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# Effects of Biomechanical Stress on Bones in Animals

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**The signals that allow bone to adapt to its mechanical environment most likely involve strain-mediated fluid flow through the canalicular channels. Fluid can only be moved through bone by cyclic loading, and the shear stresses generated on bone cells are proportional to the rate of loading. The proportional relation between fluid shear stresses on cells and loading rate predicts that the magnitude of bone's adaptive response to loading should be proportional to strain rate. For lower loading frequencies within the physiologic range, experimental evidence shows this is true. It is also true that the mechanical sensitivity of bone cells saturates quickly, and that a period of recovery either between loading cycles or between periods of exercise can optimize adaptive response. Together, these concepts suggest that short periods of exercise, with a 4–8 h rest period between them, are a more effective osteogenic stimulus than a single sustained session of exercise. The data also suggest that activities involving higher loading rates are more effective for increasing bone formation, even if the duration of the activity is short. (Bone 30: 781–786; 2002)** 

**Key Words:** Exercise; Mechanotransduction; Sensitivity; Saturation; Adaptation.

## Introduction

The idea that bone senses its mechanical environment and can adapt to it is not new, although the specific mechanical signals detected by bone cells, and the way in which the signals are converted into cellular activity that results in bone remodeling, is still unknown. Wolff[27] is routinely given credit for the idea that bone adapts its form to its function, but how this occurs is more problematic.

The general concept that bone adapts by responding to load-induced mechanical deformation was originally proposed by Thompson,[22] who stated in 1917 in his classic treatise *On Growth and Form:*

> . . . the very important physiological truth [is] that a condition of *strain*, the result of a *stress*, is a direct stimulus to growth itself. This is indeed no less than one of the cardinal facts of theoretical biology. (p. 238)

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And later:

> The origin, or causation, of the phenomenon [ie, mechanical adaptation during growth] would seem to lie partly in the tendency of growth to be accelerated under strain: and partly in the automatic effect of shearing strain . . . an automatic effect which we can probably trace as working on all scales of magnitude, accounting therefore for the rearrangement . . . of the trabeculae within the bone. (p. 241)

Not only had Thompson implicated strain as a mechanical signal controlling bone adaptation, but proposed that shear stress produced as the result of bone deformation (or strain) may form a component part of the mechanical signaling mechanism.

It is most likely that strain produces these effects indirectly by causing fluid movement within the fluid-filled spaces surrounding osteocytes and canalicular processes, creating fluid shear stresses on the osteocytes, rather than through direct deformation of the cell membrane itself.[15] When bone is deformed in bending, fluid in the bone fluid compartment[12] is moved away from surfaces of greater concavity toward surfaces of greater convexity (**Figure 1**). This fluid movement provides a mechanism to explain why periosteal and endocortical bone surface adaptation corresponds to changes in surface curvature, as Frost[3] proposed in 1964, rather than just to the polarity of the tangential wall stress (i.e., tension or compression).

The indirect role of bone strain on bone adaptation, mediated by fluid flow within the bone fluid compartment, leads to several predictions about how bone should behave. Fluid can only be moved through bone by cyclic loading and relaxation; static loads will not create such movement, nor will they create the requisite shear stresses on cells that allow an adaptive anabolic response. If bone adaptation is driven by shear stresses on cells, then adaptation should occur only in response to dynamic loads. As fluid is moved through the bone fluid compartment, shear stresses are generated on bone cells that are proportional to the rate of fluid flow. As bone is loaded more quickly, at a higher strain rate, fluid velocity and consequent shear stresses increase. The proportional relation between fluid shear stresses on cells and strain rate predicts that the net bone response to loading should be proportional to strain rate. This concept does not predict another property of bone's adaptive response that is becoming increasingly evident: The osteogenic response saturates quickly in response to mechanical loading, and cells require a recovery period to reestablish their mechanical sensitivity before they can fully respond again to their mechanical environment.

Evidence for each of these concepts is discussed in the following sections.





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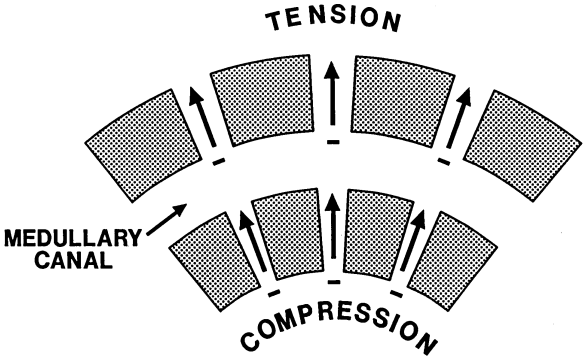


**Figure 1.** When bone is subjected to bending strains, fluid is forced to flow through the canalicular channels from regions of greater compression toward regions of lesser compression (or from more concave surfaces to more convex surfaces). This gradient of flow is proportional to the strain gradient across the cortex of the bone. The magnitude of the fluid shear stress on osteocytes lying within the lacunae is proportional to the rate at which fluid is forced through these channels, which in turn is proportional to the strain rate. Reproduced from ref. 12.

### Bone Responds Only to Dynamic Loading

Since the classic experiments of Hert and Liskova 30 years ago, it has been clear that bone adapts only in response to dynamic loads and not to static loads.[4–6,10,11,20] However, the situation is made somewhat more complex by recent observations that static loads not only fail to elicit a response, but may suppress normal appositional growth. In a recent experiment, we applied a compressive end-load to the ulnae of growing male rats for 10 min/day for 2 weeks.[19] The rats received one of three loading treatments: static loading at 8.5 N; static loading at 17 N; or dynamic loading (2 Hz) at 17 N. Dynamic loading increased osteogenesis significantly on both periosteal and endocortical surfaces, as expected from Hert's previous work and as predicted by the concept that fluid flow mediates the mechanical signal. Static loading at either load magnitude had no effect on endocortical bone formation rate, but actually suppressed periosteal bone formation (**Figure 2**). Dynamic loading is required not only to stimulate appositional bone growth, but apparently also to prevent suppression of appositional bone growth on the periosteal surface.

### Rate-related Phenomena Are Critical to Bone's Response

The observation that dynamic, cyclic loads are required to initiate an adaptive response implies that bone must be responsive to more than strain magnitude. It is now clear that rate-related phenomena are critical to bone's adaptive response. O'Connor et al.[14] first showed the association between strain rate and new bone formation, but failed to show that increased rate caused the bone formation. More recently, Mosley and Lanyon,[13] using the rat ulnar bending model, showed that strain rate provides a greater osteogenic stimulus for bone formation than strain magnitude. However, this work did not consider the viscoelastic response of bone to the loading,[8] and was performed using immature rats loaded through an open growth plate.

Prior to Mosley and Lanyon's work, Turner demonstrated cause and effect between strain rate and bone adaptation with two experiments. Strain rate is linearly proportional to strain magnitude and frequency. Using the four point tibial bending model, Turner et al.[24] showed that increasing the frequency of loading while maintaining a constant strain magnitude caused a

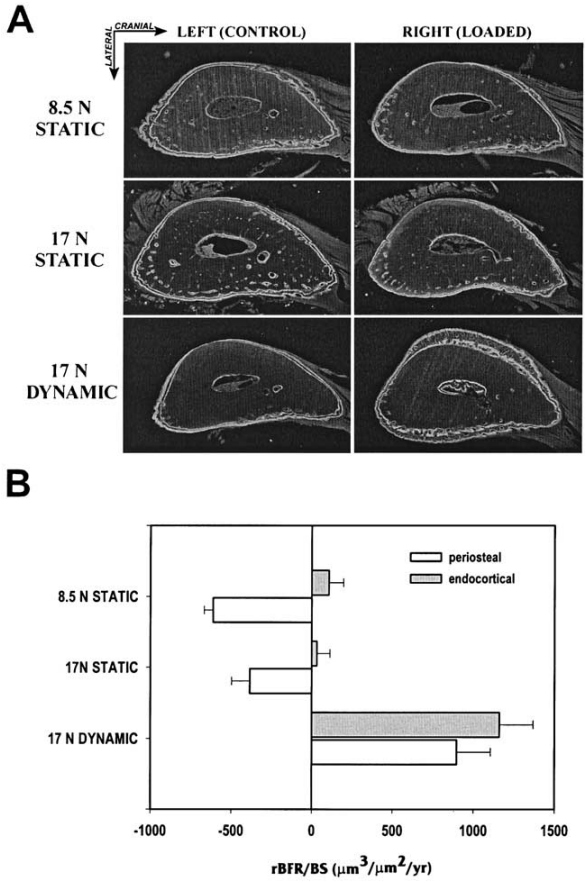


**Figure 2.** (A) Fluorochrome-labeled sections through the left (control) and right (loaded) ulnar (3 mm distal to midshaft) from growing male rats subjected to static loading at 8.5 N (top panels) or 17 N (center panels), or to dynamic loading at 17 N (bottom panels) for 10 min/day. Note in the two static groups the suppression of periosteal apposition in the loaded limbs (compared with the control limbs), which contrasts to the markedly enhanced periosteal apposition in the 17 N dynamic group. (B) Histomorphometric measurements reveal the potent osteogenic effects of dynamic loading on both endocortical and periosteal surfaces, whereas the periosteal formation rate is suppressed significantly by both magnitudes of static loading. Static loading appears to have little effect on endocortical bone formation. Panel (A) reproduced from ref. 19 with permission.

significant increase in bone formation rate at frequencies of 0.5–2.0 Hz. Because all animals received 36 cycles/day of loading, the duration of the loading varied in this experiment. To correct this, Turner et al.[25] performed another experiment in which strain rate was altered, but the frequency, duration, and peak strain magnitude were kept constant by altering the range of strain. This experiment showed that bone formation rate was directly proportional to strain rate. Based on these experiments, Turner[23] deduced that the strain stimulus ($E$) that creates the adaptive response is proportional to strain magnitude and frequency:

$$E = k_1 \sum_{I=1}^{n} \varepsilon_i f_i$$

When this equation is applied to the two Turner experiments, it is evident that the strain stimulus is highly and linearly correlated

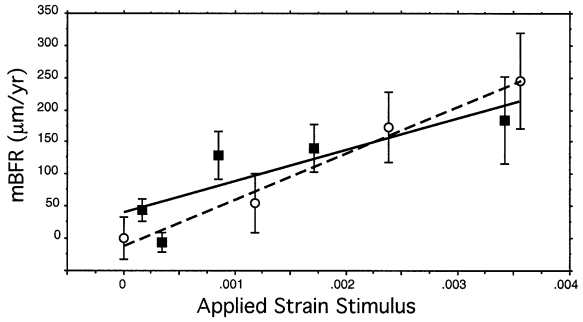


**Figure 3.** Based on two separate experiments in which loading was applied daily for 36 cycles/day over 10 days,[24,25] Turner showed that bone formation rate (mBFR) is directly related to the strain stimulus, which is proportional to strain magnitude and frequency. In one experiment (filled squares, solid line), loading frequency was varied from 0.1 to 2.0 Hz. In the second experiment (open circles, dashed line), cyclic strain range was varied from 0 to 54 N. The similarity in the relationship between BFR and strain stimulus in the two experiments suggests that bone adaptation is controlled by a combination of strain magnitude and frequency (i.e., strain rate). Reproduced from ref. 23 with permission.

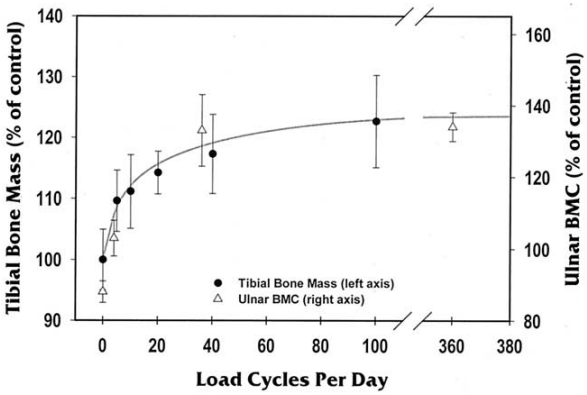


**Figure 4.** Published experiments from externally loaded turkey ulnae (open triangles, right axis),[20] and from tibiae of rats trained to jump up to an elevated platform (filled circles, left axis),[26] show that few loading cycles are necessary to elicit an osteogenic response. Moreover, the response to loading quickly diminishes after inception of the loading bout; cycles that occur beyond cycles 50–100 in a single bout are largely ineffective in stimulating any further osteogenic response than that triggered by the first 50–100 cycles. A similar saturation curve has been demonstrated in the rat tibia.[24]

with the bone formation rate (**Figure 3**). This indicates that the adaptive response is determined, and can be predicted, by a combination of strain magnitude and frequency—that is, by strain rate alone.

This is true, however, only for loading frequencies of <2 Hz (which, nevertheless, includes most physiologic loading frequencies).[7] At higher loading frequencies, bone cells become less sensitive to loading, or the products of loading such as fluid shear stresses, due to increasing cellular stiffness.[9] Therefore, for higher frequencies, the viscoelastic properties of the cells and extracellular matrix must be considered. Nevertheless, most loading of bone occurs at lower frequencies, and thus for all practical purposes the idea that loading frequency affects the dose-response relationship between the mechanical environment and the adaptive response is a useful concept.

## Bone Response Saturates Quickly

Very little mechanical stimulation is required to initiate an adaptive response in bone. Nearly 20 years ago, Rubin and Lanyon,[20] in a now classic experiment, showed that only 36 cycles/day at physiologic strain magnitudes (2000 με in compression) were just as effective in promoting bone formation as 1800 cycles/day at the same strain magnitude. The magnitude of the bone response was not enhanced by additional loading cycles beyond 36, implying that the cellular response to mechanical loading saturates quickly. More recently, Umemura et al.[26] also showed that only a few strain cycles are required to saturate bone response. In a study in which rats were trained to jump between 5 and 100 jumps/day for 8 weeks, they found that, whereas only 5 jumps/day were sufficient to cause a significant increase in cortical area and bending rigidity, area and rigidity were not increased significantly more by 100 jumps/day than by 10 jumps per day. When these data are plotted together with the data from Rubin and Lanyon's study (**Figure 4**), the resulting graph shows that the relationship between number of cycles and tibial bone mass in the Umemura et al. study is identical to that between number of cycles and ulnar bone mineral content in the Rubin and Lanyon study. In both cases, the response is logarithmic in the 1–100 cycle range, at which point it reaches an asymptote.

Data from studies using the rat four point tibial bending model[24] are consistent with the principal of mechanosensory saturation. In these studies, there was an asymptotic approach to saturation as the duration of loading was increased from 36 to 720 cycles/day. Increasing the duration of a loading bout therefore results in diminishing returns in bone formation, again suggesting that cells may become "deaf" to repeated mechanical stimuli.

## Mechanical Sensitivity Following Saturation Is Reestablished in 4–8 h

If bone response saturates quickly, it is reasonable to ask how long it takes for bone to recover its mechanical sensitivity. Information on bone cell saturation and recovery can be used to optimize physical activity programs aimed at maintaining or improving bone mass. We recently reported the results of an experiment in which the rat tibial four point bending model was used to investigate bone saturation and recovery.[17] Rats were subjected to mediolateral bending of the right tibia every other day for 5 days. In all loaded groups, identical mechanical inputs were administered—360 cycles/day using a peak force of 54 N delivered in a 2 Hz haversine wave—but the temporal distribution of load cycles was varied. One group received six separate bouts of loading each day, each bout consisting of 60 cycles (60 × 6) and separated by a 2 h recovery period from the previous bout. The three remaining bending groups received 90 cycles four times per day (90 × 4), each bout separated by 3 h; 180 cycles twice per day (180 × 2), each separated by 6 h; or 360 cycles in a single loading bout each day (**Figure 5**). A separate control group received no loading. The relative bone formation rate (rBFR, loaded − nonloaded limb) was significantly greater in all loaded animals than in control animals. However, the 90 × 4 and 60 × 6 loading groups exhibited significantly greater (~80%) rBFR than the 360 × 1 loading group (Figure 5), even though all groups were subjected to identical loads and cycles over each 24 h period. A similar experiment was performed using the rat ulnar loading model, showing similar results for the periosteal surface of the bone. These results demonstrate that allowing a recovery period of at least 2–3 h between each load

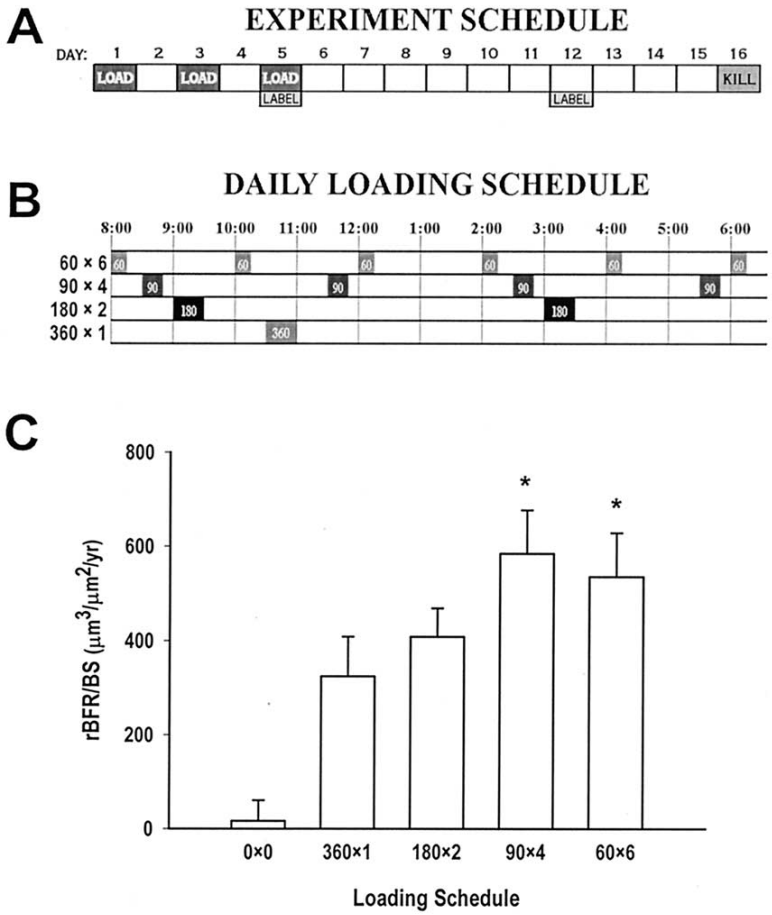


**Figure 5.** (A) Right tibiae of adult female rats were loaded in four point bending every other day for 1 week, labeled with calcein, and then the animals were killed on day 16. (B) The loading schedule on each of the 3 load days comprised 360 cycles, divided into 1, 2, 4, or 6 bouts/day. Rats in the 6 bouts/day group received 60 bending cycles per bout (60 × 6); rats in the 4 bouts/day group received 90 bending cycles/bout (90 × 4); the 2 bouts/day and 1 bouts/day groups received 180 and 360 bending cycles/bout, respectively (180 × 2, 360 × 1). A nonloaded, age-matched control group (0 × 0) and two sham-bending groups (60 × 6 and 90 × 4) were included (not shown). (C) Dividing the 360 cycles into smaller bouts, separated by recovery periods, significantly enhanced the osteogenic response to loading on the endocortical surface. *Significantly different from 0 × 0 and 360 × 1 groups (Fisher's PLSD; $p < 0.05$). Redrawn after ref. 17.

session provides a more osteogenic stimulus than applying all 360 cycles at one time. Bone cells become increasingly desensitized to repeated mechanical loading after as few as 60 cycles. By allowing a recovery period between loading bouts, the effectiveness of the loading can be increased.

Using the same model and mechanical parameters, a separate experiment was performed to determine the period of time required for optimum recovery of mechanosensitivity by varying the temporal spacing of four identical daily loading bouts of 90 cycles/bout. The daily loading bouts were administered with 0, 0.5, 1, 2, 4, or 8 h between each of the bouts. Loads were applied every other day for 5 days. Relative BFR/BS in the 8 h recovery group was more than 100% greater than that in the group allowed no recovery between loading bouts ($p = 0.0005$), and 88% greater than that in the 30 min recovery group ($p = 0.005$). The 4 h recovery group also exhibited significantly greater ($p = 0.013$) rBFR/BS than the group allowed no recovery between loading bouts. A dose-response relationship was found between recovery time and rBFR/BS (**Figure 6**), suggesting that allowing even a short recovery period would probably improve the osteogenic response somewhat. However, the data suggest that response can be optimized by allowing a recovery period of 4–8 h between loading bouts. Comparisons of the magnitude of rBFR/BS in the 8 h recovery group with bone formation rates determined after 24 h of recovery from other experiments[2,24] suggest that 8 h is sufficient to reestablish complete mechanical sensitization.

We have proposed that this loss of mechanical sensitivity, and subsequent resensitization following recovery, is mediated in part by the actin cytoskeleton in bone cells. When cells are subjected experimentally to fluid shear stresses using a laminar flow device, the actin filaments of the cytoskeleton reorganize into thick, oriented stress fibers.[1,16] Preventing this cytoskeletal assembly prevents the expression of certain genes known to be associated with bone formation, suggesting that shear-induced reorganization of the actin filaments into stress fiber bundles is required for mechanical signal transduction and gene expression. If the same cells are removed from the fluid flow environment, the cytoskeleton disassembles after a load-free recovery period lasting between 4 and 8 h.[18] These data from cell culture

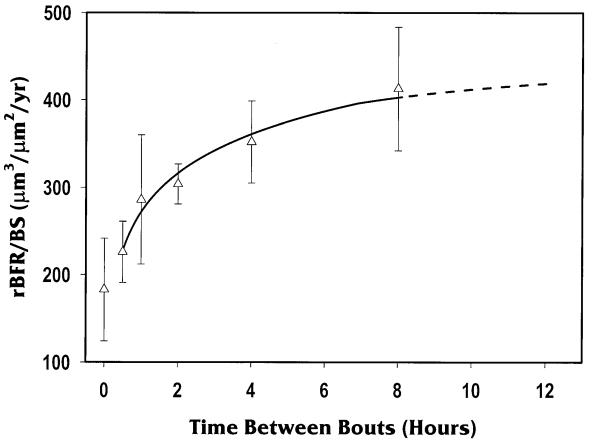


**Figure 6.** Allotting recovery periods between loading sessions allows bone cells to regain mechanosensitivity lost as a result of a previous session. Adult female rats were loaded according to the 90 × 4 schedule shown in Figure 2, but the amount of time between each of the four daily bouts ranged from 0 to 8 h. Mechanosensory recovery was nearly completely restored after 8 h of "rest." Note the trend similarity for mechanosensory loss (Figure 5) and recovery (this figure). Redrawn after ref. 18.

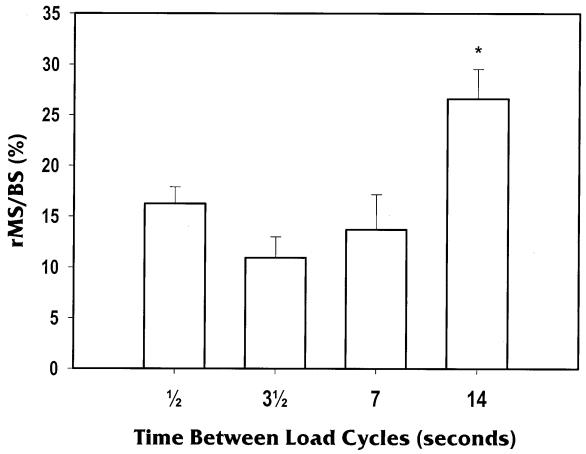


**Figure 7.** Rats subjected to four point bending of the right tibia for 36 cycles/day showed a significant increase in relative (loaded limb − control limb) mineralizing surface (rMS/BS) compared with the other loaded groups if 14 sec of recovery were allotted between load cycles. Rats allotted 0.5 (back-to-back cycles), 3.5, and 7 sec between cycles exhibited a significant increase in rMS/BS as a result of loading, but no recovery effect was detected among these three groups. *Significantly different from 0.5, 3.5, and 7 sec recovery groups (Fisher's PLSD; $p < 0.05$). Redrawn after ref. 18.

experiments correspond remarkably well to the in vivo data, tending to confirm the notion that 4–8 h is sufficient to reestablish the mechanical sensitivity of the cell.

Shorter intervals — on the order of seconds — between individual loading cycles within a single loading bout period can enhance bone's adaptive response. Srinivasan and Gross[21] recently tested this idea by comparing periosteal surface bone formation using the isolated avian ulnar model[20] in turkeys loaded at 1 Hz for 100 cycles/day for 1 week using a sawtooth waveform, and those in which the loads were separated by a 10 sec rest period. They found that the insertion of a rest period between individual load cycles significantly enhanced bone formation response ($p < 0.05$) compared with those animals loaded without an interval between cycles.

We made similar observations in a larger sample using the rat tibial four point bending model.[18] In this experiment, four bending groups were subjected to 36 loading cycles per day (54 N peak force) 5 days/week for 2 weeks. The bending groups were allowed 0.5, 3.5, 7, or 14 sec between each loading cycle. Groups allowed 0.5, 3.5, or 7 sec between loading cycles exhibited significantly greater mineralizing surface (MS/BS) and bone formation rate (BFR/BS) than sham-loaded or nonloaded controls ($p < 0.05$), but no significant differences in response were found among these three groups. The group allowed 14 sec between loading cycles, however, not only showed increased MS/BS and BFR/BS compared with controls, but demonstrated a 50% increase in BFR/BS compared with the other loaded groups ($p < 0.05$). This increase was mainly the result of increased MS/BS (**Figure 7**). These data indicate that longer spacing even between cycles within a single loading session can improve the osteogenic response over that obtained with shorter recovery intervals between cycles. The reasons for this are unclear, but may involve mechanisms of intracellular calcium signaling.[1]

In combination, these experiments show that bone response saturates quickly in response to mechanical loading, and that a period of recovery either between cycles or between loading sessions can optimize the adaptive response.

## Conclusion

These data have important implications for the design of exercise programs that can maximize bone gain or prevent bone loss. They show that short periods of exercise, with rest periods between them, are a more effective osteogenic stimulus than a single sustained session of exercise. Furthermore, they suggest that a recovery period of 4–8 h is sufficient to completely reestablish a fully mechanically sensitive state in bone. Finally, high strain magnitudes are not required to stimulate bone adaptation if the strain rate is sufficiently high. This suggests that activities involving higher loading rates will be more effective for increasing net bone formation, even if the duration of the activity is short.

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