REVIEW ARTICLE

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# Regulation of mechanical signals in bone

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**Structured Abstract**

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***Objectives –*** Response of the skeleton to application and removal of specific mechanical signals is discussed. Anabolic effects of high-frequency, low-magnitude vibrations, a mechanical intervention with a favorable safety profile, as well as the modulation of bone loss by genetic and epigenetic factors during disuse are highlighted.

***Methods –*** Review.

***Results –*** Bone responds to a great variety of mechanical signals and both high- and low-magnitude stimuli can be sensed by the skeleton. The ability of physical signals to influence bone morphology is strongly dependent on the signal's magnitude, frequency, and duration. Loading protocols at high signal frequencies (vibrations) allow a dramatic reduction in the magnitude of the signal. In the axial skeleton, these signals can be anabolic and anti-catabolic and increase the structural strength of the tissue. They further have shown potential in maxillofacial applications to accelerate the regeneration of bone within defects. Bone's sensitivity to the application and removal of mechanical signals is heavily under the control of the genome. Bone loss modulated by the removal of weight-bearing from the skeleton is profoundly influenced by factors such as genetics, gender, and baseline morphology.

***Conclusions –*** Adaptation of bone to functional challenges is complex but it is clear that more is not necessarily better and that even very low-magnitude mechanical signals can be anabolic. The development of effective biomechanical interventions in areas such as orthodontics, craniofacial repair, or osteoporosis will require the identification of the specific components of bone's mechanical environment that are anabolic, catabolic, or anti-catabolic.

**Key words:** bone adaptation; bone morphology; disuse; mechanical signals; vibrations

## Introduction

Mechanical signals can positively influence physiologic processes critical to human health – from accelerating tooth movement and craniofacial repair to preventing and treating osteoporosis. With mounting evidence from clinical and animal studies that mechanical signals can be potent regulators of bone mass, morphology, and material properties, the challenge becomes to determine which specific components of the loading

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milieu are anabolic (and anti-resorptive) to bone tissue, and how to translate this information to the clinic where physical interventions might be tuned to most efficiently and effectively harness the skeleton's responsiveness to mechanical signals.

This paper is not intended to be a broad review of bone's adaptive capacity to physical signals but will emphasize specific topics within this area. Specifically, we will describe that the ability of physical signals to influence bone morphology is strongly dependent on the character of the signal. Because of their favorable safety profile and potential clinical relevance, the effects of high-frequency but low-magnitude mechanical signals will be discussed in particular. We will also show that bone loss modulated by the removal of weight-bearing from the skeleton is profoundly influenced by factors, such as genetics, gender, and baseline morphology. An improved understanding of which components of bone's mechanical milieu are anabolic, catabolic, or anti-catabolic will allow the development of biomechanical interventions in areas, such as orthodontics, craniofacial repair, or osteoporosis.

## Review

### Clinical evidence that mechanical signals are anabolic

While links between mechanical forces and bone morphology were suggested as early as 1638 by Galileo (1) and developed in the following centuries by scientists including Wyman (1857) (2), Roux (1885) (3), and Wolff (Wolff's Law, 1892) (4), much of the clinical evidence that mechanical forces are anabolic to bone has come from exercise studies performed in the twentieth century. Cross-sectional studies in humans reveal that bone morphology can change markedly in response to long-term exercise. In professional tennis players, the cortical wall thickness of the humerus in the dominant playing arm can be up to 45% larger than the non-racquet arm (5). Similar evidence of bone hypertrophy has been reported in a range of athletes and locations from ballet dancing to soccer, weightlifting, speed skating, squash, dancing/gymnastics, or physical activity in general (6–10).

Longitudinal prospective studies that have rigorously worked towards quantifying the effects of exercise regimens on bone mass and morphology without self-selection bias have produced more ambiguous results. While some studies have provided encouraging evidence that exercise can rapidly and effectively produce large increases in bone mass (11–13), much of the data have been equivocal (14–20), perhaps a reflection of our limited understanding of which specific components of the mechanical signal are perceived as osteogenic by resident bone cells populations, such as osteocytes, osteoblasts, osteoclasts, or bone marrow cells. The separation between mechanical signals relevant to bone and those which are irrelevant byproducts is difficult to determine in human exercise studies where the individual contribution of variables, such as exercise mode, duration, frequency, or intensity are difficult to separate.

When stratified for the different exercise interventions used there are only hints that certain types of exercise are more effective than others in stimulating bone formation or inhibiting resorption. High-load, high-impact exercises using few repetitions (e.g., weightlifting or jumping) may be superior to those exercises using lower loads and high repetitions (e.g., swimming or walking) (21–24) but this could not be confirmed in all studies (25–29). Inevitable factors difficult to control, such as genetics, gender, body habitus, nutrition, compliance, or interactions between a systemic (stress) response and the local, site-specific mechanical adaptations, may have accounted for some of the discrepancies (30). Perhaps to an even greater extent, it may stem from a failure of most studies to fully characterize and evaluate bone's local mechanical milieu engendered by the specific exercise protocol, effectively precluding the ability to identify distinct aspects of the functional loading environment that promote tissue growth. In animals studies, in contrast to clinical studies, bone's mechanical loading environment can be more comprehensively quantified and controlled. Variables such as genetics, gender, nutrition and compliance, can be readily accounted for, and a host of assays can be used to determine the tissue, cellular, and molecular response to a given signal.

### Functional strains in the skeleton

To gain insights into the structural demands that are placed onto the skeleton, the mechanical environment that bone is subject to during functional load bearing has been characterized. Mechanical strain ($\epsilon$) is the

most common measure to quantify mechanical deformations in the bone matrix and is expressed as a change in length ($\Delta L$) normalized to the original length ($L$) of any given specimen ($\epsilon = \Delta L/L$) in microstrain ($\mu\epsilon$, $10^{-6}$ strain). Thus, 1% deformation corresponds to 10 000 $\mu\epsilon$. The magnitude of strain and its derivatives, such as s*train rate* (temporal change in strain magnitude within the tissue), *strain gradients* (spatial change in strain magnitude across a volume of tissue), or s*train frequency* (number of strain events that occur within 1 s) can be determined during functional activities when strain gages are surgically implanted onto bone's surface.

In cortical bone, strain gages have been used to quantify activity-related bone strains in a great variety of species, including humans (31, 32), dogs (33, 34), primates (35, 36), roosters (37, 38), horses (39, 40), sheep (41, 42), and rats (43, 44). Interestingly, vigorous physical activity induces similar peak strains in the 2000–3000 $\mu\epsilon$ (microstrain) range across species (45–47), indicating that bone loading and architecture is finely tuned to achieve a safety factor of 2–3 against mechanical failure. The rigorous quantification of bone's mechanical milieu facilitated the correlation of mechanical strain parameters to the resulting adaptive tissue response in exercise models. It also guided the development of external loading models, such as the functionally isolated avian ulna (48), the rat tibia subjected to axial loading (49), or the mouse tibia subjected to bending (50), in which the forces and moments applied to the bone can be precisely controlled over a large range. The following examples for mechanical parameters that may modulate bone (re)modeling are specific to loading regimes that were applied at frequencies of less than 10 Hz.

#### Association of strain parameters with the biologic response

Perhaps, influenced by Wolff's Law which implies a force-form relation, early investigations focused primarily on strain magnitude as the dominant determinant of bone mass and morphology. Indeed, when keeping strain frequency and the number of loading events constant, variations in strain magnitude can explain differences in the osteogenic response (49, 51, 52); the larger the deformations generated in the bone, the greater the increases in bone mass. When strains fall below a certain magnitude, they are permissive to bone loss. This association is reflected in the concept of the mechanostat (53), a simplistic model that, while highly cited, is incapable of explaining the full range of bone's adaptive response.

During functional loading, the complete strain state of any given piece of bone tissue is typically very complex but it can be described in general terms by two predominant components; normal strains cause volumetric changes in the tissue while shear strains cause angular deformations. When changes in remodeling events were compared between loading regimes inducing predominantly shear or predominantly normal strains, it became clear that bone tissue can readily differentiate between different kinds of deformation; even though bone cells were responsive to both normal and shear strains, only normal strains increased the degree of intracortical turnover (54).

While strain magnitude appears to be an important determinant of bone mass, it is critical to realize that dynamic but not static strains have osteogenic potential. At the extreme, static loading (strain rate = 0) at strain magnitudes capable of stimulating formation when applied dynamically produces a remodeling response similar to disuse, resulting in bone resorption (55, 56). Several studies support the notion that bone is sensitive to the applied strain rate, with higher strain rates being more osteogenic (57–61). Extrapolated to the design of mechanical interventions, these results imply that loads should be applied rapidly.

A threshold behavior exists for the number of loading cycles. At low loading frequencies (<10 Hz), the full response can be triggered after only a limited number of loading cycles (48, 62). Further, recent studies have indicated that the manner in which loading cycles are distributed plays an important role in defining the magnitude of the anabolic response. Partitioning a given bout of loading cycles into several discrete loading sessions can increase bone's response to the mechanical intervention (63). Going one step further, this concept can be exploited to produce a mechanical intervention that is more efficacious despite providing fewer loading cycles (64). This loading paradigm, labeled rest inserted loading, adds as little as 10 s of rest after each loading cycle within a bout and has been shown to transform an otherwise ineffective loading regime into a highly osteogenic stimulus. The mechanisms by which the sensitivity of cells to mechanical signals is increased by rest inserted loading may be

associated with high cell refractory periods, enhanced bone fluid flow, synchronized osteocytic activity, and/or enhanced cellular communication (64, 65).

The parameters described above were primarily tested at the organ level. In other words, the region of the bone that was studied in response to a given mechanical stimulus was large (e.g., the mid-diaphysis of a long bone) and encompassed a large range of strain magnitudes. For instance, applying peak strain magnitudes of 2500 $\mu\epsilon$ in bending may produce 2500 $\mu\epsilon$ in compression on one side of the mid-diaphyseal cortex, 2000 $\mu\epsilon$ in tension on the other cortex, and very low strains at the midline between these cortices. Rather than simply considering the peak magnitude of the stimulus and averaging the morphological response across a section, one could investigate whether new bone is actually deposited in those regions where the applied stimulus is the largest.

To address this issue in an exercise model of bone adaptation, adult roosters were run on a treadmill for 9 min/day (~1500 gait cycles) for 3 weeks (38). Strain gages were attached to the tarsometatarsus to determine the distribution of candidate mechanical parameters across a mid-diaphyseal section. High-speed running induced a very non-uniform mechanical environment across a transverse section of the mid-diaphysis with the anterior cortex of the bone subjected to compressive strain and the posterior cortex subjected to tensile strains (Fig. 1). It is interesting to note that the shape of the bone, with the larger diameter in the medial-lateral direction, accentuates strains rather than minimizing them. For instance, if the bone was shaped in such a way that its distribution is rotated by 90° (Fig. 1), peak strains engendered during running could be reduced to levels similar to those induced by walking (38, 66). Clearly, the functional goal of (long-term) bone adaptation, if there is one, is not centered on the minimization of strains.

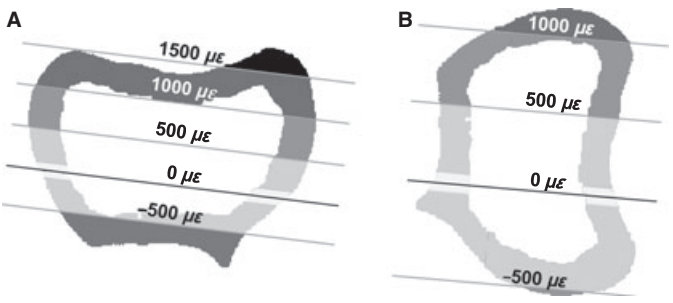


*Fig. 1.* Strain distribution across the rooster metatarsal cortex during high-speed running (A) for the anatomically correct transverse section and (B) for a section rotated clockwise by 90°. The much smaller peak strains in the rotated section demonstrate that minimization of strains is not the primary goal of genetically and mechanically adaptive bone (re)modeling.

In the group of running roosters, exercised-induced periosteal activation was quantified in several sectors of a transverse mid-diaphyseal section, enabling a site-specific correlation with the induced mechanical environment. Interestingly, the amount of periosteal mineralizing surfaces per sector was only weakly associated with strain magnitude ($R^2$ = 0.24, negative correlation) but to a much greater extent with circumferential strain gradients ($R^2$ = 0.63), consistent with data from an external loading model (67). While it is counterintuitive from an engineering perspective that new bone formation is activated at sites subjected to low strains rather than large strains, physiologically, it is important to point out that strain gradients are proportional to fluid flow in bone (68), a byproduct of strain which has been implicated in playing an important role in mechano-transduction in bone. These data imply that bone is possibly not sensitive to strain magnitude *per se* and that the process of bone adaptation may simply present a biologic response of cells to a given stimulus without an overarching functional goal in mind.

#### Skeletal sensitivity to low-level high-frequency mechanical signals

The weak correlation of new bone formation with the specific sites of peak strain magnitudes suggests that other mechanical factors may also be relevant for defining bone mass and morphology. Indeed, there may be a non-linear interdependence between cycle number, strain frequency, and strain magnitude. In the turkey ulna model of bone adaptation, peak strains necessary to maintain bone mass when loaded at 1 Hz can be drastically reduced when the loading frequency is increased to 30 or 60 Hz (69). While the reduction in strain threshold could be associated with an increase in cycle number, it is likely that the increase in frequency at which loading occurred played a large role. Indeed, over the past decade, we and others have demonstrated that bone can sense and respond to even extremely small mechanical signals if they are applied at high frequencies. Below, we have summarized examples of studies suggesting that the skeleton can benefit from these small mechanical signals under normal as well as

disturbed physiological conditions. It is important to note that the magnitude of the vibrations used in these studies was well below 1 g, somewhat in contrast with other studies in which accelerations in excess of 5–10 g were produced, levels that may potentially present a health hazard (70).

#### Vibrations induce extremely small tissue deformations

Most of our experimental studies used a vertically oscillating plate to induce whole body vibrations as a stimulus. In animal experiments, rodents remain free to roam during the stimulation (Fig. 2), and do not appear to be negatively affected by the vibration. Readings from a surgically implanted strain gage on a mouse subjected to whole body vibrations showed that the dynamic strain range induced in the tibia was extremely small – in the order of 5 $\mu\epsilon$ (Fig. 2) (71). Even when considering that the strains were recorded from a single site and that the magnitude of strains at the cortical periosteal surface may differ significantly from matrix strains within trabeculae, it is readily apparent that bone strains produced by the device are exceedingly small, several orders of magnitude smaller than the peak strains generated during locomotory activities.

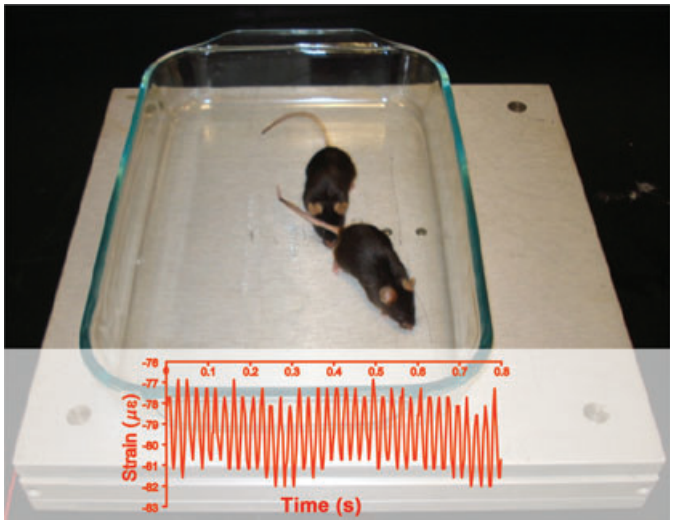


*Fig. 2.* A vibration plate on which the mice are allowed to roam freely. Not surprising given the small amplitude of the signal, the mice are not distracted or show any sign of discomfort while subjected to the mechanical treatment. For a signal frequency of 45 Hz and an acceleration amplitude of 0.3 g, the dynamic strain range recorded from a strain gage implanted on the tibia is in the order of 5 $\mu\epsilon$ (inset).

#### Vibrations can produce high-quality bone and decrease resorptive activity

To investigate potential changes in indices of bone formation, adult BALB/cByJ (BALB) mice were subjected to brief daily periods of whole body vibrations at 0.3 g, 45 Hz (72). The mechanical stimulus increased bone formation rates by 32%. Any increase in formative activity becomes structurally relevant only if the material properties of the newly formed bone are of high quality. To this end, high-resolution *in situ* analysis of collagen and mineral content and composition was performed on newly formed metaphyseal cortical and trabecular bone by synchrotron infrared microspectroscopy (71). No significant differences in the major chemical constituents were found between control and vibrated mice, suggesting that the mechanical treatment improved bone's structural strength. We then tested the effects of these mechanical signals on bone's resorptive activity in mice at an age at which the levels of modeling and remodeling are relatively high (8 week). After a 3-week exposure to the low-level vibrations, osteoclastic activity in the trabecular metaphysis and epiphysis of the tibia was 30% lower in vibrated mice than in age-matched controls. These studies demonstrated that the output of different cells residing in bone can be modulated by extremely low-level vibrations.

#### Vibrations enhance the musculoskeletal system

With evidence that bone's anabolic and catabolic activity can be altered by the vibratory mechanical signal, its impact on the musculoskeletal system was investigated (73). Eight-week-old mice subjected to the mechanical treatment described above had a 14% greater trabecular bone volume in the tibial metaphysis while periosteal bone area, bone marrow area, cortical bone area, and the moments of inertia of this region were all significantly greater (up to 29%). The soleus muscle also realized gains with up to 29% greater total cross-sectional area as well as type I and type II fiber area. The small magnitude and brief application of the non-invasive intervention emphasized that the mechano-sensitive elements of the musculoskeletal system are not necessarily dependent on strenuous, long-term activity to initiate a structurally relevant response in the adolescent musculoskeletal system. If

maintained into adulthood, the beneficial structural changes in trabecular bone, cortical bone, and muscle may serve to decrease the incidence of osteoporotic fractures and sarcopenia later in life.

#### Clinical evidence that low-level mechanical signals are anabolic to the musculoskeleton

To establish if brief, daily exposure to extremely low-level mechanical stimuli were anabolic to musculoskeletal development in young females, each of whom was in the lowest quartile of bone density in this age cohort and had already sustained a fracture, half of the 48 enrolled subjects were subject to 10 min/day, low-level whole body vibrations, with the remaining women serving as controls (74). Using an intention to treat analysis, cancellous bone in the lumbar vertebrae and cortical bone in the femoral midshaft of the experimental group increased significantly by 2.1 and 3.4%, while no changes were observed in controls. Cross-sectional area of paraspinous musculature was 4.9% greater in the experimental group vs. controls. Further analysis revealed that the benefit of the mechanical intervention when compared with controls was realized once the device was used for at least 2 min/day. Thus consistent with animal studies and two previous small clinical trials (75, 76), short bouts of extremely low-level mechanical signals, several orders of magnitude below those associated with vigorous exercise successfully enhanced skeletal properties.

#### Can bone differentiate between two high-frequency signals?

To determine if the responsiveness of bone to low-magnitude, high-frequency parameters is modulated by endocrine imbalance and whether one high-frequency signal may be more effective than another, ovariectomized (OVX) Sprague–Dawley rats were subjected to low-level vibrations applied at either 45 or 90 Hz and compared with OVX age-matched controls (44). Five additional rats were used *in vivo* to establish the induced bone surface strains. Following a 28-day protocol, bone formation rates in the metaphysis of the proximal tibia were 159% greater in 90 Hz rats when compared with age-matched controls, but 45 Hz rats were not significantly different from controls. Bone morphology of 90 Hz rats indicated significantly greater trabecular bone volume (22 and 25%) and thicker trabeculae (11 and 12%) over either controls or 45 Hz rats in the epiphysis of the distal femur respectively. Despite the enhanced sensitivity of the skeleton towards the 90 Hz signal, strain magnitudes and strain rates induced by this frequency were significantly lower than during 45 Hz vibration indicating that the efficacy of the low-level mechanical signal is maintained even in the absence of estrogen and that factors other than matrix strain are driving the anabolic response.

#### Are accelerations the component of the high-frequency signal that bone is sensitive to?

If matrix strain is not a critical factor in bone's response to low-level, high-frequency vibrations, the question arises whether the mechanical signal could be simplified and introduced in a manner that does not require weight-bearing. During whole body vibrations, the weight of the subject effectively acts against the vertically upwards moving plate, thereby inducing deformations in the weight-bearing skeleton. If deformation *per se* is not a prerequisite for mechano-transduction, cells may be equally sensitive to simple oscillatory motions (shaking) applied to skeletal segments. Indeed, a mechanism that would allow a cell to sense mechanical signals directly without reliance on matrix strain would obviate the need for compensatory tissue-level amplification mechanisms (77), reduce complexity in the system, and may provide cells with mechanical information without the potential for damaging the surrounding tissue.

#### Accelerations are anabolic in the tibia

A loading apparatus was developed to accelerate specific segments of the murine skeleton without loading them (Fig. 3). In other words, bone was subjected to oscillatory motions without the direct application of deformations to the tissue. The left tibia of eight anesthesized adult mice was exposed to small (0.3 or 0.6 g) 45 Hz sinusoidal accelerations for 10 min/day, while the right tibia served as internal control. After 3 weeks, trabecular metaphyseal bone formation rates were 88% greater in tibiae accelerated at 0.3 g than in their contralateral control, similar to the 66% increase in formation rates of bones accelerated at 0.6 g. Stimulated tibiae also displayed significantly greater cortical area (+8%) and thickness (+8%), together suggesting

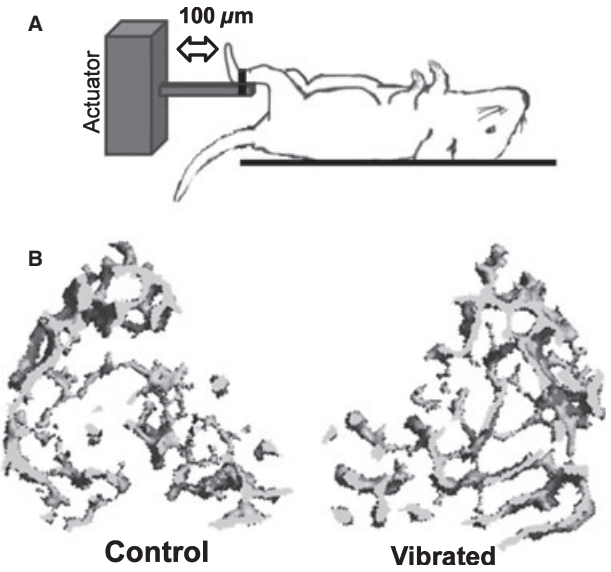


*Fig. 3.* (A) Schematic of the apparatus developed to deliver high-frequency oscillatory accelerations to the rodent tibia. At a frequency of 45 Hz and acceleration of 0.4 g, the total amplitude of the displacement is of the order of 100 $\mu$m. (B) Differences in tibial trabecular bone morphology between a hindlimb that was exposed to 45 Hz vibrations for 3 weeks and its contralateral control.

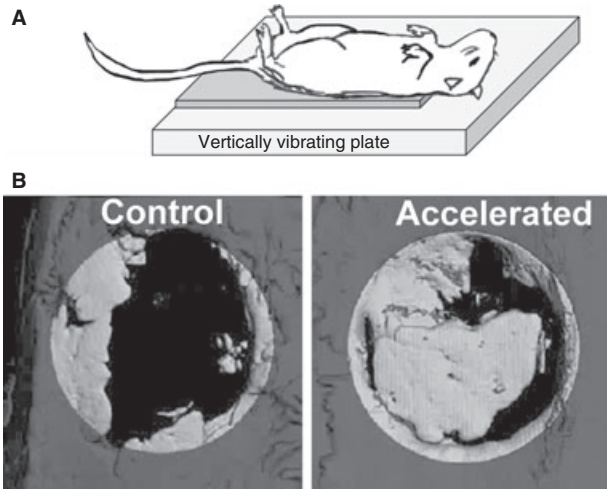


*Fig. 4.* In contrast to whole body vibrations, accelerations can be readily delivered to any skeletal segment, weight-bearing or not. (A) Schematic of the experimental setup used to expose to the cranium to high frequency oscillatory vibrations. Animals are placed on padded mats to isolate the body from the vibrations and to focus the signal on the cranium. (B) Bone regeneration in a control and vibrated cranial defects 8 weeks after surgery.

that tiny acceleratory motions – independent of direct loading of the matrix – can influence bone formation and bone morphology. In subsequent studies (78, 79), we confirmed these findings in a model in which habitual background loading was removed from the tibiae and the only loading component consisted of the applied high-frequency signal. Oscillatory accelerations, applied in the absence of weight-bearing, resulted in 70% greater bone formation rates in the trabeculae of the metaphysis, but similar levels of bone resorption when compared with contralateral controls. Quantity and quality of trabecular bone also improved as a result of the acceleration stimulus (Fig. 3), as evidenced by significantly enhanced morphological and mechanical properties. These *in vivo* data indicated that mechanosensory elements of resident bone cell populations can perceive and respond to acceleratory signals, and perhaps point to a more unifying means by which physical signals are transduced to cells and tissues of an organism.

#### Can accelerations accelerate craniofacial repair?

In regenerative medicine, improving the limited or delayed bone forming ability of osteoconductive bone materials is of significant concern for orthopedic or maxillofacial surgeries (80, 81). The hypothesis that the application of these mechanical signals could accelerate bone regeneration in both scaffolded and non-scaffolded calvarial defects was tested. The cranium of experimental rats, in which the bilateral defects either contained a collagen scaffold or were left empty, received oscillatory accelerations (45 Hz, 0.4 g) for 20 min/day for 3 weeks (Fig. 4). Compared with scaffolded defects in the untreated control group, defects with a scaffold and subject to oscillatory accelerations had a 265% greater fractional bone defect area 4 weeks after the surgery (82). After 8 weeks of healing (1 week recovery, 3 weeks of stimulation, 4 weeks without stimulation), the area (181%), volume (137%), and thickness (53%) of the regenerating tissue within the scaffolded defect were greater in experimental than in control animals (Fig. 4). In unscaffolded defects, mechanical stimulation induced a 84% greater bone volume and a 33% greater thickness within the defect. These data provided preliminary evidence that the application of extremely low-level, high-frequency accelerations could enhance osseous regenerative processes, particularly in the presence of a supporting scaffold.

#### Bone loss induced by disuse is interdependent on genetics, gender, and baseline morphology

Consistent with the anabolic effects of mechanical stimuli, the reduction or removal of mechanical loads

results in bone atrophy, altering the mass, morphology, cellular activity, and material properties of the tissue. In humans, much of our knowledge of disuse-induced bone loss stems from investigations documenting the effects of space flight (microgravity) (83–85) and prolonged bed rest (84, 86, 87) on bone properties. During space travel, the removal of gravitational and most functional loads triggers pronounced bone atrophy, with astronauts losing bone mineral at a rate of approximately 1–2% per month (85, 88). The atrophy was site-specific, with greater decay generally observed in the lower appendicular skeletal than the spine, and type-specific, with trabecular bone removal three to five times greater than cortical bone (85). Bed rest studies have yielded similar results. For instance, the BMD of healthy males confined to bed rest for 17 weeks decreased by 0.9–1.3% per month in the tibia, femur, and lumbar spine (86, 89).

The skeletal response to disuse varies greatly amongst humans. In cosmonauts, the extent of bone loss ranges between 0 and 23% for trabecular bone and 0 and 4% for cortical bone after 6 months of space travel (83). The absence of significant variability in baseline, nutritional, or physical status suggests that genetic factors contribute to differences in mechanosensitivity, a hypothesis that is supported by experiments with inbred mouse strains which allow the disassociation of genetic from environmental factors (90–93). Comparing the magnitude and spatial distribution of bone loss in the femur of female C57BL/6J, C3H/HeJ, and BALB/cByJ mice showed that BALB mice lost as much as 60% of their trabecular bone in the femoral metaphysis, with smaller losses in B6 mice and a nearly undetectable response in C3H mice (91) (Fig. 5). While bone loss was highly site-specific in BALB mice, with a threefold variation between the metaphysis and the epiphysis, it was more uniform across different trabecular regions in the B6 strain. Thus, genetic make-up is a critical modulator of both the magnitude and location of bone loss, consistent with the ability of the genome to influence the sensitivity of bone to the application of mechanical signals (72).

Using the two genetic strains that showed the greatest difference in their response to unloading (C3H and BALB), we then tested whether this relation holds true across genders. Surprisingly, male C3H and BALB mice when exposed to disuse showed distinct patterns of bone loss. In contrast to female mice from the same

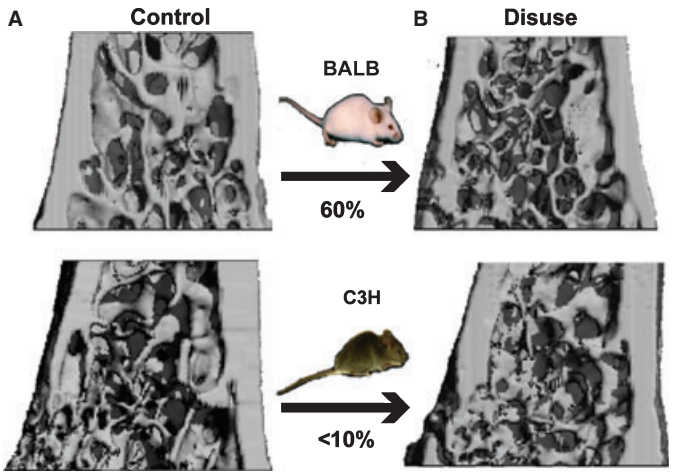


*Fig. 5.* MicroCT images of the femoral metaphysis of BALB (top) and C3H (bottom) mice subjected to either (A) control or (B) disuse conditions. The large differences in baseline trabecular morphology and disuse-induced bone loss between the two inbred genetic mouse strains emphasize the strong influence of genetic make-up on the regulation of bone mass and architecture.

strains, disuse caused similar amounts of bone loss in male BALB and C3H mice; metaphyseal trabecular bone volume fraction was reduced by 17 and 19% respectively, and epiphyseal bone volume fraction decreased by 10 and 13%. Compared with their female cohorts, metaphyseal trabecular bone loss was twice as much in male C3H mice and only half as much in BALB. In both populations, only females experienced significant loss in morphometric indices, with a 26% (BALB) and 13% (C3H) decrease in trabecular thickness between control and disuse mice respectively (91, 93). Taken together, these data reveal complex interactions by which gender, genotype, and anatomical location may determine bone's response to the loss of mechanical stimuli.

Indices of baseline bone morphology, prior to disuse, are distinct both across genetic strains for a given site as well as within inbred strains across different anatomical sites (92–94). These disparities are likely to arise from the spatial differences and interactions in both mechanical loading patterns and genetic regulation. The site specificity of both the effects of disuse and baseline morphology suggests, at least in part, that differences in phenotypic baseline bone morphology may regulate susceptibility to disuse. We recently addressed this question in male and female mice of a hybrid mouse strain subject to 21 days of hindlimb unloading (94). Bone loss was found to be modulated only by two parameters; it was inversely related to baseline bone volume fraction ($R^2 = 0.51$ for females

and 0.43 for males) and directly related to baseline bone surface to volume ratio ($R^2$ = 0.69 for females and 0.60 for males). These results highlighted that baseline morphology, independent of genetics, plays a role in predicting the patterns of disuse induced bone loss.

## Conclusions

Functional activities expose bone to a wide range of mechanical signals, from low- to high-frequency strains, normal- and shear strains, and compressive and tensile strains. The critical role that biophysical stimuli play in achieving, maintaining, and manipulating a structurally and biologically optimal bone mass is clear. Studies examining the ability of specific components of the mechanical milieu to stimulate bone formation demonstrate that osteogenic mechanical stimuli neither have to be large in magnitude nor do they have to be applied over a long duration. The precise catabolic consequences of the removal of mechanical signals from the skeleton are currently difficult to predict and are strongly dependent on interactions between genetics, gender, and the specific anatomical site. While our understanding of bone adaptation has increased dramatically since the treatises by Galileo (1) and Wolff (2), clearly, there is much to learn about how to translate this information to the clinic for orthodontic, craniofacial, or orthopedic applications.

## Clinical relevance

Mechanical signals are non-invasive and non-pharmacological growth factors in bone, and therefore have the potential to serve as a safe treatment for a number of clinical conditions. Unfortunately, the mechanisms by which bone senses and responds to changes in its mechanical environment are incompletely understood, hampering our ability to design efficacious interventions in areas such as orthodontics or orthopedics.

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