Minireview

The skeleton in a physical world

Janet Rubin and Maya Styner

Department of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
Corresponding author: Janet Rubin. Email: jrubin@med.unc.edu

Impact Statement

The skeleton represents an example of a tissue that interacts with and is molded by the dynamic physical environment. We here consider how the output of the mesenchymal stem cell responsible for forming the skeleton is regulated by mechanical force. We review the physical forces generated with loading exercise and the response of the stem cell to these forces, first at the cell membrane, and then how force is translated into control of gene expression. This type of fundamental biological information is now being used to guide exercise regimens to strengthen the skeleton and promote quality of life.

Abstract

All organisms exist within a physical space and respond to physical forces as part of daily life. In higher organisms, the skeleton is critical for locomotion in the physical environment, providing a carapace upon which the animal can move to accomplish functions necessary for living. As such, the skeleton has responded evolutionarily, and does in real-time, to physical stresses placed on it to ensure that its structure supports its function in the sea, in the air, and on dry land. In this article, we consider how those cells responsible for remodeling skeletal structure respond to mechanical force including load magnitude, frequency, and cyclicity, and how force rearranges cellular structure in turn. The effects of these forces to balance the mesenchymal stem cell supply of bone-forming osteoblasts and energy storing adipocytes are addressed. That this phenotypic switching is achieved at the level of both gene transactivation and alteration of structural epigenetic controls of gene expression is considered. Finally, as clinicians, we consider this information as it applies to a prescriptive for intelligent exercise.

Keywords: Mechanical force, exercise, mesenchymal stem cell, actin, cell structure, adipose

***Experimental Biology and Medicine* 2022; 247: 2213–2222. DOI: 10.1177/15353702221113861**

Introduction

"It is a truth universally acknowledged" (with thanks always to Jane Austen) that physical force impacts and regulates the form and function of existing organisms: wind or water alters the work of walking, flying, and swimming, physical impact bursts skin and breaks bone, and heat induces fluid loss – all these forces must be dealt with for an animal to survive. Inescapable interaction with the physical environment has guided evolution, from the beginning of life in primordial seas to determining structure of organisms leaving the sea for land. This truth brings us to the complementary fact – that living organisms in a physical world must be able to interpret and adapt to that environment. Specific to our topic, it is the vertebrate skeleton that allows organisms to move rapidly through a landscape to find food and shelter. And indeed, the skeleton is highly adapted for the multiple physical jobs that it has, melding its role allowing mobility and work with a second responsibility as a reservoir of calcium, phosphorus, and magnesium, as well as a location for hematopoietic precursors. The basis by which the cells of the skeleton sense and respond to the physical world is largely conserved between most cells in the body, and it can be traced evolutionarily to analogous pathways in single-celled organisms from which complex organisms evolved. As such, the skeleton is an excellent tissue to infer lessons of shaping and adapting to a physical environment.

Here, we will consider that the skeleton responds to loads which generate discrete mechanical signals to allow remodeling of form at a macro – or tissue – level, and we will scale down many layers to examine how the cells responsible for adding and removing bone material are guided at a molecular level by these physical forces. As the skeleton enlarges from mouse to elephant, evolution optimizes the balance between efficiency (lightness for locomotion) and strength (withstanding force). It is important to recognize that body size is scaled up: structural competence cannot be achieved if the relative proportions are unchanged. D'Arcy Thompson, the Scottish mathematical biologist writing "On Growth and Form" in 1917 emphasized that, for a larger structure, "the thing will fall to pieces of its own weight unless we either change its relative proportion . . . or else we must find new material, harder and stronger than was used before." Thompson foresaw the need for large animals to reconfigure proportions to avoid structural collapse by changing the shape of bones. Despite anatomical change, however, elephants cannot compete with mice in running up walls. In terms of the forces received by both small and large

skeletons, we will consider how and what types of forces are recognized by bones at the macro level, and by bone cells at a molecular level. Ultimately, we will consider how cells' perception of mechanical forces cause remodeling of cellular architecture all the way into the nucleus where changes in nuclear structure alter gene expression through effects on the nuclear epigenetic landscape. To round out this discourse, we will briefly consider how a person might utilize force/exercise to improve skeletal morphology and strength.

Effect of exercise on the skeleton

Bone is a composite material of a stiff hydroxyapatite mineral built on a protein matrix primarily comprised of fibrous proteins such as type I collagen, as well as protein-linked polysaccharides and proteoglycans. Bones can be flat, such as the pelvis and skull, developing without a cartilage anlage (an intermediary structure), or long, such as the humerus and femur. These types of bone contain cancellous (trabecular or spongy) and the dense cortical bone. Both types of bone respond to the presence and absence of loading.[1] During growth, the skeleton increases in length and breadth, both modeling (making new bone) and remodeling to achieve adult endpoints. The interstices of the skeleton harbor the major site where elements of the bone marrow reside, both the progenitors of specific stem cells that form bone (osteoblasts) and resorb bone (osteoclasts), and the hematopoietic precursors such as the oxygen carrying red cells and white cells for protection against exogenous threats.[2] The skeleton thus protects itself, and the cells residing within bone, as it performs its primary function of providing the framework structure upon which its organism relies to live. The skeleton needs to achieve both lightness and strength while experiencing loads that are compressive and tensile, and forces which cause bending and torsion.[3] An individual animal can increase bone mass and lose bone mass through adaption to experienced loads. The ability of bone to remodel to meet functional demands was recognized by Wolff in 1892 as the "law of bone transformation."[4] Studies in humans and animals have shown that loading can increase bone density and alter macro- and micro-skeletal structure.[5] Similarly, the loss of load leads to loss of structure.

Since bone apposition and resorption are accomplished by osteoblasts and osteoclasts, respectively, cells themselves must be able to sense loads conveyed through strain of the skeleton. Functional activity generates deformation in bone, pressure in bone cavities and transient pressure waves, shear forces through canaliculi, and electric fields as interstitial fluid flows past bone crystal.[6] Such physical signals can be described by a combination of magnitude, cyclical nature, rate of increase, distribution across the bone and repeated loading – reminiscent of the dosing of pharmacological agents. Responding to these signals within bone are other components, such as the endothelial vasculature carrying blood, which through stretch and shear, can produce soluble factors such as nitric oxide, which also affect bone cells through cognate ligand receptors.[7]

Current dogma suggests that the terminal phenotype in osteogenic lineage, the osteocyte, as it is embedded in the bony matrix is particularly well situated to assess distribution of load and control relevant processes.[8,9] However, most cells studied have the ability to respond to physical force based on *in vitro* investigations in which mechanical force has been directly applied. Marrow stromal cells, osteoblasts, and osteoclasts have all been shown to generate signals and genetic responses, as well as changes in physical structure to most (if not all) of the forces with which they have been challenged,[6,10] as well as to generated soluble factors generated after loading in the local microenvironment[2,11] (Figure 1). It is important to note that in the living organism, cells *in situ* are not simply monolayers, but also attend to organizational cues from their experienced three-dimensional (3D) space, interconnections with each other, bone surface and other cell types, and as such are primed to receive incoming physical signals which are reflected through the lacunar and marrow spaces of both flat and long bones. This complicates precise understanding of *in vivo* response to mechanical load.

Our lab has specifically studied how mesenchymal stem cells (MSCs) respond to mechanical load, the *in vitro* analogue to physical exercise. The multipotent MSC resides in the bone marrow and is different than MSCs found in, for instance, adipose depots or muscle, in that, perhaps by virtue of its geography, it is primed to become a bone-forming osteoblast.[12] As well the bone MSC can readily switch course to become an adipocyte to serve in part as a local energy depot.[13,14] *In vivo*, a lack of loading, along with an excess of calories, increases bone marrow adipose tissue (BMAT) accumulation,[15] not only through expansion of extant adipocytes but also through driving progenitors into adipocyte phenotype.[14] In contrast, increased loading increases numbers of osteoblasts and ensuing bone formation[16–19] while decreasing adipocyte formation.[20,21]

The mechanical attenuation of adipogenesis led us to become interested in the BMAT, and to ask whether this adipose depot was relevant to bone health as well as to the skeletal benefits of mechanical and exercise stimuli. BMAT was initially viewed as a marrow space filler, and even was proposed to lack a physiologic purpose; however, this was well before significant work had been performed investigating adipocytes in other depots (white and brown adipose tissues) which are now known to have significant physiologic and pathophysiologic roles in health.[22] Bone biologists' interest in BMAT stemmed from observations that it increased during aging, concomitant with the inevitable aging-associated decline in bone volume.[23]

Attention to a potential role of BMAT during aging and osteoporosis was slowed by inadequate 3D quantification of BMAT in histologic sections.[24] In 2014, the lab of Horowitz at Yale added the lipid binder osmium to mouse bone sections prepared for micro computed tomography (CT), using region of interest analyses.[25] This was, indeed, a step forward and allowed our lab to quantitate the marrow adipose depot and to appreciate how histology might miss changes, particularly in younger animals that have smaller adipose depots confined to the metaphysis. We added advanced image analysis methodology that quantified BMAT volumetrically in the whole bone as well as in prespecified regions[14] by computing the 3D volume of osmium within such regions (Figure 2). After finding that mechanical strain repressed MSC

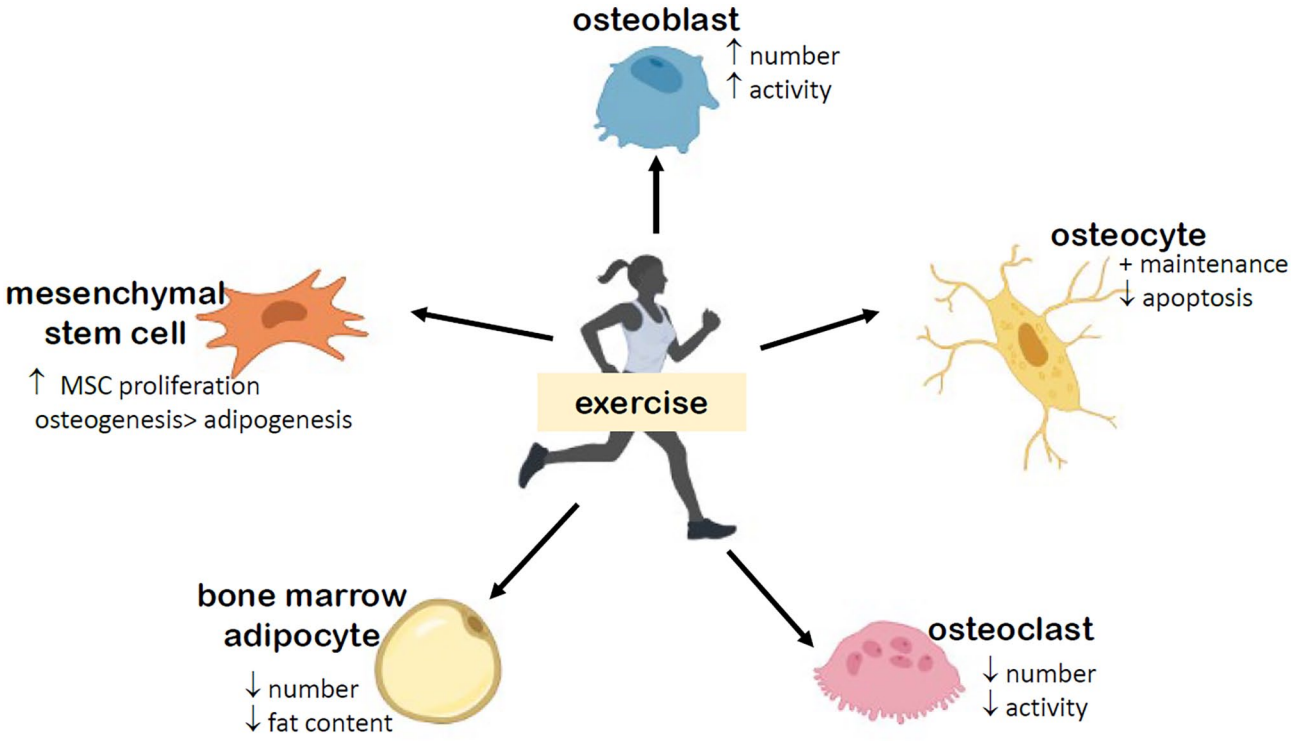

Figure 1. Bone cells are impacted by physical load. Bone cells involved in modeling and remodeling the skeleton include MSC and its output of osteoblast, osteocyte, and adipocyte cells as well as the osteoclast, from a hematopoietic precursor. Each of these cell types responds to physical force which, under physiological conditions, leads to formation and remodeling of bone to improve bone quantity and quality. (A color version of this figure is available in the online journal.)

adipogenesis *in vitro*,[26–29] we assessed the effect of exercise with respect to marrow fat after voluntary, wheel-based running exercise over six weeks.[13,14,21,30] We found that running lowers BMAT significantly, as quantified via histomorphometry, osmium-μCT and a third technique, 9.4T magnetic resonance imaging (MRI) imaging. In the same animals, running simultaneously served to increase trabecular and cortical bone.[14,21] It remains unclear as to whether decreased bone marrow adiposity and increased bone formation are due to directing MSC differentiation away from adipogenesis and toward osteogenesis – or whether the decreased BMAT stores are predominantly through use to fuel anabolism.[30]

Interestingly, not all bone marrow adipocytes are equal – nor do they function purely as a depot for energy. There are two situations where we would expect that bone marrow adiposity be depleted. The first is in the case of severely decreased calories: we limited mouse diet to 70% calories needed for "ideal body weight." In these calorie-restricted mice, we found that despite significant loss of peripheral white adipose depots, a large BMAT depot persisted,[31] suggesting that there is some basal requirement for the presence of marrow adipocytes, potentially to support hematopoiesis.[2] However, such restriction of calories and hence of peripheral white adipocyte lipid stores, prevented the effect of exercise to promote bone formation.[31] This is similar to the situation in young humans with anorexia, who have long-term problems with low bone density and increased fracture risk.[32] A second model of decreased BMAT is the *Bscl2* (SEIPIN) deficient mouse, which mimics the human Berardinelli–Seip congenital lipodystrophy having little to no adipose stores,[33] ectopic lipid in the liver (steatosis), and metabolic

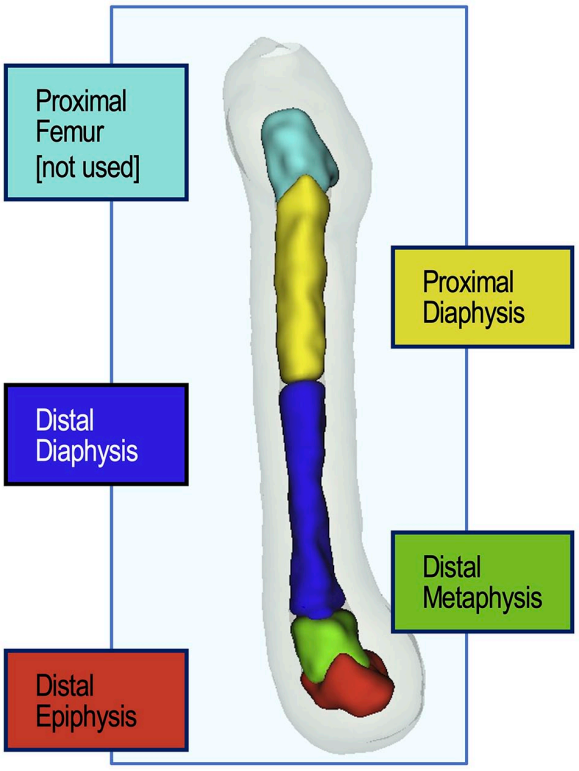

Figure 2. Bone marrow adipose tissue (BMAT) regional quantification. The figure demonstrates isolation of bone masks as detailed in McGrath *et al.*,[34] where voxel-wise correspondence allows direct comparison of intensities. Average fat maps for each experimental group were computed in the common space and superimposed on the common, average water image for visualization of group fat maps. (A color version of this figure is available in the online journal.)

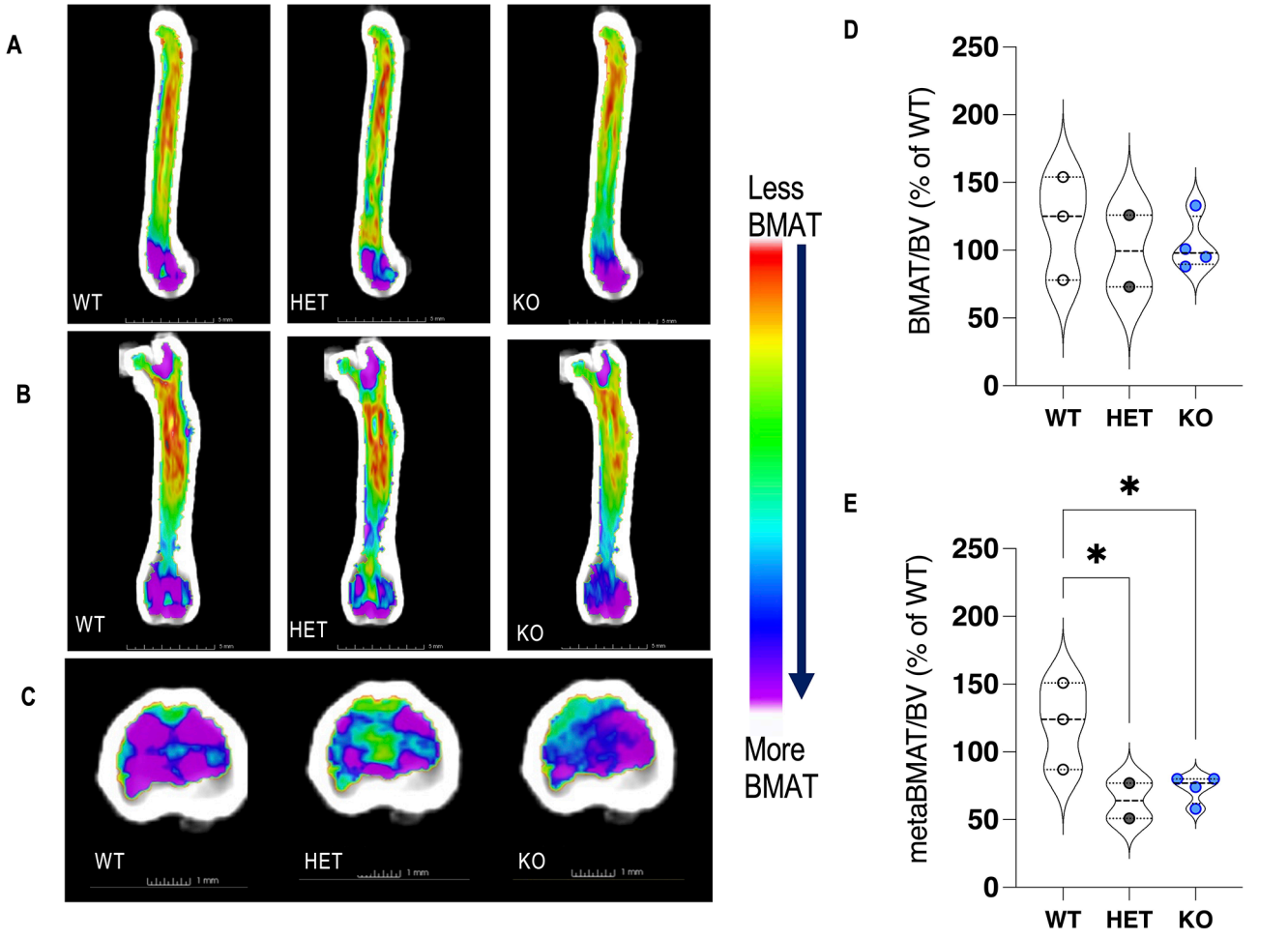

Figure 3. Bone marrow adipose tissue (BMAT) quantification via high-resolution MRI with advanced image analysis. This displays our quantitative method for measuring BMAT via 9.4T MRI with advanced image analysis, validated in Styner *et al.*[30] Fat map intensities were represented with a colored heat map in 3DSlicer for visualization (open-access: www.slicer.org). For BMAT quantification, we created a regional label map of the femur, excluding cortical bone regions, with regions for the epiphysis, metaphysis, and diaphysis. Intensity-weighted volume of BMAT was then quantified via regional fat histograms. Twelve-week-old male (WT, $n=3$), SEIPIN heterozygotes (HET, $n=2$), and SEIPIN KO (KO, $n=4$) were analyzed. Data are plotted as individual values in violin plots. Total as well as metaphyseal BMAT was quantified relative to bone volume. One-way analysis of the variance demonstrated a significant overall difference between the groups ($P=0.04$). The between group differences were obtained via posthoc analysis with $^*P<0.05$. These data have not appeared in the previous publications. (A color version of this figure is available in the online journal.)

derangements such as insulin resistance and diabetes. We were surprised to find that in the homozygous SEIPIN knockout (KO) mouse as well as its heterozygote (HET), that BMAT was still present – despite the lack of extra-marrow lipid depots, although lower than in wild-type animals. This is shown in Figure 3. Interestingly, the SEIPIN KO mouse, despite the lower BMAT, has a robust anabolic bone response to exercise,[34] in contrast to the calorie-restricted model. This highlights unknowns in exercise responses that may have to do with local energy stores or effects of BMAT on osteoblast differentiation; anabolic response to exercise requires *enough* calories, not necessarily local calories.

Thus, an objective of our work has been to understand how the MSC orchestrates its differentiation in response to physical signals: loading promotes osteoblast function and represses adipocyte differentiation and unloading decreases osteoblast formation and allows adipocytic progression. We would like to bring to attention the vast panoply of processes involved in the regulation of skeletal remodeling.

Alternative targets respond to physical forces

It would be too single-minded to propose that all exercises are anabolic to the skeleton through direct effects on cells of the skeleton. Here, we will briefly mention some of the many alternate targets impacted by physical force that indirectly modulate skeletal adaptation.

Indeed, it is difficult to separate effects of exercise on muscle from those on the skeleton, as exercise will promote muscle health and hypertrophy at the same time. For instance, it has been shown that grip strength predicts distal radius bone size and strength in women and men.[35] Furthermore, a proof-of-concept experiment (as to linkage of muscle and bone quality) can be performed with myostatin-deficient mice which have larger muscles in the absence of exercise. Myostatin is an inhibitor of muscle growth, and when deficient, as memorably shown in a family of professional athletes to increase muscle and decrease adipose shown in a

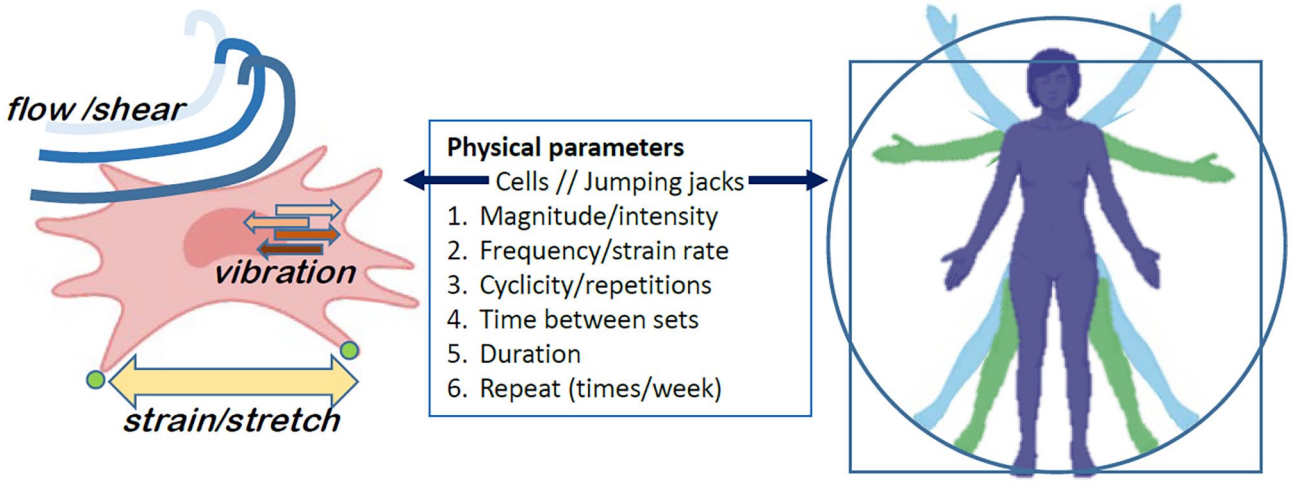

Figure 4. Parameters of experienced or applied mechanical load in cells and organisms. Exercise generates forces within bones in the form of strain as a cell is stretched between its connections to its substrate, flow of interstitial fluid over the cells causing distortion and, in some cases, electrical fields with flow over charged local molecules, and vibration of the dense nuclear body contained within the less massy cell cytoplasm. These forces are experienced as applied parameters, similar to dosing of drugs, including the magnitude (intensity) of force, and the frequency or strain rate. The force might be repeated within seconds or hours, and re-dosed over days with rest periods respecting the cell's ability to mount a response. These ideas can be represented at the macro level during exercise in terms of weight lifted, speed of performance, repetitions, and sets – and how many times per week, the individual succeeds in getting back to the gym. (A color version of this figure is available in the online journal.)

proband,[36] or to loss-of-function mutations in the "double-muscled" Belgian Blue cattle.[37] Comparing effects of 30 min/day treadmill exercise, Hamrick *et al*.[38] showed that structural and biomechanical parameters were significantly increased in myostatin deficient compared to wild-type mice, confirming that muscle mass has some effect on bone quality.

The effect of exercise on neurological and psychiatric health might contribute as well. Exercise is known to improve mood, even during the COVID pandemic.[39] What has always struck us is the desire of laboratory mice, restricted to a cage, to run. Our animals run an average of 10 km/day,[14] and concomitant obesity, hunger due to calorie restriction, illness due to SEIPIN deficiency, or frailty due to a laminopathy[40] does not impede their desire to be active.[30,31,34] Perhaps in this regard, mice are different than humans? Or perhaps exercise is a way to provide the caged animal with an enjoyable pastime.

Exercise improves the immune system as well, decreasing inflammation. This may at least partially be at the level of the hematopoietic niche in bone marrow, which houses the precursors of inflammatory cells.[41] Loss of skeletal elements leads to a loss of B-cell lymphopoiesis decreases B-cells.[42] Inflammation, as is seen in obesity, promotes osteoclastogenesis, thus loss of bone.

An acute bout of exercise enhances the immune system, mobilizing immune cells – for example, CD8 + T lymphocytes and natural killer cells – in the hours following exercise.[43] This may at least partially be at the level of the hematopoietic niche in bone marrow, which houses the precursors of immune cells[41] which are stimulated by catecholamines to exit the niche.[44] Although some lymphopenia can follow transiently in this setting, it is thought to be due to a redistribution of T lymphocytes in tissues that require immune protection. Mechanical stimuli such as vibration have been shown to improve other aspects of the immune response including B-cell lymphopoiesis.[45] Inflammation, as seen in aging and arthritis, promotes osteoclastogenesis, thus loss of bone.[2] Exercise inhibits osteoclastogenesis through a variety of mechanisms and has also been noted to interact with other immune cell population such as monocytes and neutrophils.[46] Much, although, remains to be learned about exercise interaction with the immune system as this relates to skeletal and whole-body health.

Recently, it has been shown that the make-up of the gut microbiome demonstrates sensitivity to exercise: a systemic review suggested that gut microbiome diversity is associated with aerobic exercise and training duration.[47] While results like this beg for further research, the gut microbiome has been shown to respond to exercise, and the changing phyla have correlated with bone volume.[48] Besides being part of the complex biology that determines skeletal health,[49] the gut also certainly has a role in regulating immune system effects on the skeleton.[50]

Exercise also controls the mineral homeostatic parathyroid hormone (PTH), which activates bone turnover, promoting both resorption and formation.[51] PTH has been found to increase in the late phase of long-term exercise, as well as during recovery.[52] Different intensity and duration of exercise have effects on gonadal hormones, cortisol, and multiple myokines, all which are known to affect bone and muscle, and likely modulate the macroscopic response to exercise in animals and humans.

Signaling pathways activated by physical loading in MSCs

In vivo studies in humans and animals have shown that the degree of response to exercise is determined by the magnitude of a cyclical load;[53] static loading does not induce bone remodeling and growth.[54] The intensity of the signal generated,[53] the frequency, and the cycle number[55] all have similar scalable effects in vertebrates[56] (Figure 4).

Physical signals are received by a multitude of cellular receptors which cause alterations in cell structure. These have been covered in multiple reviews, that is,[6,57] and can be generated by effects of force at multiple loci. These include strain or flow that activates integrins that connect the plasma membrane to the substrate, thus activating intracellular signaling cascades (as we will show below), or force-induced alteration of protein topology, or force-induced acceleration of the massy nucleus within the cell.[58] It is well accepted that activation of intracellular signaling pathways can have distal effects on gene activation, but we also know that physical alterations in structural components of the genome can alter gene availability – all leading to variable regulation of gene expression in MSCs.

Experiments in which physical force is applied to cells *in vitro* have demonstrated that virtually all parameters of force are sensed by the cells that model and remodel bone. The force that cells experience *in vivo* has been broken down to multiple parameters including shear and pressure – as interstitial fluid and marrow liquid flow over bone cells and through stem cell niches, electric fields generated over the charged bone crystals, and strain as cells adherent to bone stretch as the macro-structure bone bends and twists. As examples, we have shown that electric fields decrease osteoclastogenesis,[59] as does increased hydrostatic pressure,[60] by repressing soluble and cell-associated factors that control differentiation of cells of macrophage lineage. Oscillatory flow[61] and stretch[62] have direct effects on osteoblasts, altering their function. In several decades of study, we have specifically focused on effects due to strain (stretch) in bone marrow MSCs *in vitro*.

The self-renewing MSCs in bone that are abundant in youth, and sparse in old age, are inflected by location to be already primed toward osteoblast lineage.[12] Under culture conditions where ascorbic acid is available, they secrete an extracellular matrix that allows them to break their monolayer and develop into nodules, where they express the phenotype of the osteocyte.[9] MSC from bone leaving the self-renewal osteoprogenitor clone has, besides the osteoblast differentiation pathway, the capacity to completely switch into an adipocytic lineage. Adipocyte differentiation can be induced by a medium which contains dexamethasone, indomethacin, and CAMP promoters, among other media additions, causing a large proportion of cultured cells to express fat markers and accrue lipid. Adipocyte differentiation of bone marrow MSCs requires a virtual track change with up- and down-regulation of substantially more genes that are altered during osteoblast lineage.[12] We showed that we could repress phenotype switching to adipogenesis by applying dynamic stretch at levels substantially lower (2%) than had been demonstrated to affect cells from systems exposed to higher stretch (vascular smooth muscle, cardiac myocytes, alveolar cells).[26] The applied stretch regimen could also could repress elaboration of RANK-ligand (receptor activator of nuclear factor kappa-β ligand), which underwrites the ability of physical force to restrain osteoclast maturation from adjacent cells of macrophage lineage.[63]

Tracing the strain signal inwards from the plasma membrane, we found that repression of the adipogenic switch was dependent on preservation of β-catenin from destruction in the proteasome.[12] Preserving β-catenin levels with strain required a series of signaling events emerging through integrin signaling tweaked through focal adhesions: activation of FAK kinase to activate mTORC2[64] resulting in AKT activation to inhibit the glycogen synthase kinase 3 beta (GSK3β) that phosphorylates β-catenin targeting it to the proteasome.[65,66] Each of these constituents was necessary for the mechanical signal to be effective. With respect to the mechanisms of β-catenin action to restrain adipogenic differentiation, we more recently showed that this involved β-catenin activation of EZH2, the effector of polycomb repressive complex-2, to alter gene accessibility within the epigenetic landscape.[67] Here, we found that β-catenin promoted the earlier stem state of the osteoprogenitor state – and as such, prevented terminal differentiation of both adipocyte and osteoblast. This suggests that β-catenin signaling might be a close cousin of YAP in MSCs, which is stimulated by different physical signals.

As to components of the physical signal other than intensity, we were able to show that cyclical strain was effective at only 20–50 cycles – we had previously applied continuously cycling dynamic strain for more than 4 h (>2500 cycles). Repeating the short 50-cycle bout again 2 h later further reinforced the signaling pathway, to effects on signal generation and decrease in adipocyte differentiation at least equivalent to the 4-h cyclical strain.[29] The signal enhancement measured after applying a second daily bout was due to sensitization at the focal adhesion/integrin attachments in the form of new and more mature focal adhesions. Focal adhesions, which contain integrins along with many other signaling and scaffolding proteins, are where polymerized actin fibers are anchored to the membrane before coursing through the cytoplasm, to end at other focal adhesions or connectivity sites on the cell nucleus.[68] The ability of strain to reinforce, or auto-tune, its downstream effects through increasing focal adhesions and associated actin structure led us to ask what force generated signal pathways control actin polymerization. We found that the same proximal signaling pathway leading to inhibition of GSK3β – the proximal activation of FAK/mTORC2/AKT – also leads to activation of the LARG-GEF necessary for activating actin polymerization via Rho-A.[69]

We further compared static versus dynamic load as it is well known that static load does not promote bone remodeling and formation.[54] The signaling molecule YAP is a known mechanoresponsive target: studies of YAP activation and nuclear import have focused on the response of MSCs to stiff substrates which are widely known to induce changes in cellular actin fibers.[70] Our studies show that YAP and β-catenin are differentially activated by force; we confirmed that YAP is activated by static strain, but that β-catenin is activated only during dynamic strain.[71] We considered whether this was due to differences in the actin structure generated by these strains and thus far have not yet been able to distinguish differences between polymerized actin structures resulting after a single static strain versus a dynamic (cyclical) strain. What is, however, very different between these two types of strain is that dynamic strain induces actin transport into the nucleus, and this inward transfer sweeps up β-catenin along with it to enable nuclear access.[71] Static strain, however,

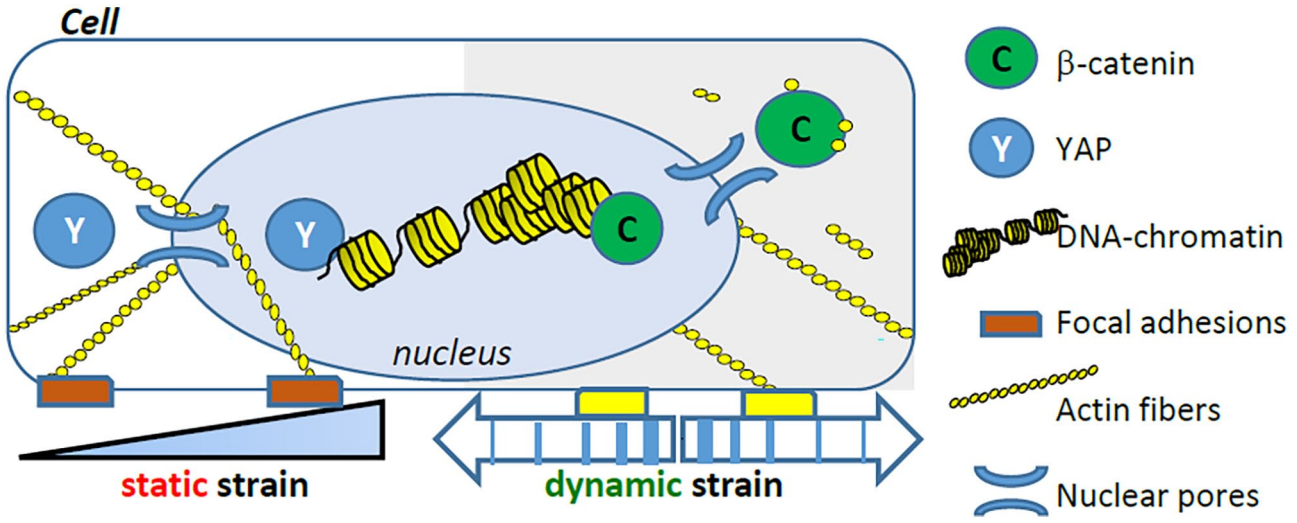

Figure 5. The mechanosensors YAP and β-catenin are activated by different components of force. β-catenin and YAP represent the mechanosensing molecules that, when activated, are translocated into the nucleus where they regulate gene expression. β-catenin entry into the nucleus occurs with dynamic load, but not static load, while YAP responds to static, but not dynamic, load. β-catenin nuclear entry in MSCs depends on the concurrent translocation of actin monomers, which are thought to alter the epigenetic landscape. (A color version of this figure is available in the online journal.)

induces YAP nuclear transfer but not β-catenin nuclear entry.[70] Clearly then, cell structure, including the ability of structural molecules, to enter the nucleus through nuclear pores,[72] responds specifically to parameters of mechanical force (Figure 5).

Mechanically controlled cell structure defines the epigenetic landscape

In this review of mechanical force guiding MSC cell fate and bone structure, we come finally to cell structure. The skeleton can be viewed as a hierarchical structure moving reductively from shape and size through many levels to the mesostructures of trabeculae, to microstructure of collagen fibrils, and to nanostructure of apatite crystal.[73] Cells also are structured hierarchically by structures describing the whole cell in 3D space, to the actin polymers and microtubules defining compartments of the cytoplasm, to structural aspects of the nucleus that are responsible for maintaining the geography of the epigenetic landscape.

After recognizing that the entire actin tool box is present within the nucleus,[74] and that actin can be found in filamentous[75,76] and rod-like forms[77] within the nucleus, we began to explore whether altering nuclear actin structure could change gene expression in MSCs. Treatment of MSCs with the actin de-polymerization agent, cytochalasin D (CytoD), induced mass transfer of actin monomers into the nucleus causing a rapid and nearly complete osteoblast differentiation of treated cells.[78] As CytoD does not enter the nucleus (there is no mechanism to transport through the nuclear membrane), the potential for nuclear actin polymerization remained, and thus suggested that actin appearing within the nucleus might be responsible for regulating differentiation. Preventing secondary polymerization with the Arp2/3 inhibitor CK666 (which does enter the nucleus) not only prevented osteogenesis but also directed the MSC population into the adipogenic lineage.[79] Complicating the picture of nuclear actin structure affecting gene expression, when we knocked out mDia2, the major formin present in the undifferentiated MSC nucleus, to limit primary actin polymerization, osteogenesis was enhanced.[80] Whether the structure of actin alters activity of effectors of gene availability such as EZH2, sequesters transcription factors, or ties up gene enhancers, remains an open question. Regarding mechanical control of actin structural elements, however, we can implicitly state that dynamic force is different than static force. At the start, dynamic force increases the level of actin within the nucleus as noted above,[81] thus increasing substrate for subsequent primary and secondary polymerization. This may be key to the skeleton's blindness to non-dynamic force.

Mechanical force requires structure for its impact to be felt – the skeleton transmits force through hard structure, and cells respond. Cell response likely engages changes in structure, and certainly in actin structure within and without the nucleus. The role of nuclear structure and how it regulates the status of hetero and euchromatin offers a rich area for study, and a potential gateway to intelligent directing of appropriate types of exercise to improve health.

Prescribing exercise as medicine

In the absence of war, a lethal pandemic, or famine (in 2022 no longer to be absent), humans are enjoying unprecedented extensions to functional life. The so-far, however, inevitable result of aging in the skeleton is osteoporosis, where women over 50 years in the USA have an even chance of having a fragility fracture during their remaining lifespans. Pharmaceuticals have been particularly helpful in addressing fracture risk, but many medical providers overlook the potential for a non-pharmacologic intervention such as exercise, to maintain, strengthen, and even build bone. In contrast, many of our patients ask the doctor, rather than vice versa, how to use exercise to strengthen their bones.

Exercise had been prescribed to promote health thousands of years before the Common Era by Chinese and Indian practitioners. The teachings of the Greek Herodicus in the 5th century led to his being heralded as the father of Sports Medicine: he wisely considered the levers of diet and physical activity to be critical in determining health.[82] In the 21st century, science has provided extensive irrefutable evidence that failure to exercise leads to skeletal loss – whether you are floating in the space station, lying on the couch, or performing only a limited range of exercise. Exercise prescriptions to improve fracture risk involve multiple types of activities.

In retrospect, the more we have exercised when young, the greater our bone strength will be.[83] As we age, bone density and quality will decline. A failure to dynamically load our skeletons as we age will hasten the decline in bone strength. But what is the dose of exercise? What intensity, how many repetitions, what kinds of input, how many times per week should we exercise to improve our skeletal strength?

As noted above, exercise studies have demonstrated that certain parameters of loading must be exceeded to elucidate remodeling to increase – and maintain – bone density. First, load must be applied cyclically, as static loading does not induce remodeling.[54] Second, the degree of response is related to the intensity of the signal[53] as well as the frequency and cycle number.[55] In this way, walking, a time honored and beloved activity of people everywhere, would appear to be a strategy to at least prevent bone resorption. Furthermore, if done with enough intensity, walking is a good cardiovascular improvement strategy;[84] however, the loading experienced by the skeleton during walking is not enough to increase bone density in postmenopausal women. Regimens that augment load intensity and loading rate to increase impact have more effect as shown by the LIFTMOR trial (Lifting Intervention For Training Muscle and Osteoporosis Rehabilitation), one of the few trial that have shown exercise can alter bone density in postmenopausal women.[85] However, we also should also strengthen muscles to change the way we fall,[86] and to protect the vertebrae which are at risk of compression as we age, strengthening of paraspinal muscles is a goal.[87] As to the spine, exercises need to be performed with neutral spine toward extension to decrease compressive loads.[88] Finally, we should not ignore balance and agility in an effort to prevent fragility fractures, as improving these will prevent falls.[89]

Physical therapists and trainers are skilled at setting goals and developing exercise plans for patients. One plan will not fit all: a patient who can barely rise from sitting will have different immediate goals than a hale 60-year-old woman training for the Ironman. Devices which mimic skeletal tissue effects of exercise may be useful, such as vibration,[90] or electric fields to enhance bone healing[91] when the subject is not able to load their skeleton. For osteoporotic patients wanting to increase bone density, high intensity resistance and impact training will be useful.[92] For the rest of us, reaching personal goals – whether they are cardiometabolic, endurance, or strength – is achievable with consistent application.

Conclusions

The skeleton gives us the ability to move freely in the material world. The stem cells giving rise to skeletal elements experience physical force during movement as instructions to increase – or decrease – skeletal mass and shape. Force at the level of the stem cell directs dynamic actin structure that modulates signaling intracellularly, with structural controls reaching into the nucleus to regulate gene expression. Gene expression in the bone stem cells regulates terminal differentiation as osteoblast or adipocyte, and exercise can improve the balance away from the adipocyte lineage and toward increased osteoblast numbers. To improve skeletal outcomes, paying attention to exercise, including both cardiovascular and resistance forms at best, and avoidance of unloading at least, can protect against fragility fracture and its associated loss of determination in old age.

AUTHORS' CONTRIBUTIONS

Both authors contributed design, writing, and research for this manuscript.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases (AR075803 and AR073264).

ORCID IDS

Janet Rubin https://orcid.org/0000-0003-3534-8667
Maya Styner https://orcid.org/0000-0001-9790-8645

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