DDT Vol. 6, No. 16 August 2001

# Inhibition of osteopenia by low magnitude, high-frequency mechanical stimuli

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**The identification of anabolic agents for the treatment of metabolic bone disease is a highly prized, and elusive, goal. In searching for the osteogenic (bone-producing) constituents within mechanical stimuli, it was determined that high frequency (10–100Hz) and low magnitude (<10 microstrain) stimuli were capable of augmenting bone mass and morphology, thereby benefiting both bone quantity and quality. Using animal models, it is shown that these mechanical signals can double bone-formation rates, inhibit disuse osteoporosis and increase the strength of trabecular bone by 25%. Considering that the magnitude of these mechanical signals are several orders of magnitude below those which cause damage to the bone tissue, it is proposed that this modality could be useful in the treatment of metabolic bone diseases.**

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▼ The World Health Organization characterizes osteoporosis as a disease when bone mineral density falls 2.5 standard deviations below the average value of a young adult. As the prevalence of this debilitating disease escalates with the increasing mean age of our population, so does the incidence of fractures. According to recent estimates, acute annual health care costs for the treatment of osteoporotic fractures surpass US$10 billion in the USA alone, and they are predicted to exceed US$250 billion over the next 50 years[1].

Considering the pandemic proportions of the disease and the severity of its symptoms, it should not be surprising that several treatment regimens exist – ranging from nutritional interventions for preventing bone loss to pharmacological cocktails for treatment of the manifested disease. However, it must also be stated that a large proportion of patients are hesitant to commit to a drug-based treatment. This treatment requires a commitment of several decades and could carry with it considerable side effects, such as weight gain and breast cancer (estrogens) or gastrointestinal complications (calcitonin and bisphosphonates). Although there is a great need for effective interventions for the prevention and treatment of osteoporosis, they must be devised such that they do not cause complications, either instantaneous or long term (e.g. fluoride compromising bone's structural properties, or hormone-replacement therapies potentiating the risk of cancer). Considering the strong sensitivity of bone to mechanical stimuli, one potential avenue for intervention would be to harness the regulatory components of this physical signal and apply it in a clinical setting. A biomechanically based intervention, therefore, would be native to the tissue (strain arises from load bearing), self-regulating (the signal would diminish as bone was laid down), self-targeting (the signal would be greatest in the load-bearing aspects of the skeleton), and would incorporate all aspects of the bone-remodeling cycle, rather than augmenting (osteoblasts) or defeating (osteoclasts) any specific aspect of it.

To build support for a biomechanical treatment approach to osteopenia, it is important to define the functional environment of bone and, subsequently, to demonstrate that physically based signals within these physiological constraints can indeed influence bone mass and morphology. Extrapolating these basic science studies to the clinic, evidence from both animal and human will be presented, which demonstrates that non-invasively induced, low-level mechanical signals are capable of inhibiting the rapid bone loss which



1359-6446/01/$ – see front matter  PII: S1359-6446(01)01872-4

typically follows the menopause. Although this approach is controversial, and indeed many aspects of this review report very preliminary results, the anabolic potential of mechanical stimuli cannot be questioned.

## Conceptual framework for a biomechanical intervention for osteoporosis

The symptoms of osteoporosis occur most frequently at specific load-bearing sites of the skeleton (e.g. femoral neck, lumbar spine). Nevertheless, the most accepted treatment protocols are administered systemically. Not only is this treatment strategy indiscriminant towards the entire skeleton, it typically approaches the disease by statically retaining the bone mass or density that is present at any given point in time. With this in mind, it is important to emphasize that bone quality is as important as bone quantity. Therefore, the ideal therapy will be one that incorporates all aspects of normal bone turnover, not one that annihilates a single component of it. An optimal treatment would target a site-specific regimen for the inhibition and/or reversal of bone loss and achieve this without interrupting the delicate interplay between the cells responsible for bone remodeling. The anabolic potential of load bearing, as well as the site-specific loss of bone density in osteoporosis, implies that an improved understanding of how mechanical signals regulate bone formation and resorption will facilitate their effective application to the treatment of these diseases.

The foremost task of the skeleton is to support the loads and bending moments that arise during activity. To a large extent, the skeleton's ability to accommodate the extremes of functional load-bearing arises through the bone tissue's ability to adapt to these functional demands by altering its mass and morphology, an attribute that was recognized well over a century ago, and is referred to as Wolff's Law[2]. The sensitivity of bone to mechanical stimuli is readily evident in clinically based studies that show the regulatory potential of exercise[3,4], as well as the bone lost through spaceflight[5,6], and also extended bed-rest[7]. The adaptive nature of the skeleton is controlled focally as demonstrated by the local hypertrophy[8,9] or resorption[10,11] following site-specific activities. However, the complexity of functional loading patterns within bone, have made it difficult to define those specific components of the mechanical environment that regulate bone mass and morphology. Over the past two decades, analytical and empirical models have hypothesized that strain magnitude[12], strain rate[13], electrokinetic currents[14], piezoelectric currents[15], fluid-shear flow[16], strain-energy density[17] and strain gradients[18] are all important in defining the skeleton.

Although each of these components is promising, it has proven difficult to identify a unifying principle of bone adaptation. Certainly, to optimize a structure to withstand load, minimizing strain for a given load would be a realistic goal, and thus the presumption would have to be that bone recognizes that it is a structure and is trying to keep strain within a certain level. However, it is just as reasonable to presume that bone cells are responding to 'biologically relevant' parameters of the functional milieu (e.g. tissue perfusion), that are not necessarily linked to peak structural challenges (matrix strain or microdamage). To identify the mechanical criteria that control adaptive processes, it is necessary to look beyond bone as a structure subject to load, and consider the biologic benefit of a viable tissue exposed to functional levels of strain.

## Characteristics of bone strain in the functionally loaded skeleton

Peak strain-magnitudes measured in diverse vertebrates are remarkably similar, ranging in amplitude from 2000 to 3500 microstrain[19,20]. Whether measured in the metacarpal bone of a galloping horse, the tibia of a running human, the humerus of a flying goose, the femur of a trotting sheep or the mandible of a chewing macaque, this 'dynamic-strain similarity' suggests that skeletal morphology is adjusted in such a way that functional activity elicits a specific (and perhaps beneficial) level of strain to the bone tissue[21]. The fact that strains of this magnitude are a factor of two below the yield-point of bone material emphasizes that a significant safety factor reigns and that the skeleton can survive the occasional mis-step. Two-thousand microstrain (2000 με) might sound like an ominously large number but in reality it represents an exceedingly small change in length from a material's original length. Cartilage is subject to 25% compressive deformations, tendons experience functional tensile strain upwards of 20%, and ligaments could stretch 4–5% during the extremes of functional loading. The 20% strain of these connective tissues is two orders of magnitude greater than the 0.2% (2000 με) peak strains experienced by bone. Admittedly, even 0.2% strain seems unwieldy for a bridge support or skyscraper, yet, by the time a 10-micron bone-lining cell is subject to 2000 με, such deformation is on the order of angstroms. Clearly, if deformations of this order are to affect cell metabolism, the bone-cell mechanosensory system must be exceedingly sensitive.

Although peak strains might be similar, *in vivo* strain-gauge data demonstrate the spatial distribution of peak normal and shear strains, as well as strain-energy density (an aggregate of the stress or strain state), to be highly variable across the bone section[22,23]. Measurements from the appendicular skeleton during functional activity demonstrate that the predominant (>85%) component of strain is generated by bending moments rather than axial loads, even

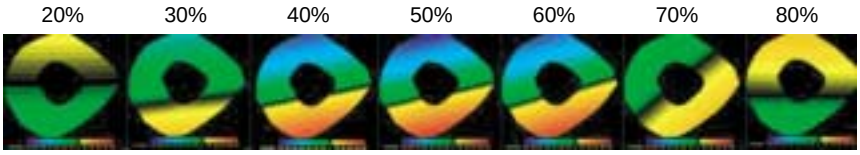


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**Figure 1.** The distribution of strain in the macaque ulna during the stance phase of a walk. This is longitudinal (normal) strain, at different phases of the gait cycle, beginning at 20% of the stance phase and continuing at 10% intervals to 80% of the gait cycle. Strain data were obtained using three triple-element rosette strain gauges, attached around the circumference of the bone. This demonstrates that, although the strain magnitudes change rapidly as the animal puts more weight on the bone (peaking at 50% of the stance phase), the manner of loading remains constant. Although the distribution of strain is non-uniform, the neutral axis of strain (where there is essentially zero longitudinal strain, as depicted by the black bar across the bone) shifts very little throughout the stride. This figure was adapted from Ref. 69.

though less bone mass would be required to support the same loads if the bone were loaded only in an axial condition[19,24,25]. Furthermore, committing one surface to tension and another to compression means that the transition between these two areas creates a region of the cortex that experiences very low peak-strain magnitudes. Although this 'neutral axis' is far removed from the area of the cortex subject to the peak strains, somehow tissue is retained. Clearly, bone cannot be presumed to be solely a compressive element, and strain cannot be presumed to be uniform across the cortex.

### *Strain memory*

Perhaps a more uniform strain stimulus is achieved via integration of strain information over time[26,27], and that bone cells have a 'strain memory', with non-collagenous matrix proteins 'responding' to load by changing their morphology[28]. Over time, a uniform strain-signal could be achieved by ensuring that all areas of the cortex are subject to the peak strain milieu. Unfortunately, the concept of equilibrating strain is not consistent with *in vivo* data, which show that the manner in which the bone is loaded remains the same through the stride. This causes strain levels to become even more disparate as the strain energy is summed over the course of a stride, approaching differences of two orders of magnitude. Summing the functional strain milieu over an entire 24 h period demonstrates the range of total strain experienced between areas of the cortex to be huge[29], approaching three orders of magnitude (Fig. 1).

Alternatively, strain information could be spatially integrated via a cell network facilitated by gap junction intercellular communication[30], such that the area of the cortex subject to low strains (e.g. neutral axis) resists resorption, because regulatory signals arise from adjacent areas subject to higher strain. This hypothesis is supported by the uniformity of the circumferential bone loss that follows disuse, although the net change in bone strain varies widely[31]. Considering that this intercellular communication is somewhat dependent on gap junctions, the reduced expression of these proteins with aging might contribute to the etiology of osteopenia[30].

As a rather unique means of focally 'adapting' to changes in a bone's physical environment, we believe that each osteocyte regulates its own local environment by modulating the architecture of its individual lacunae[32]. The bone cell would autoregulate its perception of strain by modifying the level of its attachment to the matrix, as well as the size of the periosteocytic space, thereby controlling the amount of direct deformation and/or shear to which the cell would be subjected. This works in both a positive and a negative manner: with the osteocyte capable of enhancing its attachment to the matrix via upregulation of collagen, integrins and/or osteopontin expression, or detaching from the matrix through expression of collagenase, it is able to accommodate new mechanical stimuli, whether they are increased or decreased[33]. Importantly, the cell's own ability to alter its specific mechanical environment emphasizes that bone can 'adapt' without necessarily requiring formation or resorption of tissue (no change in bone density). Thus exercise regimes that do not increase bone density *per se* might still improve the viability of the tissue (bone quality) without altering bone quantity. Recognizing that bone is a tissue first and a structure second, it is important to consider the biological implications associated with mechanical stimuli. Rather than peak events that are easily identifiable, bone adaptation might depend on some camouflaged subset of the mechanical milieu, such as the mechanical strains induced by muscle.

An interdependent correlation between muscle and bone is indisputable, yet the concept of one defining the other is not often considered[34]. Because muscle contraction imposes far smaller strains on the skeleton than those caused by ground-reaction loads (e.g. impact), their role in defining bone morphology directly is not given much credence. However, it is also important to point out that muscle-induced strains, although perhaps small, are sustained for extended periods of time, and thus might dominate the strain history of bone, the mechanical environment of bone integrated over time. By examining strain data collected from a variety of animals over extended

periods of normal activity, including simple standing (perhaps our predominant activity), a broad frequency range of strains in the appendicular skeleton is evident[29]. Furthermore, spectral analysis of these data show significant mechanical information extending out to even 50 cycles per second (Hz). Although the magnitudes and frequency content of gait-related strains change transiently as a function of speed and gait, the time-averaged strains (strain history) might be dominated by the standing strain spectra and are, therefore, stable over time and more uniform. From a stimulus standpoint, these persistent, low-amplitude signals might, when summed, be at least as important as the seldom occurring, and somewhat unpredictable, strain events that arise from vigorous activity[35].

## Bone adaptation to mechanical stimuli

Defining those parameters within the mechanical milieu that regulate bone mass and morphology requires that the tissue itself be evaluated, rather than the whole organ. Further, to ensure that the adaptive modeling is uniquely the product of those mechanical parameters applied, it is helpful if the bone is exposed to a minimal number of spurious (and unmonitored) loading events. These conditions have been met through several different animal models, ranging from the loading of cancellous bone in the distal femur of dogs[36], to the loading of tail vertebrae in rats[37]. In our laboratory, a principal approach to studying the adaptation of cortical bone to biophysical stimuli has been the use of functionally isolated turkey ulna[38]. The advantage of this model is that the bone tissue is subject only to the mechanical[39] or electrical[40] regimen prescribed by the investigators, with no aberrant biophysical signals entering the preparation.

The turkey ulna model has demonstrated that eight weeks of functional isolation alone will consistently result in a 10–15% loss of bone in eight weeks. Subject to an externally applied mechanical-strain regimen, physiological in strain magnitude, shows that mechanical signals are strongly anabolic, as dependent on the magnitude[12] and distribution[41] of strain within the bone tissue. However, to be osteogenic, the strain must be dynamic (time-varying) in nature, as static loads do not influence bone morphology[42]. The full osteogenic potential of a high-strain amplitude regimen is realized following only an extremely short (<1 min) exposure to the stimulus[38].

When an exogenous signal is anabolic, whether mechanical or chemical, the character of the response is often hyperplastic and disorganized in nature, diminishing the quality of bone and thus its potential to combat osteoporosis. To determine if this 'woven' tissue-type persists over time (or disappears altogether), a series of longer adaptation studies were performed[43]. Following 16 weeks of a load regimen of only 100 cycles per day, inducing a peak-compressive strain of 2000 με, new bone stimulated in the turkey ulna model was lamellar, composed of primary and secondary osteons toward the original cortex and circumferential lamellae at the periphery (Fig. 2). Remnants of the initial woven-bone response seen at four weeks remained clearly visible at both eight and 16 weeks as diffusely labeled interstitial elements within the newly formed lamellar construct. The presence of secondary osteons, circumferential lamellae, and an osteocyte density and organization similar to that seen in controls suggests that the presence of woven bone in the initial stages of the adaptive process is not necessarily a pathological or transient reaction to injury, but could instead represent a normal stage in response to a potent mechanical stimulus.

## Sensitivity of the anabolic response to stimulus frequency

As described previously, the bone-tissue modeling or remodeling response is sensitive only to dynamic strains; static strains are not perceived as anabolic[42]. This observation indicates that, in addition to strain magnitude, the total number of strain events, the number of strain events per unit time, and/or the strain rates involved in the loading regimen could be crucial to bone mass and morphology. In cortical bone, 2000 microstrain induced at 0.5 Hz (cycles $sec^{-1}$) maintains bone mass and achieves this with just four cycles of loading, encompassing eight seconds per day[38]. Reducing this strain to 1000 με at 1 Hz requires 100 cycles, and 100 seconds, to maintain bone mass[12]. Raising the loading frequency to 3 Hz, bone mass can be retained with 1800 cycles (600 seconds of load) with peak-induced strains of only 800 με (Ref. 44). With the same 600 seconds per day loading regimen, only 200 με is necessary to maintain cortical bone mass if the strain is applied at 30 Hz, a protocol employing 18,000 cycles of loading. When these 30 Hz mechanical signals are induced for one hour per day (108,000 cycles), only 70 με is necessary to inhibit bone loss. These data indicate that the sensitivity of cortical bone to mechanical loading goes up quickly with frequency, and thus much lower strains are necessary to maintain bone mass. Another view of these data is that the magnitude of strain necessary to maintain bone mass diminishes as cycle number increases. Therefore, thousands of low-magnitude cycles could be as important as a few high-magnitude events (Fig. 3), and thus the constant barrage of strain caused by muscle not only exists, it might be the key to defining bone mass.

These data indicate that bone is certainly responsive to even extremely low-level mechanical events, as long as there

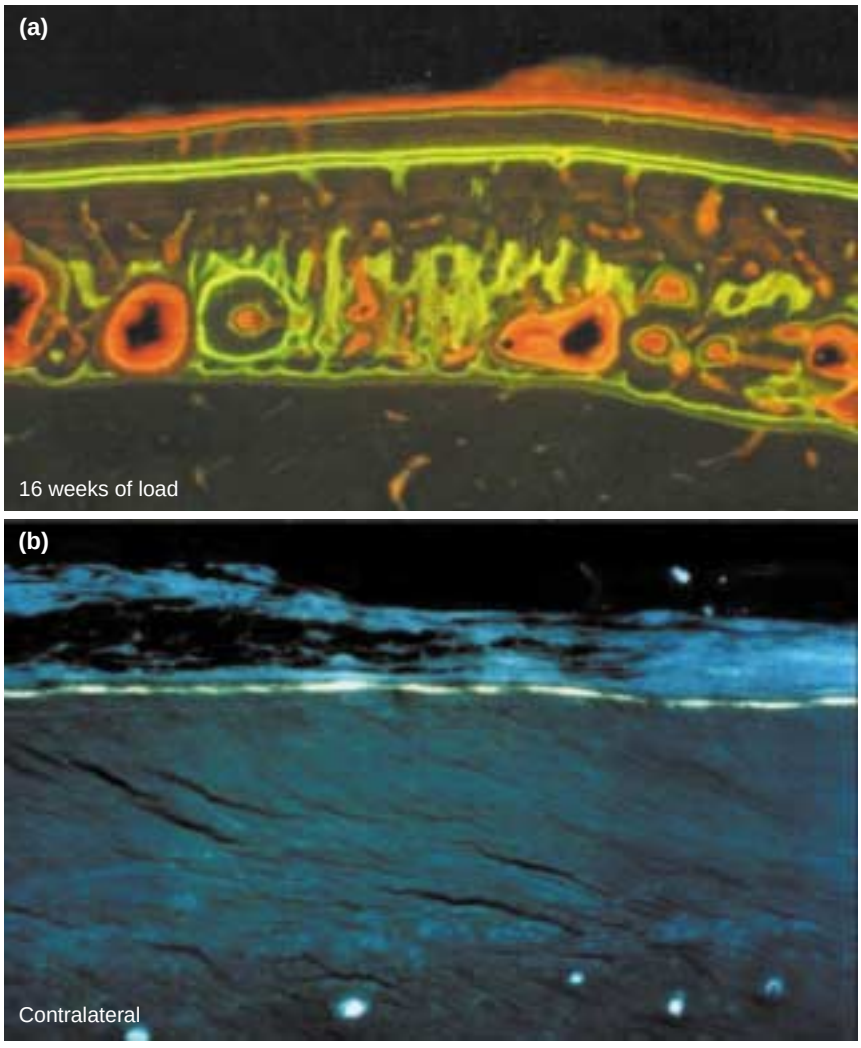


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**Figure 2.** A fluorescent photomicrograph of the periosteal surface of a turkey ulna diaphysis following 16 weeks of a mechanical regimen sufficient to cause a peak of 2000 microstrain (με). Remnants of the original woven response can be seen serving as interstitial elements of primary and secondary remodeled bone **(a)**. In essence, the woven bone response has served as a strategic stage in the achievement of a structurally appropriate increase in bone mass. Certainly, mechanical stimuli are strong, site-specific growth factors. The absence of a response in the contralateral control **(b)**, emphasizes that mechanical modulation is locally, not globally, mediated. This figure has been reproduced, with permission, from Ref. 42.

are enough of them. Using whole-body vibration as a means of inducing low-level mechanical signals into the skeleton, our first studies used turkeys but, rather than invasive studies on the ulna, these animals simply stood on a small oscillating plate[45]. Over a two-month period, each animal was subjected to a 30 Hz sinusoidal vibration for ten minutes each day, five days per week. Five animals were in each of four groups to test acceleration intensity of 0.1 g, 0.2 g, 0.3 g, and 0.9 g (where 1.0 g = earth's gravitational field, or 9.8 $ms^{-2}$). Eight control animals remained unstimulated. When not being loaded, animals freely roamed their pens. At 0.3 g, this stimulation induced approximately 5 με on the cortical surface of the tibia. Dynamic indices of bone formation [(labeled surface (LS), mineral apposition rate (MAR)] were determined for all animals via pulsed, double labels of tetracycline.

The trabeculae within the proximal tibial metaphysis of the eight control animals showed 1.9% LS, with no measurable MAR, whereas trabeculae in the femoral head of controls showed 1.8% LS, with no detectable MAR (Fig. 4). These data demonstrate that the skeletons of these adult animals were in a state of low bone-turnover. The LS in trabeculae following eight weeks of stimulation, encompassing 18,000 cycles of load per day, demonstrated a linear dose response with an increase in vibration intensity, extending to 50.7% in the tibia, and 44.2% in the femur, at 0.9 g. By contrast, the MAR, when turned on at 0.1 g (1.47 μm $d^{-1}$ in the tibia, 1.36 in the femur), failed to increase further by rising intensity.

These results indicate that extremely small strains – two orders of magnitude below the peak strains experienced by the skeleton during vigorous activity – if induced at sufficiently high frequency and cycle number, can also be influential in the design of the skeleton. Furthermore, strains non-invasively induced were far less than those in the invasive protocols (e.g. the isolated turkey ulna), suggesting that trabeculae could be even more sensitive to strain than cortical bone.

To determine if long-term (12-month) stimuli will ultimately improve the structural status of the bone, we have used dual-energy X-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT) and histology (static and dynamic histomorphometry, μCT) to evaluate the skeletal effects of a low-magnitude mechanical stimulus[46]. Eighteen adult female sheep, 5–7 years of age, were randomized into two groups, experimental and untreated controls. For 20 minutes per day, five days per week, the experimental sheep stood constrained in a chute such that only the hind limbs were

subject to a vertical ground-based vibration, oscillating at 30 Hz, to create peak–peak accelerations of 0.3 g. Following one year of stimulation, the animals were euthanized and the femora and ulnae removed. When compared with untreated controls, bone mineral density (BMD) of the proximal femur in stimulated animals was 5.4% greater, but this difference was not significant ($p<0.1$). Although pQCT also failed to demonstrate a significant difference in the total density of the proximal femur (+6.5%; $p<0.1$), when this assay was used to selectively evaluate cortical and cancellous bone at the lesser trochanter, a 34.2% increase in bone density was observed in mechanically stimulated sheep ($p<0.01$).

To determine whether the stimulus had any benefit to bone quality, high-resolution three-dimensional models were made of 1 cm cubes of trabecular bone harvested from the medial condyle of the femur, composed of 300 micro-tomographic slices for each cube[47]. Trabecular bone pattern factor (TBPf), an index of connectivity, was decreased by 24.2% in the animals subject to the non-invasive stimulus ($p<0.03$), reflecting a significant increase in connectivity and thus an improvement in the quality of bone. True physical property measurements of the bone samples, as performed using ultrasound and material testing, substantiated these findings, showing that the elastic modulus and stiffness of the bone subject to the low-level mechanical stimulus increased stiffness by 12% and strength by 26% in the longitudinal direction ($p<0.01$; Ref. 48). Both the quantity and quality of bone were enhanced by an extremely low-level mechanical stimulus, in the 'orientation' in which the stimulus was directed.

## How mechanical signals influence bone mass and morphology

Considering the requirement that the physical signals must be dynamic

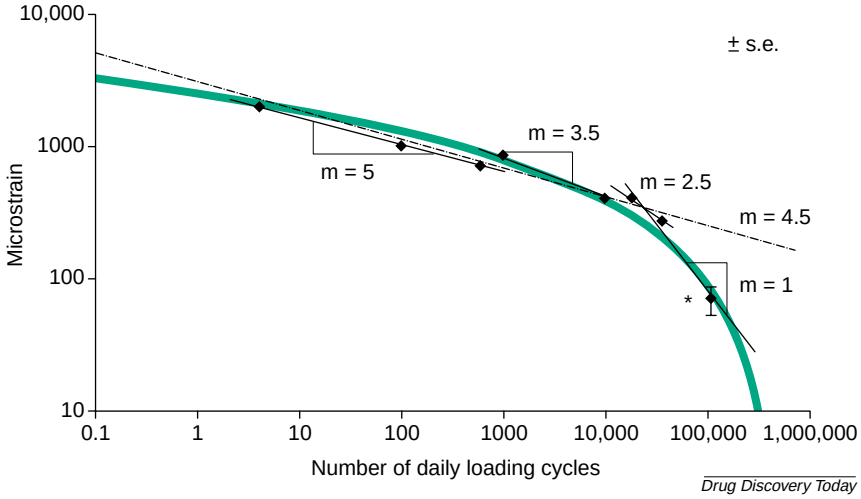


**Figure 3.** Strain threshold (microstrain) required to maintain bone mass as a function of daily loading cycle number. A curve fit to the data permits extrapolation to daily loading cycle numbers of less than one cycle per day and greater than 100,000 cycles per day; with this, the strain necessary to maintain bone mass will decrease as the daily loading cycle number increases. Of course, the strain necessary to maintain bone at one cycle per day exceeds the yield strength of bone and fracture is inevitable. With hundreds of thousands of loading cycles, only very low strains are required. It is hypothesized that any point above the line is anabolic and any point below the line will stimulate resorption. This figure has been reproduced, with permission from Ref. 44.

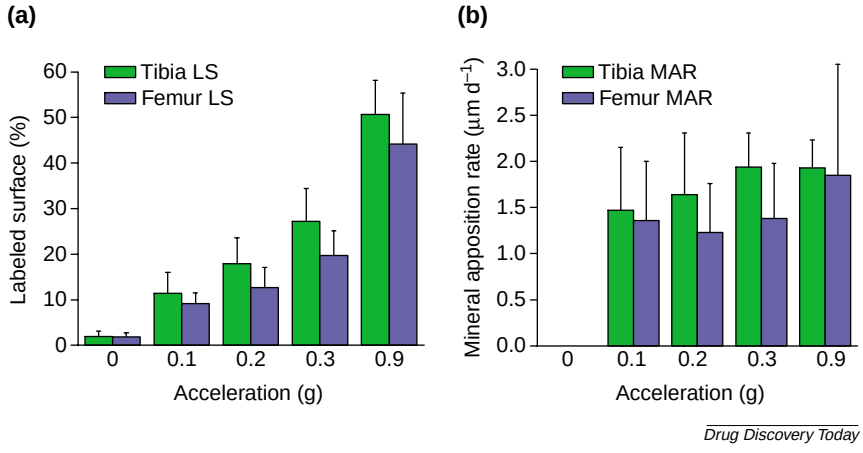


**Figure 4.** Dynamic histomorphometry of trabecular bone with the proximal tibial metaphysis and the trochanteric region of the femur of 1.5-year-old turkeys following 30 days of 5 min per day 30 Hz accelerations from 0.1–0.9 g (1 g = 9.8 $ms^{-2}$). The mineral apposition rate (MAR) and labeled surface (LS) were essentially zero in the skeletally mature controls, whereas these dynamic indices of formation rose to 41 ± 3.4% of surface actively LS in the loaded group, with MAR rising to 1.9 ± 0.22 μm per day ($N = 4$). By small fluctuations in gravity, which are induced at the proper frequency, trabecular bone formation is stimulated noninvasively in the weight-bearing skeleton. For each successive level of stimulation, the bone LS is significantly larger ($p<0.01$). At 0.2 g ($p<0.03$) and 0.3 g ($p<0.02$), the amount of LS in the tibia is significantly larger than the femur. MAR is greater in stimulated specimens than controls, but there is no significant MAR differences between groups stimulated at different levels of acceleration. This figure was adapted from Ref. 45.

(intermittent) to be osteogenic, it is important to consider byproducts of mechanical load, rather than necessarily the strain itself. For example, perturbations of intramedullary (IM) pressure, and the resultant intracortical fluid flow within the bone, would be a reasonable means by which dynamic strain could influence the cell population. To examine this hypothesis, an avian model was used to determine whether dynamic loading of the ulna resulted in significant IM hydraulic pressure. Surprisingly, when loaded to the same degree, the IM pressure rose monotonically with increasing loading frequency in the range from 0.1 to 100 Hz. Indeed, in the axially loaded mode, the loading-generated pressure at 30 Hz was approximately 10 times higher than that measured at 0.1 Hz (Ref. 49). Because of the sensitivity of bone to high-frequency stimuli, this suggests a physical mechanism whereby the extremely low-level signals generated by muscle could have a disproportionate influence on bone mass and morphology.

Considering the potential influence of fluid movement caused by pressure, in contrast to that caused by deformation of the matrix, a model was developed that enabled fluid loading without matrix strain, by oscillating IM pressure. A sinusoidal fluid pressure was applied to the ulna with the magnitude of 60 mm Hg, 20 Hz, 10 minutes per day for four weeks. Whereas IM pressure generated a spatial fluid-pressure gradient distribution through the cortex, fluid loading maintained bone mass at the endosteal surface and stimulated new bone formation at the periosteal surface (12.2 ± 4.2% increase in bone area). In the animal group subjected to disuse alone, the cortex showed a significant decrease in cross-sectional area (6.1 ± 3.0%), indicating a net 'benefit' of pressurization of 18% (Ref. 50). The bone loss in the disuse animals was primarily achieved by intracortical resorption and an increase in porosity of the cortical bone. These results indicate that the osteogenic potential of mechanical signals are derived, at least in part, by the byproducts of matrix deformation, rather than the strain of the tissue directly.

To determine if these anabolic signals could inhibit disuse osteoporosis, six-month-old rats were subject to either 10 minutes per day of low-level high-frequency mechanical stimulation (0.25 × g whole body vibration), 24 h per day of tail suspension-related hind-limb disuse, or 23 h and 50 minutes of hind-limb disuse interrupted by 10 minutes of mechanical stimulation[51]. After 21 days, hind-limb suspension significantly decreased trabecular bone-formation rates by 92%, compared with that quantified in the tibiae of control animals ($p<0.05$). The ten minutes per day of the 90 Hz low-level stimulation was strongly anabolic, as bone-formation rates increased by 97% ($p<0.05$). When this anabolic stimulus was used to combat disuse, the impact of the intervention served to normalize the response back to control values (no significant difference from the control).

Examining interdependence of the tissue-level response with the molecular activity of the cells, the expression of the receptor activator of NFκB ligand (RANKL) a cytokine involved in osteoclastogenesis, was quantified[52]. It was hypothesized that RANKL expression would be inversely related to altered bone-formation rates, including the depressed formative activity anticipated with disuse (i.e. disuse osteopenia is not simply a suppression of formation but an enhancement of resorption). When evaluating the 'molecular mechanism' of the adaptive response, northern analysis showed that disuse increased the expression of RANKL by 72% compared with control values ($p<0.1$). Mechanical stimulation for 10 minutes per day decreased RANKL mRNA levels by 78% ($p<0.1$), whereas disuse interrupted by 10 minutes of daily mechanical stimulation muted this decrease to 49% (not significantly different from the control). When linear correlation was used to relate bone-formation rates to RANKL expression levels across groups, the $r^2$ value was 0.79 (inverse correlation) (Fig. 5). In other words, mechanical signals that form bone (and prevent bone loss) also influence the activity of molecules that ultimately stimulate both bone formation and resorption.

### *Disrupting Wolff's Law by aging*

Evidence is building that systemic distress such as age[53], calcium deficiency[54], or endocrine imbalance[55] will dramatically affect the interaction of biophysical stimuli with the modeling or remodeling response. For example, mechanical signals that are osteogenic in the young skeleton fail to stimulate bone formation in the old skeleton. Although the skeletal system appears to be sensitive to mechanical stimuli, it is confusing that osteopenia should become symptomatic at skeletal sites subject to the greatest mechanical demand; something must happen either to the nature of the strain signal (e.g. suppressed streaming potentials caused by age-induced change in interstitial fluid viscosity), the perception of that signal by the cells within bone (disruption of intercellular communication), or the means of responding to those signals (suppressed cell metabolism). Perhaps it is this attenuation of bone's response to mechanical signals that could explain why exercise is not considered an ideal osteoporosis therapy for the postmenopausal or aging population[56]. Indeed, the risk of increasing signal strength to induce a response in the frail skeleton could induce the failure that one is trying to prevent.

To examine the inability of older bone to perceive mechanical stimuli, a series of experiments was devised to quantify the osteocyte population's perception of exogenous signals. Clearly, the amplitude of the strain itself does not

diminish in the older skeleton. If anything, it gets larger as bone mass disappears. Perhaps instead, it is the means by which the cells perceive this deformation as a regulatory signal that has been affected. This hypothesis was addressed in the turkey ulna model using *in situ* reverse transcript-PCR (RT-PCR). These experiments show that the mRNA expression of many matrix proteins (e.g. type I collagen, osteopontin) and cell-adhesion molecules (e.g. integrins) are reduced in the osteocytes of aging cortical bone[32]. For example, 12.8% of the osteocytes in the one-year-old bone showed positive labeling for osteopontin, which dropped 84% to 2.1% of osteocytes in the old bone and positive labeling for integrin $\beta_3$, which dropped from 5.3% in young bone to 0.8% in old bone, an 85% decrease in message for this protein[33].

These data reflect a diminished cell–matrix interaction; whereas the matrix still experiences strain, the regulatory information never reaches the osteocyte. Measuring the mRNA abundance in osteocytes following load of old bone supports this mechanism of signal perception. Subjecting the older bone to a load regimen of 2000 με at 1 Hz, which is strongly osteogenic in young adult bone, was insufficient to stimulate any new bone formation, and protein expression remained essentially unchanged from the suppressed levels observed in the intact old bone. However, changing the mechanical signal to a low-magnitude, high-frequency signal increased the levels of expression of these crucial proteins and stimulated substantial new bone formation (+14% over the animals' intact control). A 500 με regimen at 30 Hz increased the number of osteocytes labeled for mRNA message of integrins to 7.4%, which is 2% above the basal level of young bone. Furthermore, osteopontin message increased to 13.9%, a level equivalent to that of young bone. Finally, collagen type I message increased to 92.4% of osteocytes with label, which is a 300-fold increase in active osteocytes.

This upregulation of mRNA activity is observed as early as three days into the loading regimen, long before the surfaces have begun to produce osteoid[57]. These data suggest that extracellular matrix proteins provide a means of translating the mechanical-loading dynamics of the skeleton to the osteocyte population. Age or menopause-induced reduction in the presence of these proteins would diminish the cell's ability to sense some aspects of the mechanical signals within the bone tissue.

## Using mechanical signals as a clinical intervention

Suppression of the cell–matrix interaction in aged bone indicates that strain signals will not be efficiently transduced in older bone tissue[32]. Before completely blaming the bone cells' inability to perceive or respond to the strain environment, it is worth considering whether the aged skeleton is instead lacking a crucial component of the functionally induced endogenous signal. As suggested by strain-gauge recordings from the appendicular skeleton, low-level, high-frequency strains arise directly from muscle dynamics[35]. Furthermore, these persistent, low-magnitude strains have been shown to be anabolic, and could represent a strong stimulus in defining the morphology of the skeleton[45–48]. Therefore, if there is an age- (or menopause) induced change in the dynamics of these muscle oscillations, it could be argued that bone mass declines partly because these muscle-based signals also attenuate. To determine the role of muscle dynamics in the etiology of osteopenia, the spectral characteristics of muscle activity as a function of age were obtained through measurements of muscle-surface vibration[58].

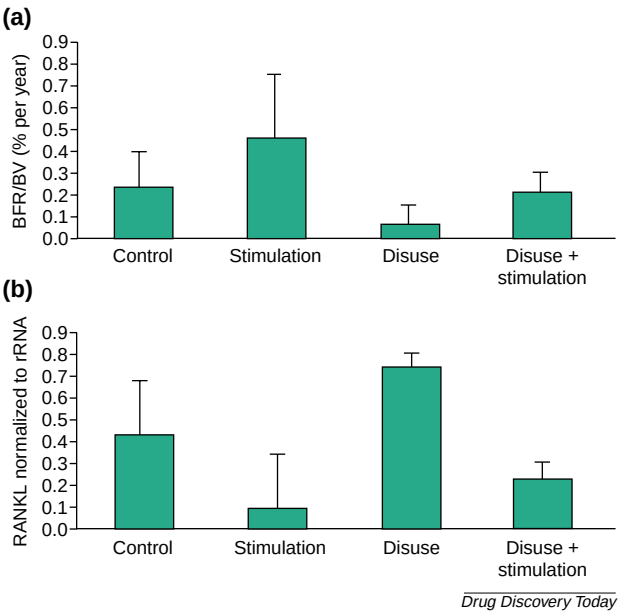


**Figure 5.** Dynamic histomorphometry and receptor activator of NFκB ligand (RANKL) mRNA expression in rats with tail-suspension induced osteopenia and simultaneous treatment with an osteogenic stimulus. Figure **(a)** shows tibial trabecular bone formation rates per bone volume (BFR/BV) of age matched controls and after 28 days of mechanical stimulation (10 minutes per day), disuse as caused by tail suspension and disuse interrupted by 10 minutes per day of low level mechanical stimulation (mean ± SD). Stimulation and disuse are both significantly different for the control and each other ($p<0.05$), whereas disuse plus simulation is not significantly different from the control. Figure **(b)** shows tibial RANKL expression in arbitrary units (mean ± SD), under identical mechanical conditions. Altered RANKL mRNA levels (with respect to control values) were inversely correlated with altered bone formation rates ($r^2= 0.79$), suggesting that mechanical stimulation inhibits RANKL expression and disuse is permissive to it. This figure was adapted from Refs 51 and 52.

During the contraction of a muscle, radial expansion of the individual fibers results in fiber collisions and the production of muscle sound or acoustic vibrations of the muscle body. The frequency of these vibrations reflects the firing rate of the motor units and, correspondingly, the force output of the motor unit, at least up to 80% maximum voluntary contraction during isometric contraction. The acoustic vibrations normal to the surface of the soleus muscle were recorded in 40 volunteers (20–83 years) using a low-mass accelerometer. Recordings were made from the left and right soleus, a principal leg muscle associated with posture, while the volunteer sat (passive) and stood (active). The recordings from each leg were averaged and integrated to obtain spectral power in the boundaries of 1–50 Hz, 1–25 Hz and 25–50 Hz.

Spectra obtained from these recordings show that muscle activity in the frequency range above 20 Hz decreases by a factor of three in the elderly, compared with young adults, a sarcopenia consistent with loss of fast oxidative-type fibers. As the high-frequency components seen in bone strain almost certainly arise through muscle activity, the deterioration of the postural muscle contraction spectra with age would contribute to a decrease in the spectral content of strain above 20 Hz. From this perspective, it can be argued that the sarcopenia that parallels aging could prove to be a principal etiologic factor in osteoporosis, as this portion of the strain spectra is anabolic. Although an association between loss of muscle dynamics and bone mass does not demonstrate a causal relationship, it provides support for the concept that the chronic activity of postural muscles could be a dominant force in controlling bone mass. Furthermore, if aging leads to the loss of specific muscle fibers crucial to the maintenance of bone mass, providing a 'surrogate' for the lost spectral strain history could presumably inhibit osteoporosis.

The weight-supporting skeleton facilitates direct transmission of mechanical energy into bone tissue. Therefore, a dynamic strain on the skeleton can be induced by modulating g-force[59]. The strains arising from dynamic alterations in g-force would be transferred into the skeleton along a normal trajectory, ensuring that the stimulus is concentrated at those sites with greatest weight-bearing responsibility (e.g. femoral neck), yet weak at sites not subject to resisting gravity (e.g. cranium). Although conceptually simple, it must be demonstrated that ground-based accelerations are indeed transmitted through the bones and joints of the lower appendicular skeleton; little is known of transmissibility of ground-based vibration at frequencies above 12 Hz (Ref. 60). To establish the relationship between acceleration at the plate surface and transmission of acceleration through the appendicular and axial skeleton, accelerations were measured from the femur and spine of the human standing on a vibrating platform[61]. Force transmission to these bones was determined using accelerometers mounted on Steinman pins transcutaneously imbedded in the spinous process of L4 and the greater trochanter of the right femur of six volunteers. To determine damping as a function of posture, data were also collected from subjects while standing with bent knees.

For a constant force input (18N), plate accelerations increased with frequency at both the femur and spinous process of L4. When the subject stood erect, negligible loss of acceleration was observed in the femur and spine in the lower-frequency bands, yet transmissibility decreased by as much as 40% when the frequency approached 40 Hz. When the subject was asked to stand with bent knees, transmissibility fell to below 20% at the femur and spine. These measurements confirm the ability of the standing adult skeleton to transmit a substantial fraction of ground accelerations to regions of the weight-bearing skeleton most susceptible to osteopenic bone loss.

The *in vivo* animal work, as well as the design and development of a prototype device suitable for humans, led to a one-year feasibility trial on a small cohort of women[62]. The ability of a low magnitude (0.2 g), high frequency (30 Hz) mechanical stimulation to inhibit post-menopausal osteopenia was evaluated in a prospective, randomized, double blind, placebo-controlled clinical trial. Sixty-two women, 3–8 years postmenopausal, enrolled in the pilot study. Thirty-one women underwent mechanical loading of the lower appendicular and axial skeleton for two ten-minute periods per day, induced via floor-mounted devices that produced the mechanical stimulus. Accelerations of 0.2 g are just perceptible and no adverse reactions were reported. Thirty-one women received placebo devices and underwent daily treatment for the same period of time.

Dual-energy X-ray absorptiometry was performed on the spine (L1-4), right and left proximal femur and non-dominant radius at baseline, and at approximately three, six and 12 months. A full complement of DXA data was obtained for 56 of the patients (28 treatment, 28 placebo; six subjects dropped out from the study for reasons not related to the device). In a *post hoc* analysis, a linear regression of the means was used to show that lumbar spine BMD declined by –3.3% (±0.83, n = 28) in the placebo group compared to only –0.8% (±0.82, n = 28) in the treated group ($p<0.03$), reflecting a 2.5% benefit of the biomechanical intervention. A 3.3% treatment benefit was observed in the trochanter region of the hip, with a –2.9% (±1.2) loss observed in the placebo group, yet with a 0.4% (±1.2) gain in the treated group ($p<0.03$). At the distal radius, no significant differences were observed as a function

of time or between groups, emphasizing the influence as being local.

Stratifying the results based on patient body-mass index (BMI; weight height$^{-2}$; Kg m$^{-2}$), end-point analysis confirms the relationship between svelte stature and a greater degree of osteoporosis[63,64]; subjects with BMI ≤25 lost 2.5% (±0.6) BMD over the course of the year, whereas those with a BMI >25 did not show any change over the 12-month treatment period. This stratification also demonstrates the ability of mechanical stimulation to inhibit this bone loss in the group at greatest risk; in subjects with BMI ≤25 who were exposed to the mechanical stimulus, the bone loss in the spine was not significantly different to zero (+0.2% ±0.7). The 2.7% difference between placebo and treatment groups was significant at $p<0.01$. Treated subjects with BMI >25 showed no apparent effect of treatment, perhaps because there was no bone loss to inhibit. Overall, these results indicate the potential of a unique, non-invasive biomechanical therapy for osteoporosis, representing a non-pharmacological means of inhibiting the rapid decline of bone mineral density that follows the menopause.

## Conclusion

Mechanical stimuli are crucial in achieving and maintaining appropriate bone mass. They have great potential for direct clinical applications, such as in fracture healing[65,66] or osseointegration[67]. In contrast to systemic, pharmaceutical intervention, such as estrogens, bisphosphonates or calcitonin, the attributes of these mechanical prophylaxes are that they are native to the bone tissue, safe at low intensities, incorporate all aspects of the remodeling cycle, will ultimately induce lamellar bone and the relative amplitude of the signal will subside as formation persists (self-regulating and self-targeting). Furthermore, they can be used prophylactically; that is, they can maintain bone as well as rebuild lost bone. Especially at the onset of osteopenia, when a pharmacological therapeutic intervention might not yet be beneficial to the patient, a mechanical treatment modality represents a long-term strategy with minimal risk to the patient. However, the widespread use of mechanical stimuli in the treatment of skeletal disorders will undoubtedly be delayed until we achieve a better understanding of the mechanisms by which they act[68].

In addition to the large amplitude strains typically associated with functional activity, a strain signal, far less than 10 με in amplitude, arises through muscular activity in the frequency band of 10–50 Hz. This signal is present in the cranial, axial, and appendicular skeleton and persists essentially at all times, including passive actions such as standing and speaking. The sarcopenia that parallels the aging process, and more specifically the attenuation of the 20–50 Hz spectral content of muscle contraction, suggests that the deterioration of these signals might also indicate the decline of a key regulatory stimulus to the bone tissue. These data suggest that osteopenia might arise not through the inability of bone cells to respond to key mechanical or chemical stimuli, but rather through the absence of a regulatory signal normally established by muscle activity. Certainly, the anabolic potential of mechanical stimuli points to their potential as a unique intervention for disorders and injuries of the musculoskeletal system.

## Acknowledgements

We are grateful for the contributions made by our colleagues; in particular Jack Ryaby, Susannah and Chris Fritton, Kenneth McLeod, Yan-Qun Sun, Steven Bain, Ted Gross, Simon Turner and Robert Recker. This work has been kindly supported by grants from The National Institutes of Health (AR39278, AR41011, AR41040, and AR43498), The Whitaker Foundation, Exogen, NASA (5390) and National Space Biology Research Institute, New York Office of Science, Technology and Academic Research, and The National Science Foundation (PYI 865105).

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