (3 years old) male turkeys. Eight weeks of 300 cycles per day of loading (each cycle generating ~3,000 microstrain) resulted in statistically significant differences between the loaded and unloaded ulnae of the young turkeys (+3.5% cortical area, -5.6% endosteal area, +17% periosteal area), with no significant differences between ulnae of the older turkeys.

In a similar study, Hoshi et al [98]., allowed 4 age groups of mice (10-70 weeks old, 10-30 weeks old, 30-50 weeks old, 50-70 weeks old) to voluntary run on a treadmill and included age-matched control groups. While they found higher bone density in all

exercise groups compared to controls, cortical thickness, maximum breaking force, ultimate stress and elasticity were all greater in exercise groups, except the oldest group (50-70 weeks old). Again, these results suggest that older skeletons are less sensitive to mechanical stimuli. However, because the mice were voluntarily run, the oldest group did not run as much as the younger groups, possibly confounding the results.

Lieberman et al[99], also found exercise to be more effective in younger animals. They studied the effects of constant speed treadmill running on the long bones of juvenile (40 days old), subadult (265 days old), and adult (415 days old) Dorset sheep. After 90 days of treadmill running for 60 minutes per day, the authors reported greater periosteal modeling of the tibial and femoral midshafts in runners compared to controls in the two younger groups. However, there was no significant difference in periosteal modeling between the adult exercise and control groups. Similar to the periosteal modeling response to treadmill running, the effects of exercise on haversian remodeling was also blunted with age.

Umemura et al [100]., tested the efficacy of running and jumping in young and old rats. The authors report that after 8 weeks of running, jumping, or remaining sedentary, jumping and running significantly increased the fat-free dry weights of the femur and tibia of the young rats, while only jumping significantly increased the fat-free dry weights significantly in old rats. These findings suggest that a greater magnitude of loading is necessary to result in a positive skeletal response in the older skeleton. A study by Turner et al [101]., supports this theory by demonstrating that the older skeleton requires a greater mechanical stimulus to result in an anabolic response. In this study, varied mechanical loads (30 – 64 N) were applied to the tibiae of young (9 months-old) and old

(19 months-old) rats. Mature rats responded with periosteal apposition to loads only at 40 N or greater, but this anabolic response occurred in a lower percentage of older rats than younger rats (59% old vs. 100% adult). In turn, relative bone formation rate in the old rats at the greatest applied load (64 N) was over 16-fold less than that reported for the younger adult rats.

Studies that suggest a differing means of bone functional adaptation with age

Not all of the animal studies of exercise in different age groups have concluded that exercise is less effective in maturity and old age than in youth. For example, Jarvinen et al[102], found that 14 weeks of progressively intensified running resulted in no significant differences in bone strength in response to exercise between 5 week-old and 33 week-old male rats compared to age-matched controls. Nevertheless, there were differences in bone structural alterations in response to exercise between age groups with the older exercised rats demonstrating greater volumetric bone mineral density (vBMD) compared to age-matched controls while the younger exercised rats demonstrated greater periosteal apposition compared to age-matched controls.

Buhl et al.[103] concluded that age has a beneficial effect on bone responsiveness to exercise. They studied the skeletal effects of resistance training in young (4-months-old), adult (12-months-old), and old (22-months-old) male rats. The rats were trained to depress a lever high on the side of a cage while wearing a weighted backpack for 50 repetitions, 3 times a week for 9 weeks. The authors concluded that low-intensity resistance training is more effective in the old skeleton due to the observation of significantly smaller medullary area and decreased trabecular spacing in the older exercise group compared to their age-matched controls. Nonetheless, as with the study

by Jarvinen et al.[104] bone strength gains with exercise were not significantly different between groups suggesting that exercise may result in different alterations in bone structure in old age compared to youth.

In support of this theory, Raab et al.[105] reported exercise-induced increases in breaking force and ultimate stress in young (2.5-months-old) and old (25-months-old) femora of rats, respectively. However, while the younger trained rats had increases in bone cross-sectional moment of inertia (CSMI) compared to age-matched controls, the older trained rats did not—suggesting an inability of older rats to initiate the periosteal modeling necessary to increase CSMI. Similar results were found in a study in which both young (5-weeks-old) and mature (17-weeks-old) treadmill-trained rats had significant differences in tibial subperiosteal area, cortical cross-sectional area, peak load, stiffness, and moment of inertia compared to age-matched controls[106]. However, while not significant, static histomorphometry at the tibial midshaft showed that immature rats had a 3% greater between-group difference in subperiosteal area and a 9% greater between-group difference in CSMI compared to mature rats. Again, these findings suggest a difference in the ability of bones from older animals to expand in diameter through periosteal apposition in response to exercise.

Studies that demonstrate enhanced bone mechanosensitivity with age

Leppänen et al [107]., randomly assigned 108 male and 101 female mature (47-weeks-old) and senescent (75-weeks-old) rats into mature exercised, senescent exercised, mature control, and senescent control groups. Exercise groups were subjected to progressive treadmill training for 14 weeks. Following training, both mature and senescent male rats demonstrated exercise-induced increases in total cross-sectional area at the femoral

diaphysis (6%, $p = 0.018$ and 19%, $p = 0.003$, respectively). However, only senescent female rats had an increase in total cross-sectional area at the femoral diaphysis in response to exercise (10%, $p = 0.001$). Treadmill running was associated with statistically significant ($p < 0.05$) increases in total bone mineral content in both genders of mature and senescent rats. Furthermore, breaking load at the femoral neck and diaphysis significantly increased (16% and 19%, respectively, both $p < 0.05$) in the senescent female exercise group compared to age- and gender-matched controls. These findings suggest that there is not only no loss of bone mechanosensitivity between maturity and old age, but that mechanosensitivity may actually increase in old age. However, as the researchers did not study immature animals, the study does not provide evidence of the difference between bone responses to mechanical stimuli between immaturity and maturity or old age.

In summary, while the studies of the ability of bones to sense and adapt to mechanical loading with age are not entirely consistent, the majority of studies in this body of literature provide evidence for declines in bone mechanosensation with age or at least a clear difference in the strategies adopted for adaptation, with bone added to the periosteal surface in response to exercise in youth—a more rapid and, arguably, a more efficient adaptation to anabolic mechanical stimuli. Overall, the predominance of the animal research of bone mechanosensitivity in different age groups indicates that exercise is more effective in youth than in maturity and senescence.

Why might exercise be less effective with age?

Exercise is often prescribed for prevention of osteoporotic fracture based on the theory of functional adaptation of bones in which bone is able to adapt to changes in the

mechanical environment. The mechanism for doing so, the mechanostat, is a dynamic regulatory system that increases bone strength when exposed to larger than typical loads and rids bones of excessive mass when exposed to lower than typical loads [36]. The mechanostat's effector cells responsible for bone formation and resorption (osteoblasts and osteoclasts, respectively) have been well-studied and characterized. However, the mechanostat's sensory cells have only recently received attention. These sensory cells are members of the mesenchymal stromal cell lineage—osteoblasts, osteocytes, and bone lining cells. Of these cells, osteocytes are abundantly embedded throughout the mineralized bone tissue. They are also highly connected to each other and to bone lining cells by dendritic processes—allowing them to sense local changes in the mechanical environment. Therefore, osteocytes are considered the primary mechanosensory cells of bone [69].

Given the importance of osteoblastic precursors, osteoblasts, and osteocytes in bone formation, alterations in the abundance and functions of these cell types with age may help explain declines in bone mechanosensitivity.

Age and the ability to sense mechanical load

As osteocytes comprise the main mechanosensory mechanism in bone, whether these cells continue to remain viable with advancing age may be a key determinant of the ability of bone to process and respond to mechanical loading in adulthood and old age. As early as 1960, Frost made the observation that the percentage of osteocyte-occupied lacunae in human cortical bone fell 25% from age 10 (95% occupied lacunae) to age 40 (70% occupied lacunae)[108]. Frost reported no further significant decline following age 40 in the prevalence of empty lacunae and noted that the number of empty lacunae was

greater in interstitial bone than in osteonal bone. The later observation is logical in that osteonal bone is younger, and therefore, has been remodeled more recently than interstitial bone. As remodeling leaves behind new osteocytes, and an osteocyte lifespan has been estimated to be about 15-25 years [108, 109], it follows that bone must be remodeled at least every 25 years to replace dead osteocytes. Considering that remodeling is a surface-dependent process, bone that is further from a surface may be at greatest risk of not being turned over in a timely fashion to maintain osteocyte viability.

Later studies of osteocyte viability by Dunstan, Wong, and colleagues [110-112] measured lactate dehydrogenase activity in osteocytes from the femoral heads of 51 cadavers, eleven to eighty-nine years of age at the time of death. The authors reported a decline in osteocyte viability of trabecular bone of the femoral head from 92% at ages 1-22yrs to 68% at ages 81-90 yrs. Like Frost's observation of primary confinement of empty lacunae to older interstitial cortical bone, Wong et al., [112] reported that dead osteocytes tended to be in the deeper areas of the trabeculae. In this study, lacunar occupancy at the lumbar spine was not affected by age. In a similar study, [113], cancellous osteocyte density (number of osteocytes per unit bone *area*) from iliac biopsy samples from individuals 20-70 years of age. Parallel with previous research, the authors report a significant decline in total lacunar density with an increase in empty lacunar density and a fall in the proportion of occupied lacunae from 20 to 70 years of age. Again, this group found that at all ages, fewer osteocytes were found in deeper bone than in superficial bone.

Interestingly, several studies have found significantly reduced osteocyte density [114, 115] in patients with spontaneous vertebral fracture compared to controls. While two studies

reported no significant differences in osteocyte density [116] or osteocyte viability [111] between fracture cases and controls, both studies reported great variability, in which a few osteoporotic fracture patients had very low osteocyte viability. For example, Dunstan et al [111] report that five fracture patients had osteocyte viability of less than 25% in the femoral head.

Overall, these studies demonstrate consistently that the number of viable osteocytes in bone decrease with advancing age with the greatest losses of osteocytes in deeper bone tissue. Furthermore, these studies show that some individuals with osteoporotic fracture have significantly fewer osteocytes than controls. Given the crucial role of osteocytes in mechanotransduction, it is plausible that loss of osteocyte numbers in bone in with advancing age may result in a suppression of the ability for bones to detect mechanical loading, and therefore, lead to a perceived disuse. This remains to be empirically tested as it may have important implications for bone fragility.

While the number of viable mechanosensitive cells decreases with age, so too does the ability of mechanosensitive cells to initiate biochemical signaling following mechanical stimulation. Donahue et al [111] used fluid-flow induced shear stress to study intracellular calcium signaling in osteoblastic cells isolated from young, mature, and old rats. The authors reported that a significantly lower percentage of osteoblastic cells from old rats responded to fluid flow by release of Ca^{2+} compared to mature rats. Furthermore, reductions in basal Ca^{2+} signaling activity of older rats were reported in this study, suggesting that bone cells from older animals are less metabolically active than bone cells of younger animals.

Age and the bone formation response to mechanical stimuli

Not only is the ability to perceive increased mechanical stimuli likely hampered with advancing age, but so too is the ability to respond to a mechanical signal with bone formation. Osteoblasts are the primary matrix-forming and mineralizing cells in bone, and an attenuated ability to form bone in response to higher than customary loading with advancing age could be due to a decrease in the supply of osteoblastic cells which differentiate from multipotent mesenchymal stem cells.

The prevalence of osteoblastic progenitors can be estimated when bone marrow stromal cells (MSCs) are cultured *in vitro* by counting the colony-forming units fibroblastic (CFU-Fs) which are colonies of cells with fibroblast morphology that exhibit osteoblastic features such as alkaline phosphatase (ALP) expression. Tsuji et al [117] investigated the effect of donor age on the production of bone-like tissue and number of ALP-positive cells after in vitro culture. These authors reported three-fold fewer mineralized bone-like nodules produced by the MSCs of older rats (18 months) than those of younger rats (5-6 weeks). Furthermore, MSC cultures from the older rats also had reduced numbers of ALP-positive cells. Quarto et al [118]., also found a fewer number of ALP-positive CFU-Fs as well as fewer overall CFU-Fs in aged rats compared to young rats.

Similar studies using osteoblastic cells harvested from human donors have also demonstrated an age-related degradation in the number of ALP-positive CFU-Fs [119-121] Nishida et al [121] cultured human iliac bone marrow cells ex vivo which were harvested from ilia of 49 women from 4 to 88 years of age. The authors reported the highest level of ALP-positive CFU-Fs from the youngest group (under 10 years of age) with a lower number of CFU-Fs after the age of 20 and a gradual decline with advancing age.

Similarly, Majors et al. [119], reported a negative correlation of ALP-positive CFU-Fs with donor age (30 subjects ranging in age from 8 - 80 years) for both female and male donors, while another study found a decreased number of APL-positive CFU-Fs with age from female donors (n = 26, 13 - 79 years of age) but not from male donors (n = 31, 15 – 83 years of age) [120].

These findings from *in vitro* cell culture studies using cells from both animal and human donors demonstrate that age is associated with a marked reduction in the prevalence of osteoblast progenitors in the bone marrow. The decline in availability of osteoprogenitors with age may be partially due to an age-related decline in the ability of MSCs to differentiate towards the osteogenic lineage [118], and along these lines, it has been suggested that MSCs may have an increased affinity to differentiate into adipocytes rather than osteoblasts [122, 123] though there is evidence that refutes this theory [124]. A decreased ability to differentiate along the osteoblastic lineage may be in part related to replicative aging of osteoblastic precursors by telomere shortening [125]. Moreover, an age-related decline in locally produced factors such as insulin-like growth factor-I (IGF-I), transforming growth factor-beta (TGFβ), and bone morphogenic proteins (BMPs) that promote osteoblast proliferation and differentiation [126, 127]. While age-related growth factors such as IGFs may help explain declines in osteoblast differentiation, they are also known to play a roll in preventing osteoblast apoptosis [127], and therefore, may help explain declines in the lifespan of osteoblasts.

The ability of mechanical loading to result in bone formation may also be a function of osteoblast location. As highlighted by Parfitt[128], the surfaces of growing long bones are subjected to modeling drifts in which osteoblasts are continually recruited to the same

location for an extended period of time. Given that osteoblasts are already present and active along the periosteum, it follows that periosteal formation drifts are likely accelerated by physical activity during growth.

Menopause and bone mechanosensitivity

Onset of menopause may also explain declines in bone mechanosensitivity with age in women. Estrogen has been demonstrates to increase human bone cell production of prostaglandins in response to pulsating fluid flow, suggesting that estrogen enhances bone cell mechanosensitivity[129]. Therefore, declines in estrogen with the onset of menopause may make bones less responsive to mechanical stimulation. Along these lines, estrogen deficiency has been shown to increase the prevalence of osteocyte apoptosis[130], and estradiol has been shown to prevent osteoblast apoptosis[131]. However, interactions between estrogen and mechanical loading are not so straightforward. Estrogen has a surface-specific interaction with exercise in which it inhibits bone formation on the periosteal surface and enhances bone formation on the trabecular and endocortical surfaces—possibly due to differences in the prevalence of alpha and beta estrogen receptors on bone surfaces[132]. As estrogen receptors are necessary for exercise to have an effect on bone, the observation that there is a loss of estrogen receptors with a lack of estrogen may also explain declines in bone mechanosensitivity with the onset of menopause.

Summary of mechanosensory cells and age

In summary, the number and viability of osteocytes, availability of osteoblast cell progenitors, the lifespan and activities of mature osteoblasts, as well as declines in availability of estrogen and biomolecules critical to bone formation may all play important

roles in attenuating the osteogenic potential of mechanical loading in maturity, and particularly, with advancing age. This decline in bone mechanosensitivity with age highlights the importance of identifying important time periods during the lifespan to optimize bone strength through mechanical loading, as well as countermeasures which may make exercise more potent in maturity and senescence.

Implications for prevention of fragility fracture

Optimal timing for skeletal loading interventions

Given the evidence that exercise is more effective at improving bone strength during youth, it follows that youth provides an optimal time to intervene with exercise. However, for exercise to offset fragility fractures in later life, skeletal benefits from loading in youth must track into old age when fragility fractures are most frequent and have the greatest consequences. Retrospective cross-sectional studies and follow-up analyses of skeletal loading interventions using dual x-ray absorptiometry (DXA) have been variable in demonstrating long-term retention of skeletal benefits[133]. Nevertheless, it has been proposed that the planar nature of DXA measurements may underestimate small changes in bone geometry which confer large changes in bone strength[134], and therefore, DXA-based outcomes may underestimate long-term bone structural changes from exercise in youth that persist throughout adulthood and into old age. Although a randomized controlled trial of exercise in youth with follow-up into old age is unpractical, a recent animal study provides some new insight into these concepts.

Warden et al.[135], used the forearm compression loading model to exercise the right forearms of 5-week-old rats, 3 days per week, for 7 weeks followed by 92 weeks of detraining. While bone mineral content and areal bone mineral density of trained ulnae

were significantly increased following 7 weeks of exercise compared to the untrained ulnae, these benefits were not retained with detraining. However, structural changes due to exercise including periosteal apposition resulted in a 25.4% increase in cross-sectional second moment of area. These structural changes persisted throughout the rats' lifespan and resulted in a 23.7% greater ultimate force (indicative of bone strength) and a 10-fold greater fatigue resistance in exercised ulnas when broken at 92 weeks of age. These findings provide evidence that exercise in youth confers lifelong benefits in bone structure, strength, and fatigue life; and therefore, supports the theory that exercise should be implemented during critical time periods in youth to prevent skeletal fragility in older age.

Optimal loading characteristics to make interventions effective in old age

From animal studies, we know that for skeletal loading to be anabolic, it should be of moderate to high magnitude[136], be dynamic in nature[88], only require few loading cycles [137], and include rest-insertion between loading cycles and bouts[138, 139]. Given that exercise is less effective in maturity and old age, these loading characteristics should theoretically be included in a skeletal loading intervention in these age groups. In particular, as indicated by the animal literature reviewed above, a higher magnitude load is likely needed in old age to initiate an anabolic response. While high intensity resistance training in elderly populations have been conducted safely and have resulted in improvements in volumetric bone mineral density and falls risk[140, 141], high magnitude loading remains controversial in older populations due to functional limitations associated with ageing and consequent risk of musculoskeletal injury. Therefore, exercise prescription for skeletal health in old age should focus on implementing other loading characteristics.

One such characteristic is rest-inserted loading. Several animal studies have demonstrated that rest in the order of S, seconds[139], hours[138], and weeks[132] inserted between loading cycles and bouts increases bone mechanosensitivity, resulting in greater gains in bone strength. For instance, Srinivasan et al[139]., demonstrated that loading turkey ulnae with 100 cycles per day with low-magnitude loading and 10 seconds of rest inserted between each load cycle resulted in 21.9% of the periosteum activated compared to ulnae loaded with the same number of cycles at the same low magnitude for the same number of days but without rest inserted between each loading cycle (3.8% of the periosteum activated).

In another study by the same group[91], the authors demonstrated that while low-magnitude cyclic loading did not result in periosteal bone formation, insertion of rest between loading cycles significantly increased periosteal bone formation to the same degree as doubling the strain magnitude. These studies suggest that rest-insertion has great potential to magnify the stimulus of low- to moderate-magnitude mechanical loading, and therefore, enhance bone strength in old age.

While the mechanisms underlying the benefits of rest-insertion remain to be elucidated and likely differ based on the amount of time of rest insertion (i.e. seconds, hours, and weeks), one such mechanism may be cellular accommodation in which mechanosensitive cells adjust the stiffness of their cytoskeletons in response to fluid flow[142], likely inhibiting continued release of important signaling molecules such as nitric oxide and prostaglandins. Following cellular loading via fluid flow, the stiffness of the cell begins to return to baseline. While this mechanism likely protects the cell from strain

damage, it also may help explain why rest-inserted between loading cycles is beneficial. Cellular accommodation may also help explain cellular “saturation” in which bone cells become less mechanosensitive with increased loading cycles in a single loading bout. Fewer loading cycles have been shown to have similar osteogenic potential as a larger number of loading cycles. For instance, Umemura et al [137]., demonstrated that rats which performed 5 jumps per day had similar increases in bone mass and strength compared to those which jumped 40 times per day. Jumping 100 times per day resulted in only slightly greater bone strength gains than 5 jumps per day. Taken together, these studies suggest that only a few low- to moderate-intensity loading cycles with rest-insertion may be adequate to increase to formation of bone in response to exercise in the mature and senescent skeleton.

Another potential mechanical modality to bypasses the need for high magnitude loading to stimulate formation and preserve bone mass and strength with advancing age is low amplitude, high frequency vibration. This technique has been shown to suppress post-menopausal bone loss[143] and likely works by stimulating mechanosensitive cells by inducing fluid-flow while circumventing the need for tissue-level strains. Further human studies are needed to test the efficacy and safety of vibration therapy; nevertheless, this modality has osteogenic potential in elderly populations.

While skeletal loading in mature and older populations has generally not been effective as in youth, implementing loading interventions that take advantage of optimal loading characteristics highlighted from the animal literature may make such interventions more anabolic during later life when bone fragility is most prevalent and has the greatest consequences.

Summary and conclusions

Mechanical loading of the skeleton appears to be less anabolic in adulthood and in old age than in youth. This may be attributed to an attenuated ability to sense mechanical load, produce and sustain biochemical signals from mechanical signals, and to respond to mechanical stimuli through bone formation with advancing age. These senescent changes are likely related to decreases in the supply, function, and lifespan of mechanosensitive and bone formation cells as well as changes in the availability of biomolecules and sex hormones.

Therefore, from a public health perspective, skeletal loading interventions should be implemented during youth when exercise is most osteogenic to offset the population burden of osteoporosis and fragility fractures. Finally, during maturity and old age, exercise interventions should take advantage of loading characteristics such as rest-insertion that have been demonstrated to enhance the response of bone to mechanical stimuli.

4

THE ROLE OF BONE FUNCTIONAL ADAPTATION IN THE PREVENTION OF OSTEOPOROTIC FRACTURES

The structure of our bones appears to be well adapted to the mechanical demands placed on them. This structure is, in part, contained in our genetic blueprint[144]. However, our bones are also able to adapt to alterations in the mechanical environment throughout the human lifespan. For example, when in a state of disuse, such as immobilization from casting or bed rest, bone is lost[65, 145]. Conversely, when bone is exposed to greater than customary loading, bone is added to the existing structure. It is this functional adaptation of bone that provides the theoretical basis for exercise prescription to optimize bone structure with the goal of offsetting risk of fracture. It has been postulated that if the role of bone functional adaptation is indeed to optimize bone structure, then the frequency of fractures should be low. Since this is not the case and fractures are quite common, it has been suggested that a flaw must exist in the mechanical adaptation hypothesis[146].

In this paper, I present an alternative way of interpreting the role of bone functional adaptation in the prevention of fracture. In particular, I use evidence form the skeletal literature to demonstrate that the goal of functional adaptation of bone is not necessarily to reduce risk of fracture from abnormal loading events (i.e. a sideways fall on the hip), but rather the goal is to prevent fractures from customary loading. I propose that these two goals may, but do not necessarily, overlap and therefore fractures remain prevalent despite the continued proper functioning of the mechanisms of bone functional adaptation.

I begin by briefly reviewing how bones adapt to changes in their mechanical requirements. I then use two clinically relevant skeletal sites as examples of how this adaptation to customary mechanical loading may not transfer to the prevention of fractures from abnormal loading events. I conclude with a discussion of why exercise

remains beneficial for preventing fractures from abnormal loading events and some practical suggestions for optimizing the effectiveness of exercise in this endeavor.

Bone Functional Adaptation

The adaptation of bone to prevailing loads has been characterized in one way or another since Galileo, and later by Wolff[147], von Meyer, Cullman, and Roux[35]. More recently, the mechanism of functional adaptation of bone was introduced by Harold Frost[148] and further characterized by Frost[36] and several others[149,88,150,39.] This mechanism, termed the "mechanostat," is a dynamic regulatory system in bone responsible for altering bone structure in response to changes in the mechanical demands placed on bone. In line with the mechanostat hypothesis, bone has been shown to decrease in mass with a lack of customary loading, while bone has been shown to be added to the skeleton when greater than customary loading is introduced[65, 151]. However, bone mass is not added randomly to the existing skeletal structure in response to increased mechanical loading (i.e. exercise), but rather, bone material appears to be added to specific locations that greatly increase the strength of bone. For example, Robling et al., demonstrated that after loading the right ulnas of 26 adult female rats for 16 weeks of 360 load cycles/day resulted in only 5-12% gains in areal bone mineral density and bone mineral content. However, when the bones were broken in a similar direction in which they were loaded, the ultimate force the bones could sustain without fracture increased by 64 - 87% and the energy to failure increased by 94 - 165%. These findings suggest that modest increases in bone mass, placed where mechanically optimal, can result in large biomechanical benefits[151].

Orientation of Bone Tissue

While addition of mass to mechanically optimal locations will increase bone strength in the direction of loading, the orientation of bone tissue may also aid in this effort. Like most materials, bone is anisotrophic which means that its mechanical behaviors vary according to the direction of loading[11]. In bone, anisotrophy is due in part to the grain of the tissue. In cortical bone, the grain of the bone is determined by the orientation of osteons, and in cancellous bone, anisotrophy is due to the orientation of the trabeculae. Because both the osteons of cortical bone and the trabeculae of cancellous bone are aligned in the dominant loading direction, it has been proposed that this alignment is mechanically regulated [152]. That is, in bone remodeling, mechanical loading determines the directions of tunneling osteoclasts (the cells responsible for resorbing bone) as well as the coupled action of osteoblasts (the cells that are introduced to the same area to deposit new bone). In alignment with this theory, in loaded bones, osteon orientation appears to be in the direction of principal stresses[153] while osteon orientation in unloaded bones appears to be deregulated[153, 154].

Given the evidence that bones become stronger in the direction of customary loading due to optimal placement of new bone tissue as well as changes in the grain of the cortical bone and the orientation of trabeculae, it follows to question whether these adaptations to mechanical loading also transfer to abnormal loads such as a fall on the side of the hip. In the next sections, I consider two examples in which bones adapt to customary loading but may not aid in the prevention of fractures from abnormal loading.

The Proximal Femur and Fracture

Fractures occur when the external force placed on bone exceeds the strength of the bone [155]. In the proximal femur (a common site for osteoporotic fractures), the strength of the bone appears to be adapted to customary loading such as walking but not adapted to the loads incurred from a fall on the side—an event that precipitates most hip fractures. In line with the mechanostat theory, at the midfemoral neck, humans have thicker cortices in the inferior region where the bulk of mechanical loads are transmitted from walking. However, the cortices are thinner in the superior region of the midfemoral neck where the stresses are greatest in a sideways fall onto the greater trochanter [156]. This discrepancy in bone distribution is reinforced with advancing age as the cortical thickness of the susceptible superior region of the midfemoral neck is preferentially thinned while the inferoanterior region of the midfemoral neck is well maintained.

Another example of the femoral neck's adaptation to mechanical loading is that, with ageing, there is periosteal expansion at the proximal femur[29]. With loss of bone mass that occurs with age, this expansion of the outer diameter of the bone maintains the bending strength of bone during customary loading by positioning the remaining bone tissue further from the bone's neutral axis[29]. However, this process also further thins the fragile cortices of bone which may render bone more vulnerable to fracture from a fall on the greater trochanter[29]. In line with this theory, Beck et al., demonstrated that the proximal femurs of elderly women who had experienced hip fracture had thinner cortices that were wider in diameter than age-matched, fracture-free controls[29].

These site-specific structural differences at the proximal femur suggest that the hip, even when rendered fragile by old age, is well adapted to customary mechanical demands such as walking but is not adapted to abnormal loading events such as a sideways fall.

Vertebrae and Fracture

Like the proximal femur, vertebral bodies are common sites of bone fragility fractures and also adapt in structure to alteration in their customary mechanical environment. Homminga et al., used finite-element analysis to study the trabecular load distribution in osteoporotic and healthy vertebral bodies under normal daily loading[157]. They found that, although an osteoporotic vertebra had 25% less bone material than a healthy vertebra, the number of highly loaded trabeculae was not higher in the osteoporotic vertebra than in the healthy one under normal daily loads. The authors were able to explain this surprising finding through their observation that the osteoporotic trabeculae were more oriented in the longitudinal direction. However, as with the example of the proximal femur, this high orientation to customary loading resulted in less resistance to abnormal loading. The researchers found that the number of overloaded trabeculae in the osteoporotic vertebra was higher than in the healthy vertebra with abnormal loading. Therefore, they conclude that osteoporotic bone is well adapted to customary mechanical loading, but that this adaptation through increased axial orientation makes vertebral structures more vulnerable to loads in unusual directions[157].

These examples of functional adaptation in the hip and vertebrae highlight that bone functional adaptation is concerned primarily with improving bone strength for customary loading. In both examples, even with bone loss, the structure is adapted to maintain mechanical competence for customary loading but is not well adapted to abnormal loading events. These observations raise questions concerning the efficacy of prescribing exercise for improving bone health.

Why prescribe exercise for bone health?

Even though the skeletal benefits from mechanical loading do not completely transfer to prevention of fractures from abnormal loading events, exercise is still critical for developing bones that are resistant to osteoporotic fracture. This is due to several reasons. One such reason is that, when bones are not loaded as they are accustomed to, bone is lost through thinning of cortices and trabeculae as well increased pitting of bone due to a higher rate of remodeling[158]. This disuse-mediated loss of bone can be particularly devastating in trabecular bone where increased bone resorption can result in perforation of trabecular elements—creating a disproportionate loss of bone strength relative to the amount of bone lost. Also, thinning of cortices with disuse will make bones less resistant to fractures in general. Therefore, at least maintaining customary physical activity is important in order to keep bones from becoming fragile.

Exercise not only protects from disuse-mediated bone loss. As reviewed above, loading of bones above the customary level also adds new bone to the skeleton—albeit with the concession that this addition of bone may not be in critical locations for protection from fracture in the case of abnormal loading events. However, exercise can be designed specifically to target areas at greatest risk for osteoporotic fracture through odd-impact loading. Lanyon and Rubin[158] were the first to demonstrate the importance of odd-impact loading on bone formation. They showed that altering the distribution of strain through artificial loading of the wing bones of turkeys resulted in greater formation of bone relative to the loading generated by the natural flapping of wings. This greater bone formation occurred despite the same peak strain magnitude and maximum strain rate during both the artificial and natural loading of the wings—indicating that abnormal distribution of strain in bones is osteogenic. These findings highlight that adaptations to

mechanical loading are site-specific, and therefore suggest the possibility that loading may be manipulated to target areas at greatest risk from abnormal loading. In support of this theory, Nikander et al.,[159] demonstrated that soccer and squash players, whose sports consist of odd-impact exercises, had ~20% thicker cortical bone at both the anterior and posterior cortex of the femoral neck compared to controls. This study demonstrates that odd-impact loading is associated with thicker cortices in areas of the proximal femur that are most susceptible to fracture, and therefore, it is important to include such activities in exercise prescription for the prevention of fragility fractures.

A final but important argument for prescribing exercise for the prevention of osteoporotic fractures, is that, not only does exercise strengthen bone, but it also decreases the risk of falling by improving muscle performance and balance. This benefit from exercise is particularly important because the strongest risk factor for fracture is falling and not low bone mass[160].

In summary, despite incomplete transfer of benefits from mechanical loading to prevention of osteoporotic fractures, exercise remains important for bone health because it prevents bone fragility, it can be targeted to specific areas that are beneficial in abnormal loading situations, and finally, exercise may prevent fracture through decreasing the propensity to fall.

Conclusion

The fact that fractures are common does not necessarily suggest a flaw in the functional adaptation hypothesis of bone. Rather, bones are able to respond to deviations from customary mechanical stimuli in order to remain mechanically competent. They are not

able to predict how one might load them in unusual or traumatic ways, and therefore, are not concerned with adapting in this way. Adaptations of the proximal femur and vertebrae to customary loading but not abnormal loading provide examples of this phenomenon. Despite these observations, mechanical loading remains critical for preventing bone fragility. In particular, diverse, odd-impact activities may help target areas of bone at greatest risk of osteoporotic fracture. Understanding this concept is important for properly characterizing the role of exercise for the prevention of fragility fractures.

5
CONCLUSIONS AND PRACTICAL IMPLICATIONS

Paper One: *The Biological Underpinnings of Frost's Mechanostat Thresholds*

Main Conclusion: Osteocytes are an important part of the cellular machinery of bone functional adaptation.

Secondary Conclusions

- Osteocytes are critical for bones to respond to increased loading through bone formation (Bone Formation Threshold).
- Osteocytes are critical for bone to respond to a decrease in loading through bone resorption (Bone Resorption Threshold).
- Osteocytes are critical for responding to microdamage formation by initiating bone remodeling.
- Bone modeling and remodeling are distinct processes that are initiated for different mechanical (and nonmechanical) reasons. The initiation for either process is reliant on osteocytes, but the pathways are different.

Implications:

- Maintaining osteocyte viability may be a key determinant in preventing bone loss.
- Osteocytes should be targeted for osteoporosis preventative measures and pharmacological therapies.
- Effectiveness of exercise interventions should be based on the effects of the intervention on osteocyte viability.

Paper Two: *Is Exercise Less Osteogenic with Age?*

Main Conclusion: The immature skeleton is more sensitive to mechanical loading than the mature and senescent skeleton.

Secondary Conclusions:

- Younger skeletons are better able to respond to increased mechanical loading through additions of bone to the periosteal surface than older skeletons.
- This age-related decline in bone mechanosensitivity is likely due to reductions in mechanosensory and osteoprogenitors cells with age—decreasing the ability of bone to sense and respond to changes in the mechanical environment.

Implications:

- Therapies for osteoporosis treatment should target osteosensory and osteoprogenitors cells.
- Exercise interventions should be initiated in youth when bones are most responsive to mechanical loading.
- In maturity, and especially in old age, exercise characteristics such as rest-inserted loading should be used to optimize bone's response to loading.

Paper Three: *The Role of Bone Functional Adaptation in the Prevention of Osteoporotic Fractures*

Main Conclusion: Skeletal benefits from exercise do not completely transfer to prevention of osteoporotic fractures.

Secondary Conclusions

- This incomplete transfer is related to how and where bone is added to the skeleton. Bone becomes stronger in the direction of customary loading and is therefore not completely resistant to loading in abnormal directions.

Implications:

- Exercise can be targeted to directly prevent fracture from abnormal loading. As an example, odd impact loading may improve bone strength of susceptible regions of the proximal femur.

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