

Three Rules for Bone Adaptation to Mechanical Stimuli

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The primary mechanical function of bones is to provide rigid levers for muscles to pull against, and to remain as light as possible to allow efficient locomotion. To accomplish this bones must adapt their shape and architecture to make efficient use of material. Bone adaptation during skeletal growth and development continuously adjusts skeletal mass and architecture to changing mechanical environments. There are three fundamental rules that govern bone adaptation: (1) It is driven by dynamic, rather than static, loading. (2) Only a short duration of mechanical loading is necessary to initiate an adaptive response. (3) Bone cells accommodate to a customary mechanical loading environment, making them less responsive to routine loading signals. From these rules, several mathematical equations can be derived that provide simple parametric models for bone adaptation. (Bone 23:399-407; 1998) © 1998 by Elsevier Science Inc. All rights reserved.

Key Words: Bone density; Mechanotransduction; Mechanical stress; Biomechanics.

Introduction

Bone architectures are elegant and structurally efficient as if they were designed based on an engineering blueprint. This blueprint for the skeleton is partially contained within the bone cells' genetic program, but there is also an epigenetic component of skeletal design that is continuously updated in response to the mechanical forces exerted on the bones. Bone cells begin with the genetic blueprint and sculpt it until the skeletal design meets the loading requirements. This process, termed *bone adaptation*, requires bone cells to detect mechanical signals in situ and integrate these signals into appropriate changes in the bone architecture. Over 100 years ago, Roux²⁸ and Wolff⁴⁰ proposed that bone architecture is determined by mathematical laws: The thickness and number of trabeculae (i.e., the distribution of mass) must correspond to the quantitative distribution of mechanical stresses, and the trabeculae must be stressed axially in compression or tension (**Figure 1**). Pauwels furthered this work to describe the effects of mechanical stresses on long bone cross-sectional shape and fracture healing.²³ These laws form the basis of our current concepts of bone adaptation and, from this basis, new concepts are emerging.

A great deal of experimental evidence has been gathered in the last 30 years, and common threads have emerged that allow

us to describe the concept of bone adaptation in mathematical terms. Of greatest importance are the following three rules:

1. Bone adaptation is driven by dynamic, rather than static, loading.
2. Only a short duration of mechanical loading is necessary to initiate an adaptive response. Extending the loading duration has a diminishing effect on further bone adaptation.
3. Bone cells accommodate to a customary mechanical loading environment, making them less responsive to routine loading signals.

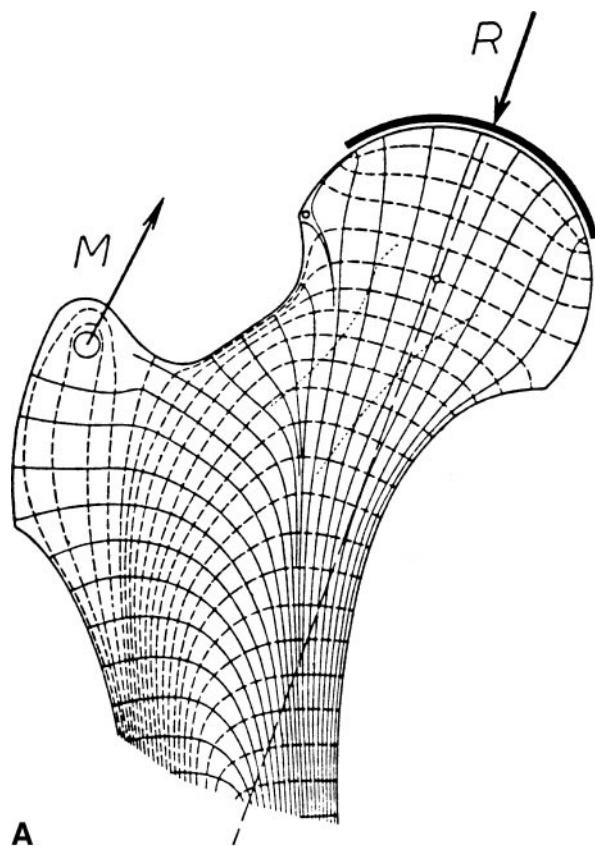
In this study, the foregoing rules are converted into mathematical formulas and the utility of these formulas is demonstrated. The body of the article is divided into three sections, each describing one of the three rules, and culminates in a general discussion.

Rule 1: Dynamic Strain Stimulus

The nature of the mechanical stimulus for bone adaptation has been debated for over 100 years. What follows is a brief chronicle of thoughts about this issue. In 1892, Wolff proposed that the stresses on the bones determined the bone architecture. Later, Thompson³⁴ pointed out "the very important physiological truth that a condition of strain, the result of stress, is a direct stimulus to growth itself." In 1964, Frost endorsed Thompson's view and asserted that not only was mechanical strain the principal determinant of bone adaptation, but that a "minimum effective strain" threshold must be surpassed before bone adaptation would occur.¹¹ A most important contribution was made in 1971 by Hert and coworkers when they showed that dynamic, but not static, strains increased bone formation in rabbits.²⁰ Dynamic strains thus appeared to be the primary stimulus of bone adaptation. In 1984, Lanyon and Rubin confirmed Hert's finding using the isolated avian ulna model. These investigators also demonstrated that bone adaptation in the isolated avian ulna model was directly proportional to the peak applied strain (**Figure 2**). Rubin and McLeod³² went on to show that frequency (i.e., the number of cycles per second) of the loading waveform was an important factor in bone adaptation. More recently, experiments in our laboratory have confirmed that both loading frequency and strain rate are important determinants of bone adaptation.^{36,38}

These results tell us the following: (a) dynamic strains drive bone adaptation; (b) the strain stimulus is increased if the magnitude or frequency of the dynamic signal is increased; and (c) increasing strain rate enhances the strain stimulus. To incorporate these facts into a mathematical formula, we must consider that peak strain rate is proportional to the frequency of the loading waveform and strain magnitude (see Appendix). Assuming that the strain stimulus is proportional to strain rate, as has been shown experimentally^{22,38}:

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A



B

Figure 1. According to Wolff,⁴⁰ bone architecture is determined by mathematical laws: The thickness and number of trabeculae (i.e., the distribution of mass) must correspond to the quantitative distribution of mechanical stresses, and the trabeculae must be stressed axially in compression or tension. This principle is demonstrated by the studies of Pauwels²³ showing the correspondence between stress trajectories (A) and trabecular architecture (B) in the proximal femur. (© Springer, New York 1980, used with permission.)

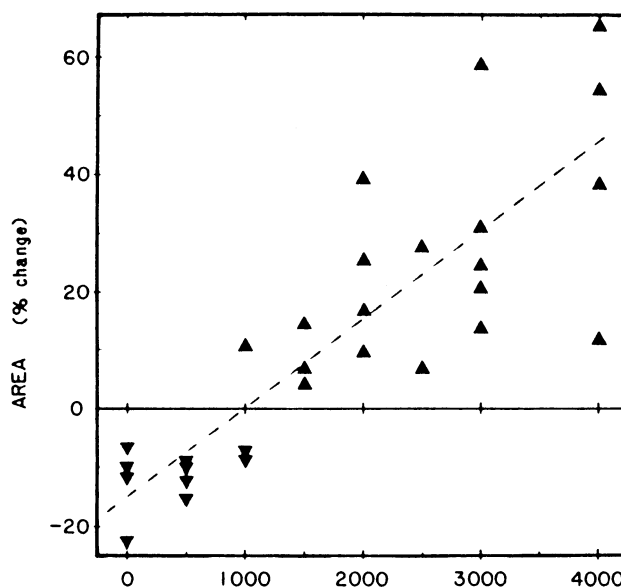


Figure 2. Rubin and Lanyon³¹ demonstrated, using the isolated avian ulna model, that newly formed bone area is proportional to applied strain magnitude. This experiment has often been interpreted, albeit incorrectly, to show that strain magnitude, rather than strain rate or the frequency of the loading waveform, is the primary stimulus for bone adaptation. However, strain rate, frequency, and magnitude of a dynamic loading signal are interrelated so the loading waveforms applied by Rubin and Lanyon varied not only in strain magnitude but also in frequency (see Appendix for further discussion of this experiment). (© Springer, New York 1985, used with permission.)

$$E = k_1 \epsilon f \quad (1)$$

where E is the strain stimulus, k_1 is a proportionality constant, ϵ is peak-to-peak strain magnitude, and f is loading frequency in cycles per second. Equation (1) gives the strain stimulus for a sinusoidal loading waveform. However, the result can be generalized using the Fourier method, which allows any periodic loading waveform to be expanded into a series of sine waves at different amplitudes and frequencies. So, in the general case, the strain stimulus is defined as:

$$E = k_1 \sum_{i=1}^n \epsilon_i f_i \quad (2)$$

Equation (2) predicts all of the results we have observed in our laboratory, as well as those derived from other experimental models. For instance, Equation (2) demonstrates that static loads should not affect bone adaptation,^{19,20} because $f = 0$; it predicts that bone adaptation should be proportional to applied dynamic strain magnitude³¹; and that higher frequency loading should be more effective at stimulating bone adaptation. It was demonstrated, using the isolated avian ulna model, that 2000 $\mu\epsilon$ applied at 1 Hz could reverse the effects of disuse and stimulate new bone formation. According to Equation (2), we should expect a similar response if 100 $\mu\epsilon$ were applied at 20 Hz. This is precisely what was observed by Rubin and McLeod³² when they showed that 150 $\mu\epsilon$ at 20 Hz reversed the effects of disuse and stimulated bone ingrowth around transcortical, porous-coated titanium implants. In our laboratory, bending loads are applied to the tibiae of adult rats to induce bone adaptation.³⁶⁻³⁸ We have shown that new bone formation is influenced by loading frequency (Figure 3) and peak-to-peak loading magnitude (Figure

Figure 3. Mechanically induced bone formation rate (mBFR) on the endocortical surface of the rat tibia increased significantly with increasing applied loading frequency ($p < 0.05$, asterisks). Loading was applied daily as four-point bending of the tibia for 36 cycles/day over a period of 10 days. Sham bending loading had no effect on mBFR. Adapted from Turner et al.³⁶

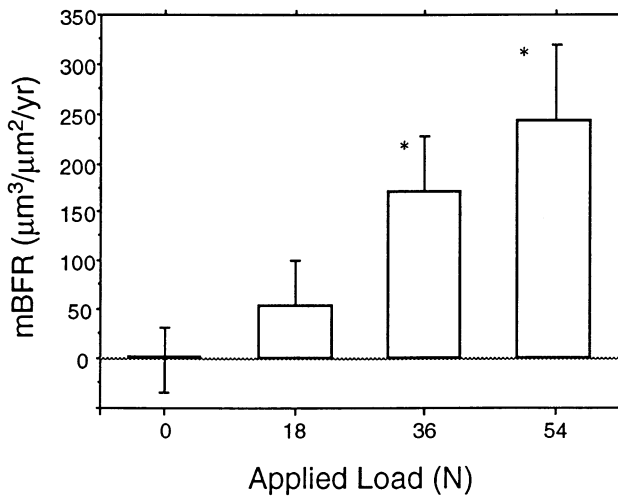
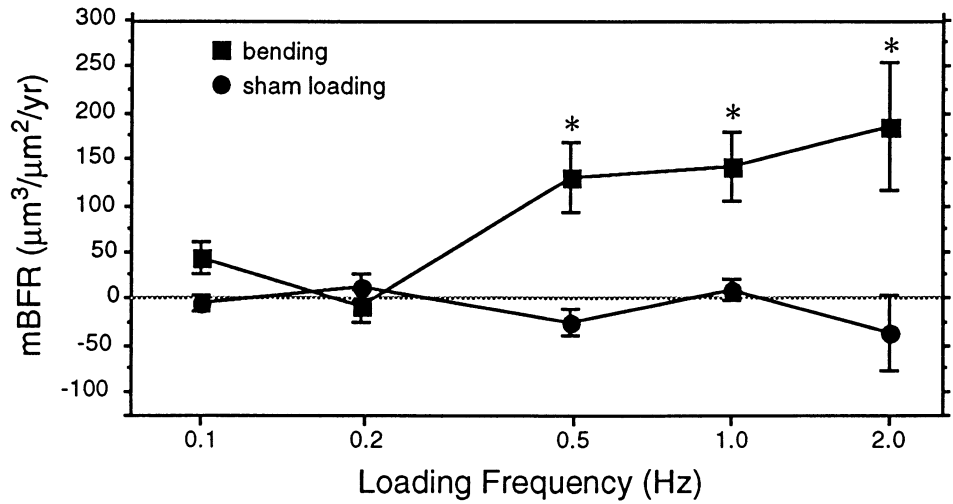
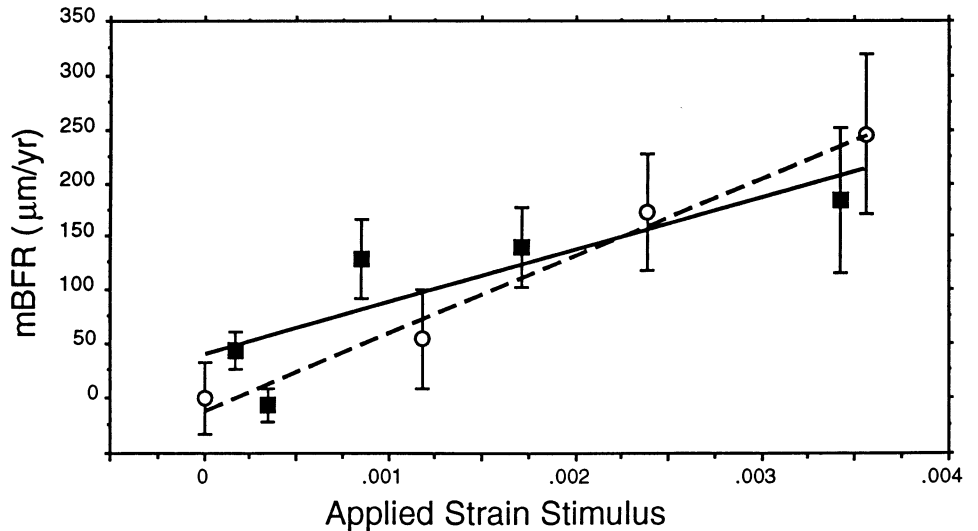


Figure 4. Mechanically induced bone formation rate (mBFR) on the endocortical surface of the rat tibia was proportional to the peak-to-peak magnitude of four-point bending applied at 2 Hz. Loading was applied daily for 36 cycles/day over a period of 10 days. Asterisks indicate a significant difference from 0 N ($p < 0.05$). Adapted from Turner et al.³⁸

Figure 5. Mechanically induced bone formation rate (mBFR) in the rat tibia from two experiments previously done in our laboratory^{36,38} (Figures 3 and 4). In both experiments, loading was applied daily for 36 cycles/day over a period of 10 days. Applied strain stimulus was calculated for each datapoint using Equation (2). The closed squares are from an experiment in which loading frequency was varied from 0.1 to 2 Hz (solid regression line), and the open circles are from an experiment in which the peak-to-peak loading magnitude was varied from 0 to 54 N (dashed regression line). The results show that mBFR has approximately the same relationship with strain stimulus in either experiment, regardless of the disparate loading conditions.



4). For each of these previous experiments, bone formation can be presented as a function of strain stimulus calculated using Equation (2). After converting to strain stimulus, the data from the two different experiments are virtually superimposed upon each other (Figure 5), demonstrating that, for the rat tibia loading model, Equation (2) can predict results from different experiments and different loading conditions.

Equation (2) may have limits in its predictive ability at high loading frequencies. It presents a linear model for the relationship between strain magnitude and frequency, while biological systems are seldom perfectly linear. For instance, Equation (2) predicts that very low strains can create a significant strain stimulus if applied at a very high frequency, yet there may in fact be a limited range of loading frequencies to which bone is sensitive.

Rule 2: Case of Diminishing Returns

Increased duration of skeletal loading does not yield proportional increases in bone mass. As loading duration is increased the bone formation response tends to saturate. This phenomenon of diminishing returns is best demonstrated in the study by Rubin and Lanyon³⁰ using the isolated avian ulna loading model, and the study by Umemura et al.,³⁹ where rats were trained to jump various numbers of times per day and changes in their tibial and

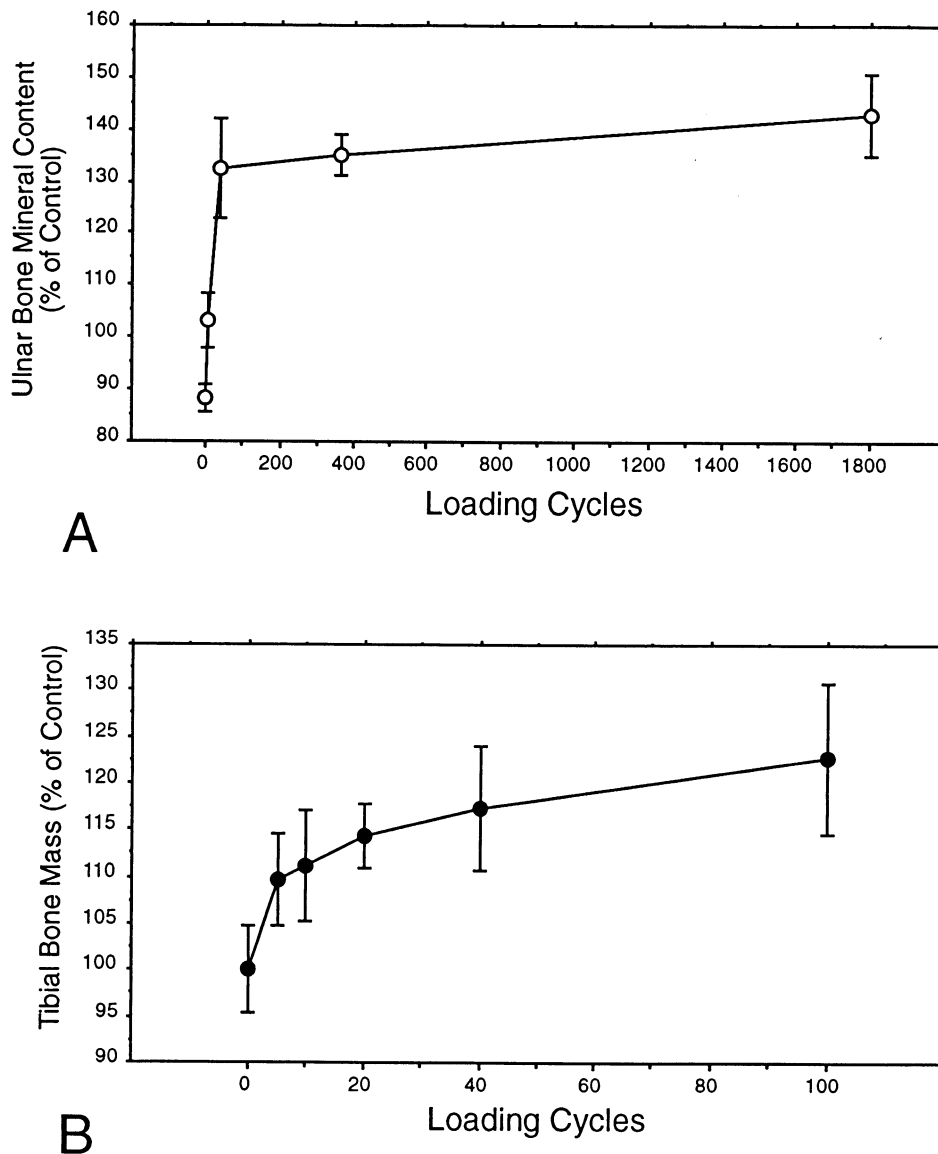


Figure 6. Increased duration of skeletal loading does not yield proportional increases in bone mass. This effect is demonstrated best by two experiments. First, Rubin and Lanyon³⁰ applied mechanical loading of different durations daily to the isolated ulna of roosters and found that the resulting increase in bone mass was not proportional to the loading duration, but that increasing loading duration past 36 cycles/day was not very effective for causing further increases in bone mass (A). Second, Umemura et al.³⁹ trained rats to jump various of numbers of times per day and found that five jumps/day were sufficient to increase bone mass, but increasing numbers of jumps gave diminishing returns with respect to bone mass (B).

femoral bone mass were measured (Figure 6). The data from each of these studies can be characterized mathematically using a logarithmic function:

$$\text{Rubin and Lanyon—ulnar BMC (\% of control)} = 93.2 + 16.8 \log(1 + N).$$

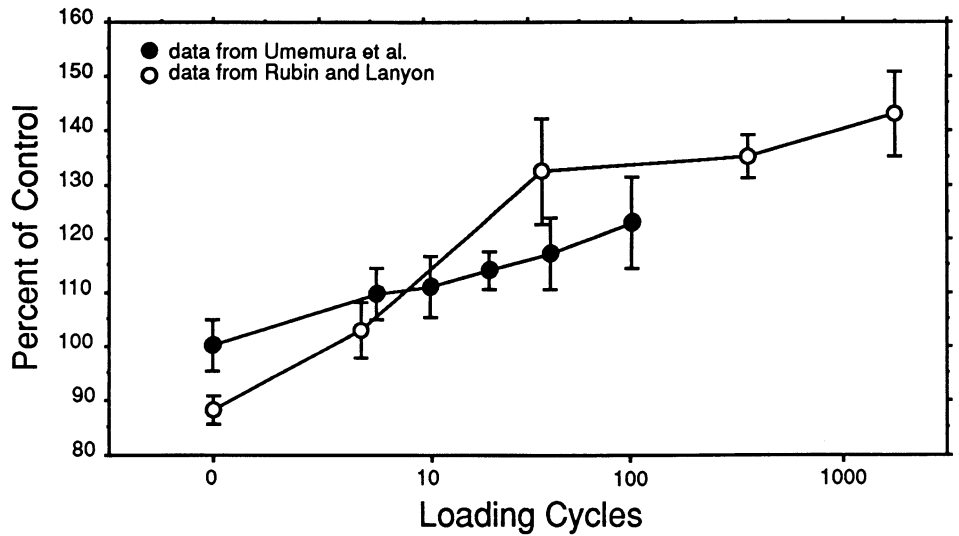
$$\text{Umemura—tibial bone mass (\% of control)} = 100.1 + 11.0 \log(1 + N).$$

where N is the number of loading cycles per day. There are differences in the two animal models that are worth noting. First, the isolated ulna loading model was in a state of disuse when no loading was applied, so bone was lost; this explains why the y intercept from the Rubin and Lanyon data is less than 100%. In contrast, the rats in the jumping model of

Umemura et al. were normally active when not jumping, so the y intercept should be, and is, almost exactly 100%. Second, the isolated ulna loading model gained 16.8% more bone mass with each incremental ($10\times$) increase in loading cycles, whereas the rat jumping model gained only 11% more bone mass per increment. The larger increase in the avian ulna may be due to the fact that this loading regimen induced woven bone formation for loading durations of 36 cycles/day and above, thus leading to more dramatic increases in bone mass than would be expected from lamellar bone formation that occurred in the jumping rats. Despite these differences, the two data sets have considerable overlap when plotted on a logarithmic scale (Figure 7).

Another mathematical approach for explaining the phenomenon

Figure 7. The relationship between mechanically induced change in bone mass and number of loading cycles is logarithmic. The data from Umemura et al.³⁹ taken from an experiment using rats and the data from Rubin and Lanyon³⁰ collected using the isolated avian ulna model follow similar trends when plotted as a function of the logarithm of loading cycles. The change in bone mass induced in the avian ulna model is slightly more dramatic, but this could be due to the fact that this model causes a wider range of responses in the bone; bone loss occurs in the absence of loading cycles and woven bone formation is induced by application of 36 or more cycles/day.



of diminishing returns was derived by Carter et al.³ They proposed that a daily loading stimulus (S) should be expressed as:

$$S \propto \left[\sum_{j=1}^k N_j \sigma_j^m \right]^{1/m} \quad (3)$$

where k is the number of different daily loading conditions applied to the bone, N is the number of loading cycles per day for each loading condition, σ is the effective stress (or strain) for each loading condition, and m is a constant. The value of m is a weighting factor for the relative importance of stress or loading cycles on S . For instance, if $m = 1$, the effects of stress magnitude and loading cycles would be equally weighted. This formula is limited because the effect of loading frequency is not taken into account, but the idea of weighting factor m was an important step forward. The experimental data clearly dictate that m should be >1 ; Carter and colleagues estimated the value of m to be 4,¹ based on a review of the available data from Rubin and Lanyon.^{30,31} From a curve fit of the more recent data of Umemura et al., I estimated the value of m to be 3.5 (Figure 8), which is reasonably close to the estimate used by Carter's group.

The interaction between the strain stimulus (ϵ) and duration of loading (number of loading cycles per day, N) can be described mathematically using either a logarithmic relationship or

the exponential relationship derived by Carter et al.³ We can combine Rule 1 and Rule 2 of bone adaptation into a new formula for the daily loading stimulus:

$$S \propto \sum_{j=1}^k \log(1 + N_j) E_j \quad (4)$$

where:

$$E_j = \sum_{i=1}^n \epsilon_i f_i$$

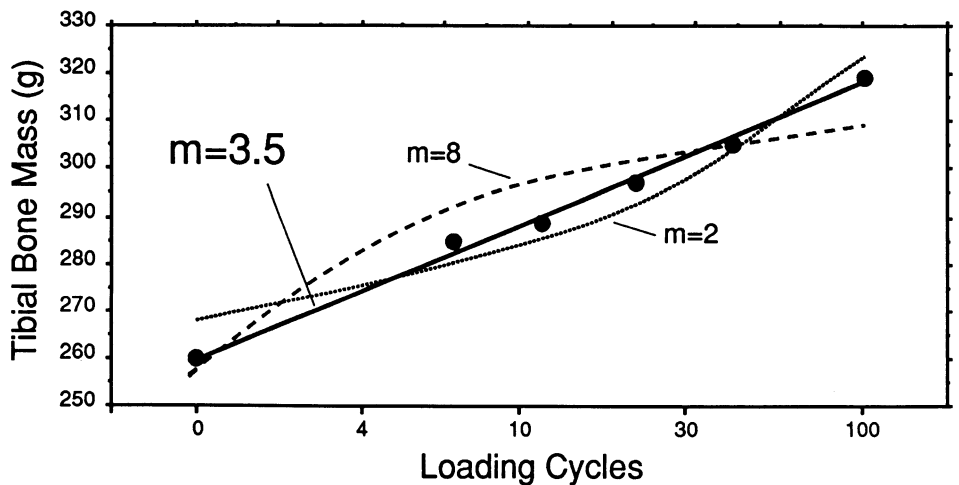
k represents the number of daily loading conditions, and n represents the number of frequency components for each loading condition. Alternatively, the daily loading stimulus can be represented using Carter's formula [Equation (3)] as follows:

$$S \propto \left[\sum_{j=1}^k N_j E_j^m \right]^{1/m} \quad (5)$$

Rule 3: Bone Cells Accommodate to Routine Loading

Bone adaptation is "error-driven," in other words the abnormal strains applied to the skeleton drive structural change. As stated

Figure 8. Carter's formula [Equation (3)] fits nicely to the data from Umemura et al.³⁹ if we choose $m = 3.5$. This indicates that the daily loading stimulus is proportional to $EN^{1/3.5}$, where E is the strain stimulus and N is the number of loading cycles.



by Lanyon,¹⁸ “the mechanically adaptive response is dominated not by the numerous cycles of ‘normal’ strain change engendered during the predominant activity but rather by far fewer cycles of relatively ‘abnormal’ strain changes produced during unusual loading situations.” This rule reflects accommodation, at a cellular level, that causes bone cells to become accustomed to “normal” strain waveforms from routine daily activities like walking or running.

By accommodation, I refer to restructuring of cells’ cytoskeletal machinery and/or genetic expression for the cell to become accustomed to its local strain environment. The biological signals that govern cellular accommodation are not well understood, yet there are several lines of evidence that suggest accommodation takes place. For example, we found that cultured osteoblasts exposed to mechanical stimuli rearrange their cytoskeletons to form actin stress fibers. During the process of stress fiber formation proteins important in the process of bone adaptation (e.g., *c-fos*, and cyclooxygenase 2) are expressed in large quantities²⁴ (and unpublished data). However the formation of these proteins is transient and, with continued exposure to the mechanical stimulus, the cells settle into a new biological steady state in which they are adapted to the predominant mechanical loading. To stimulate a new response through the cytoskeletal signaling pathway, an “abnormal” loading regimen is required.

The accommodation of bone cells to customary strain patterns in the diaphyses of many long bones should be expected given what is known about the loading typically applied to these bones. Most long bones are curved along their axis and this curvature augments rather than reduces mechanical strains due to bending.² Bones could support more weight with less mass if they were loaded instead in axial compression. Therefore, bone curvature introduces a paradox in mechanical design.¹⁷ Bertram and Biewener² proposed that bone curvature provides a benefit by increasing the predictability of strain patterns in the bone cross section under a range of dynamic loads that vary in both direction and magnitude. They showed that the mean curvature of the radius of several species of quadrupedal mammals represented a design that maximized both load carrying capacity and predictability of strain patterns. By providing strain predictability, bone curvature guarantees that the region of bone along the neutral bending axis will receive very small strains routinely, so we can assume that the bone cells have accommodated to small strains; otherwise, the bone along the neutral axis would resorb away. The strains increase in magnitude as one moves further from the neutral bending axis, thus the cells should be accommodated to different strains at each point across the long bone section. The strain state to which the cells are accommodated is sometimes called an equilibrium strain.⁵ Large deviations from equilibrium (i.e., abnormal loading states) drive adaptation. This may explain why experimental loading regimens that produce bending along an abnormal neutral axis have a dramatic effect on bone formation even though the strain magnitudes achieved are well within physiological limits.³⁰ In this scenario, the difference between “abnormal” and “normal” loading stimuli at different points within the bone tissue can be quite large.

The mathematical function that describes error-driven bone adaptation has the general form:

$$\frac{\partial M}{\partial t} = B\{\phi - F\} \quad (6)$$

where M is bone mass, t is time, ϕ is the local stress/strain state, and B and F are constants.¹⁵ F represents the “normal” loading state to which the bone cells are accustomed. Thus, $\phi - F$ is an error function that drives the system to change bone mass. If ϕ is represented by the daily loading stimulus (S) given in Equa-

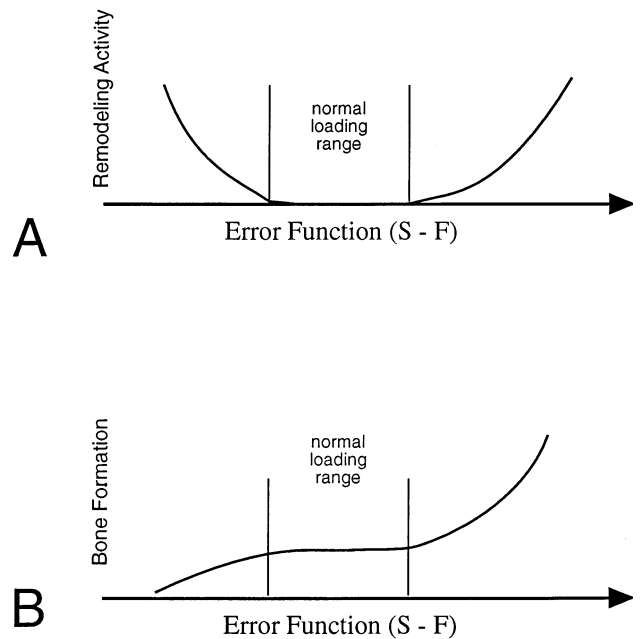


Figure 9. The error function, that is, the daily loading stimulus (S) minus the normal loading pattern (F), drives bone adaptation. This function differentially affects bone modeling and remodeling. Disuse (low values of $S - F$) increases bone remodeling on trabecular and endocortical surfaces and a mismatch between bone resorption and formation leading to bone loss. Bone formation on modeling surfaces is inhibited by disuse. Bone remodeling is minimal for mechanical strains in the normal loading range¹⁴ but increases to repair microdamage within the bone matrix for high values of $S - F$. High values of $S - F$ also cause increased osteoblast activity on bone modeling surfaces and increased bone mass.^{12,13,35}

tions (4) and (5), $S - F$ is the driving force for bone adaptation. Under normal loading patterns, bone remodeling is maintained at low levels, but if the error function ($S - F$) surpasses a lower threshold (i.e., abnormally low loading) osteoclast activity associated with bone remodeling increases. If the error function surpasses an upper threshold, osteoblast activity on bone modeling surfaces is activated, causing sculpting of the shape of long bones or trabeculae (Figure 9). Abnormally high values of S can cause increased bone remodeling of bone due to the introduction of microdamage.

Discussion

Hydrostatic or Shear Strains?

Bone adaptation, it is often said, is dependent upon strain magnitude, duration, frequency, history, type (compression, tension or shear), and distribution. The three rules presented here provide a mathematical treatment that integrates the influences of strain magnitude, frequency, duration, and, to some extent, history. The importance of strain type and distribution has not yet been discussed. Generally, principal tensile or compressive strains are considered most important for bone adaptation, and shear strains seem to have little effect.²⁶ There is a growing body of evidence demonstrating that fluid flow within the canaliculae and lacunae of bone is primarily responsible for mechanochemical signal transduction in bone cells.⁸ If true, this suggests that hydrostatic stress gradients within the bone must develop to initiate bone adaptation. Hydrostatic stress is generated by dy-

dynamic dilatational strains, but not by shear strains, suggesting that adaptation occurs in the presence of dynamic loading, dilatational strains (i.e., volume changes in the tissue), and a strain gradient. Not surprisingly, all of the studies cited in the derivation of Equation (1) involved animal models in which bending loads were applied (bending creates a dilatational strain gradient across the bone cortex). Long bones of terrestrial mammals are loaded predominantly in bending,²⁹ whereas wing bones of flying mammals such as bats are adapted to considerable shear loads.³³ Thus, it might be of interest to compare the effects of artificial loading on bone adaptation in bats to responses seen in their terrestrial relatives.

Cellular Accommodation and Cellular Mechanotransduction

As discussed earlier, mechanical stimuli can induce cytoskeletal rearrangement and cellular accommodation in cultured bone cells. This is not to say that bone adaptation cannot take place without triggering cytoskeletal rearrangement in bone cells. Other mechanosensitive second messenger pathways involving constitutive enzymes (cyclooxygenase and nitric oxide synthase) in the cell membrane have been identified.^{16,27} Once bone cells are accommodated to “normal” mechanical loading, adjustment of cellular metabolism to slightly abnormal loading might involve constitutive signaling pathways, whereas more severely abnormal loading signals could induce dramatic changes in cellular activity by causing remodeling of the cellular cytoskeleton and subsequent intercellular protein expression. Certainly, the robustness of response observed when artificial loading regimens are applied in animal models (i.e., quiescent bone surfaces are converted into active bone-forming surfaces^{4,10,25}) suggests that induction of protein expression is involved. Forwood’s⁹ finding that the inducible isoform of cyclooxygenase is involved in bone adaptation to artificial loading supports this proposition. It is worth noting also that cellular accommodation does not only involve adaptation of single bone cells to “normal” loading, but probably involves adaptation of networks of osteocytes, bone lining cells, and osteoblasts communicating through gap junctions in their cell processes.^{6,7,41}

Cellular Accommodation in Growing Bone

The description of cellular accommodation presented does not fully capture its overall complexity, especially with respect to interaction between mechanical effects and genetic programming during growth. Mechanical loading tends to enhance curvature of long bones as they grow, thus increasing bending strains.¹⁷ This may reflect interactions between bone and muscle development, as well as interactions between loading and longitudinal growth. When artificial mechanical loading was superimposed upon the normal growth patterns of the rat ulna, the resulting changes in periosteal bone formation were complex and nonlinear.²¹ Applied loading depressed bone formation in some regions of the bone, while enhancing formation in other regions. Because the observed loading results were repeatable, mathematical prediction should be possible. However, one must determine the appropriate reference strain state [F in Equation (6)]. Because growth imposes nonequilibrium conditions, the reference state must be constantly updated, thus creating a level of complexity that defies current mathematical approaches.

Because efficient bone adaptation improves skeletal performance and reduces the risk of fracture, often a fatal affliction in ancient times, the process of evolution probably preserved and enhanced the ability of bone cells to respond appropriately to mechanical loads. The current mathematical treatment of bone adaptation, although imperfect, provides insights into the mech-

anosensory system in bone. Bone adaptation can be predicted, to some extent, from three fundamental rules: bone adaptation is driven by dynamic, rather than static, loading; extending the duration of mechanical loading or exercise has a diminishing effect on further bone adaptation; bone cells and/or cellular networks tend to accommodate to a mechanical loading environment, making them less responsive to routine loading signals. The nature of cellular accommodation is not well understood, but there is evidence that it does occur. Certainly long bones lose considerable bone mass when they are not mechanically loaded, while the calvarium is ordinarily under small mechanical strains but does not resorb away, suggesting that the cells from these bones have accommodated to different loading environments. It also is possible that hormones and cytokines can cause accommodation of bone cells. Over 10 years ago, Frost¹² proposed that hormones interact with bone adaptation by altering a series of mechanical loading “thresholds,” which determine the sensitivity of bone cells to mechanical loading. This theory, called the “mechanostat theory,” implies that hormones enhance or inhibit mechanical loading effects at some fundamental level within the bone cell signal transduction machinery. Although there is anecdotal evidence to support Frost’s theory, no cellular mechanism for the mechanostat has yet been discovered. Further investigation into the nature of bone cell mechanotransduction may well provide answers to these and other fundamental questions in bone biology.

Appendix: Derivation of the Strain Stimulus Formula

In a simple experimental system, where a long bone is subjected to either static or periodic cyclic loads, the applied load is given by:

$$\text{Load} = A + B \sin \omega t \quad (\text{A1})$$

where A is the magnitude of the stationary load, $2B$ is the peak-to-peak magnitude of the cyclic load, ω is the angular frequency (2π times frequency) of the cyclic load, and t is time (**Figure A1**). If the bone is assumed to be a perfect cylinder loaded in bending, the peak strain applied to the surface of the bone is given by:

$$\text{Peak strain} = \frac{4L(A + B \sin \omega t)}{\pi E r^3} \quad (\text{A2})$$

where L is the moment arm for the applied load, E is the Young’s modulus of the bone, and r is the radius of the bone. The strain rate on the bone surface is found by taking the derivative of Equation (2):

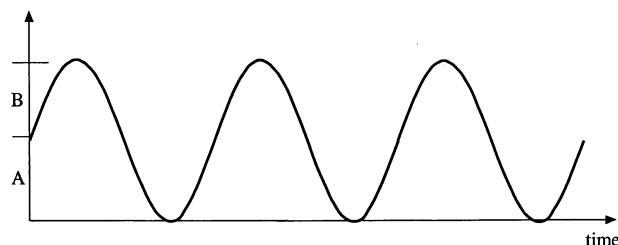


Figure A1. Any periodic loading waveform is made up of a series of sine waves. In a generalized representation of a sinusoidal loading waveform, there is a static component of the load (A) and a dynamic component of the load (B). For bone adaptation, the dynamic component of loading is most important.

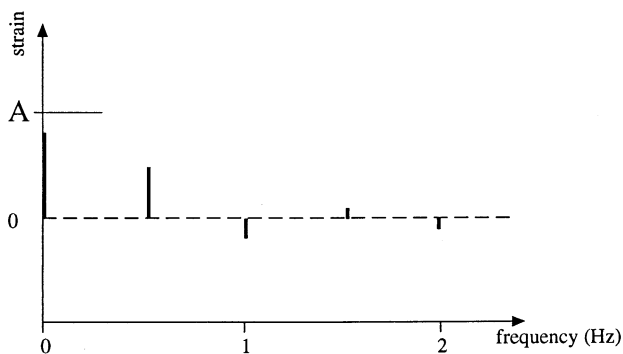
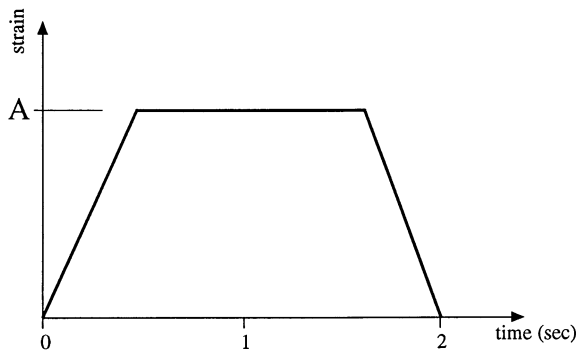


Figure A2. The trapezoidal loading waveform used by Rubin and Lanyon³⁰ can be expanded into a Fourier series, which shows a static loading component of about 0.75 A, a major dynamic component at 0.5 Hz, and several higher frequency components with progressively lower amplitudes. The strain stimulus from this waveform is the sum of strain magnitude \times frequency for each of these components [Equation (A5)].

$$\text{Strain rate} = \frac{4L\omega B \cos \omega t}{\pi E r^3} \quad (\text{A3})$$

The peak strain rate is proportional to ωB , the stationary load A does not affect the strain rate. Because the peak-to-peak dynamic strain (ϵ) is proportional to $2B$ and the loading frequency (f) is proportional to ω , the strain rate is proportional to ϵf . If we assume that the strain rate determines the tissue-level strain stimulus, an assumption supported by experimental studies,^{22,38} then:

$$E = k_1 \epsilon f \quad (\text{A4})$$

where E is the strain stimulus, and k_1 is a proportionality constant.

For a given bone under any periodic loading condition (e.g., walking, running, or experimentally applied loading) the loading waveform can be expanded into a series of sine waves at different amplitudes and frequencies using the Fourier method. For instance, the loading waveform that was used by Rubin and Lanyon, in their studies of bone adaptation in the avian ulna,³⁰ is expanded into a Fourier series in **Figure A2**. It seems a reasonable assumption that, if a single sine wave creates a strain stimulus proportional to ϵf , the strain stimulus from a series of sine waves is given by:

$$E = k_1 \sum_{i=1}^n \epsilon_i f_i \quad (\text{A5})$$

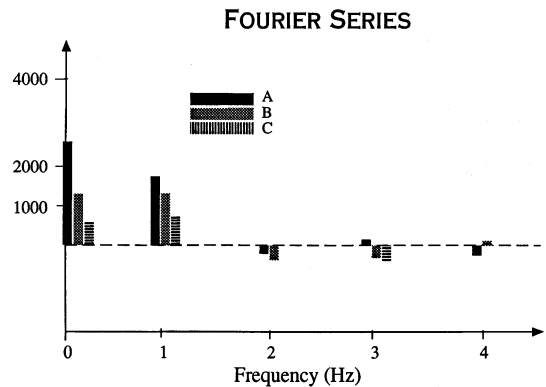
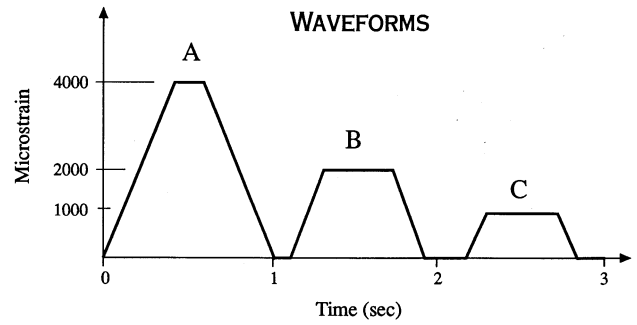


Figure A3. Three different trapezoidal loading waveforms applied by Rubin and Lanyon.³¹ Each waveform maintains constant loading and unloading strain rates (0.01/sec) and constant periods of duration (1 sec), but vary in strain magnitude (from 1000 to 4000 $\mu\epsilon$). Rubin and Lanyon postulated that these loading waveforms allowed strain magnitude to be varied, while strain rate and the frequency of the loading waveform were held constant. However, Fourier series expansions show that waveform A, B, and C each has unique frequency components above the fundamental frequency (1 Hz). Higher frequency strain components (>1 Hz) are very important in the calculation of the strain stimulus E as errors of 40%–150% result if these components are ignored. Values of E calculated from the Fourier components of each waveform correlated with the bone formation parameters measured by Rubin and Lanyon as well as did peak strain magnitude ($r = 0.83$ for peak strain magnitude, and $r = 0.82$ for E).

where $n = \infty$. However, the majority of information in a typical Fourier series is contained in the first 10–20 terms, so $n = 20$ may serve as a reasonable approximation.

It is important to note that any nonsinusoidal loading waveform always induces higher frequency loading components. Unfortunately, many investigators assume incorrectly that a trapezoid waveform, in which the loading and unloading rates are held constant, allows the application of a constant strain rate while keeping loading frequency constant. For instance, Rubin and Lanyon³¹ and Mosley et al.²¹ applied loading using a series trapezoid waveforms in which the wave heights (strain magnitudes) were varied but the slope of the rise (strain rate) for the waveforms was held constant. Although these investigators assumed that loading frequency was held constant by their loading regimen, in fact there were different magnitudes of high frequency strain components for the different loading waveforms (**Figure A3**). The experimental results were thus confounded. It is possible to vary the dynamic strain magnitude without varying loading frequency if loading is applied using a sinusoidal waveform.³⁷ However, this loading regimen also varies strain rate in

direct proportion to the dynamic strain magnitude. It is also possible to vary the strain rate while keeping the peak strain magnitude constant.³⁸ However, this loading regimen also varies the dynamic (peak to peak) strain magnitude. As shown in Figure A3, *one cannot avoid the mathematical fact that it is impossible to independently control dynamic strain magnitude, strain rate, and frequency, because these variables are interrelated.*

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