

Enabling bone formation in the aged skeleton via rest-inserted mechanical loading

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Abstract

The mild and moderate physical activity most successfully implemented in the elderly has proven ineffective in augmenting bone mass. We have recently reported that inserting 10 s of unloaded rest between load cycles transformed low-magnitude loading into a potent osteogenic regimen for both adolescent and adult animals. Here, we extended our observations and hypothesized that inserting rest between load cycles will initiate and enhance bone formation in the aged skeleton. Aged female C57BL/6 mice (21.5 months) were subject to 2-week mechanical loading protocols utilizing the noninvasive murine tibia loading device. We tested our hypothesis by examining whether (a) inserting 10 s of rest between low-magnitude load cycles can initiate bone formation in aged mice and (b) whether bone formation response in aged animals can be further enhanced by doubling strain magnitudes, inserting rest between these load cycles, and increasing the number of high-magnitude rest-inserted load cycles. We found that 50 cycles/day of low-magnitude cyclic loading (1200 $\mu\epsilon$ peak strain) did not influence bone formation rates in aged animals. In contrast, inserting 10 s of rest between each of these low-magnitude load cycles was sufficient to initiate and significantly increase periosteal bone formation (fivefold versus intact controls and twofold versus low-magnitude loading). However, otherwise potent strategies of doubling induced strain magnitude (to 2400 $\mu\epsilon$) and inserting rest (10 s, 20 s) and, lastly, utilizing fivefold the number of high-magnitude rest-inserted load cycles (2400 $\mu\epsilon$, 250 cycles/day) were not effective in enhancing bone formation beyond that initiated via low-magnitude rest-inserted loading. We conclude that while rest-inserted loading was significantly more osteogenic in aged animals than the corresponding low-magnitude cyclic loading regimen, age-related osteoblastic deficits most likely diminished the ability to optimize this stimulus.
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Introduction

Declines in bone mass accompanying the natural process of aging markedly increase skeletal fragility [1]. This age-related loss of bone mass commences beyond the third decade of life, reflects changes in gonadal status, nutrition, and physical activity, and affects both trabecular and cortical bone tissues in the appendicular and axial skeleton [2,3]. It occurs primarily due to imbalances in the bone remodel-

ing cycle (i.e., the inability of osteoblastic bone formation to keep pace with osteoclastic resorption) [4,5]. As such, a number of pharmacological agents that target osteoblastic cells (i.e., their proliferation, function, and survival) are under consideration as anabolic strategies to enhance bone mass [6,7]. Notwithstanding their promise, counteracting bone loss accumulated over a life span would likely require prolonged utilization of these agents. This is a concern considering that such therapy is invasive and expensive and has substantial potential for deleterious side effects associated with long-term use (including back pain, nausea, and neoplastic transformations) [6,7].

Mechanical loading of the skeleton holds promise as a noninvasive, nonpharmacological alternative to counter

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age-related bone loss. However, while mechanical stimuli are perceived by bone cells and tissues to be potentially anabolic [8], general physical exercise strategies have thus far proved ineffective in substantially enhancing bone mass in the elderly. Specifically, the elderly are unable to consistently comply with the strenuous types of physical activity currently required for bone accretion [9,10]. On the other hand, the mild and moderate types of loading events most successfully implemented in the elderly are ineffective in initiating and sustaining bone formation [11,12].

The ineffectiveness of mild and moderate loading in influencing bone mass is consistent with an age-related degradation in mechanotransduction (i.e., in the ability to perceive, initiate, and sustain responses to mechanical stimuli) [13,14]. While the inability to perceive mild and moderate loading events as stimulatory may reflect potential deficits in numbers and/or viability of mechanosensory (e.g., osteocytic) cells [15,16], the inability to initiate and, especially, sustain bone formation more likely reflects potential for deficits in the numbers and/or function of osteoblastic cells [17–22]. Additionally, the declining availability of biomolecules involved in coordinating and enhancing osteoblastic response to mechanical stimuli (e.g., TGF- β , IGF-1) [23,24] potentially compromises the ability of bone cells in aged tissue to perceive low and moderate magnitude loading events as being stimulatory. Last, the age-related decrease in the surface to volume ratio of bone mineral matrix [25] and increased viscosity of interstitial fluids [26] could decrease biophysical stimuli delivered to bone cells via standard exercise regimens. In effect, initiating and sustaining an osteogenic response in aged bone tissue may require a more intense stimulus than that delivered via mild and moderate types of exercise regimens that cyclically load bone.

Previously, we proposed that insertion of an unloaded rest interval between load cycles would enhance the osteogenic potential of low-magnitude mechanical loading [27]. In support of this hypothesis, we observed that impotent, low-magnitude mechanical loading regimens were transformed into potent osteogenic stimuli in both young and adult skeletons when a 10-s unloaded rest interval was inserted between each load cycle [28,29]. In this study, we extended our observations and hypothesized that inserting rest between load cycles initiates and enhances bone formation in the aged skeleton. We tested our hypothesis by examining whether (a) low-magnitude rest-inserted loading can initiate bone formation in the aged skeleton and (b) whether bone formation response can be further enhanced by increasing strain magnitude, inserting rest, and increasing the number of rest-inserted load cycles.

Methods

A total of 49 aged female C57BL/6 mice (mean \pm SE; 21.5 ± 0.16 months) underwent mechanical loading of the

right tibia utilizing the noninvasive murine tibia loading device [30]. The device fixes the proximal tibia (at the tuberosity) against motion and applies controlled loads to the distal tibia, thereby placing the tibia diaphysis under “cantilever” bending in the medial–lateral direction. Prior to in vivo experiments examining our hypothesis, a calibration study was conducted to determine the exogenous load-induced strains in the tibia diaphysis utilizing a combined strain gauging and finite element analysis approach. Immediately following sacrifice, two single-element strain gauges were attached at the medial and lateral surfaces of right tibia mid-shaft in 21-month-old female C57BL/6 mice (Fig. 1). The right tibiae were loaded and strain data were sampled over a range of loading magnitudes over five loading trials (the mouse was repositioned between each trial). The variability in induced strains was minimal between load cycles (CV <4%) and small across loading trials (CV <9%). After strain data collection, animal-specific FE models of the tibia–fibula structure were created from high-resolution μ CT scans of the tibia–fibula structure (μ CT20, Scanco Medical AG, Switzerland; Fig. 1a) using a multimodule automated software program (within PV-Wave, VNI, Inc., Boulder, CO). The FE models were solved (within Patran, MSC, Inc., Los Angeles, CA) over the range of loading magnitudes utilized in the strain data collection (Fig. 1b). Predicted strains (FE) at the medial gauge site were then compared with the medial gauge data and the model was validated by comparisons with the lateral strain gauge readings. Over the range of load magnitudes relevant to our experiments, we found that the induced strains exhibited a linear relation to applied loads. As well, FE predictions were within 9% (range 1–20%) of strain gauge data across the range of load magnitudes. From these data, a strain versus load calibration curve was determined and yielded peak strains in the range of 800–2400 $\mu\epsilon$ at the periosteal surface (and 600 to 1800 $\mu\epsilon$ peak strains at the endocortical surface) for loads of 0.4–1.2 N, respectively. We then performed the following two experiments to examine our hypothesis and the osteogenic potential of rest-inserted loading in the aged skeleton.

Initiating bone formation in the aged skeleton via low-magnitude rest-inserted loading

The first experiment examined whether inserting a 10-s unloaded rest interval between low-magnitude load cycles can initiate bone formation in the aged skeleton (Fig. 1). Two groups of aged female C57BL/6 mice underwent a 2-week loading protocol using the noninvasive murine tibia loading device. The groups were different in that: (a) the right tibia of the first group ($n = 8$) of animals received 50 cycles/day of a 1-Hz, low-magnitude loading protocol for 5-days/week (Low) calibrated to induce peak periosteal longitudinal normal strains of 1200 $\mu\epsilon$ at the tibia mid-shaft (and 900 $\mu\epsilon$ peak strains endocortically) and (b) the second group ($n = 9$) received an identical protocol with the ex-

ception that a 10-s unloaded rest interval was inserted between each of the 50 load cycles (Low–10 s Rest; Fig. 1). Animals were allowed normal cage activity in between the brief daily loading sessions. All animals received calcein labeling (ip) on days 3 and 12 of the experiment and animals were killed on day 15. Following sacrifice, the right (exogenously loaded) and left (intact contralateral) tibiae were dissected of soft tissue and three cross-sections (300 μm) spanning the tibia mid-shaft were obtained utilizing a minitome (Struers A/S, Copenhagen, Denmark). Sections were hand ground to 90 μm , coverslipped, and imaged using a Nikon epifluorescent microscope. Digital images were obtained and analyzed blinded, and standard static and dynamic histomorphometry measures were determined at the endocortical and periosteal surfaces using custom written software within PV-Wave [31]. Briefly, single labeled (s.LS) and double labeled surfaces (d.LS) and interlabel width (i.L.wd) were measured and mineralizing surface (MS), mineral apposition rate (MAR), and bone formation rate (BFR) were determined. As well, static histomorphometry parameters such as cortical area (Ct.Ar), periosteal envelope (Ps.Ar), endocortical envelope (Ec.Ar), and cortical thickness (Ct.Th) were determined. Last, using beam theory [32], boundary conditions derived from the mid-shaft section of calibration tibia were applied to the mirrored image of the mid-shaft section of intact contralateral tibia to determine animal-specific peak strains induced in the experimental groups. The University of Washington animal care and use committee approved all procedures.

Enhancing bone formation in the aged skeleton via high-magnitude rest-inserted loading

We next performed a limited study where we sought to determine whether bone formation in the aged skeleton could be further enhanced. Specifically, we examined whether the amount of bone formation response could be further enhanced by increasing load magnitude (doubled), inserting rest (10 s, 20 s), and increasing the number of rest inserted load cycles (fivefold). In this experiment, four groups of aged female C57BL/6 mice (mean \pm SE: 21.5 \pm 0.16 months) were subjected to a 2-week protocol as previously described. The right tibia of all animals received exogenous loading and animals were randomly assigned to one of four groups: (a) the first group of animals ($n = 9$) received 50 cycles/day of cyclic loading where the induced strain magnitude was doubled with strain rate held constant (high; inducing 2400 $\mu\epsilon$ peak strain at the periosteal surface and 1800 $\mu\epsilon$ at the endocortical surface of the tibia mid-shaft); (b) a second group of animals ($n = 9$) received an identical protocol but with the exception that a 10-s unloaded rest interval was inserted between each load cycle (high–10 s Rest); (c) considering the possibility that 10 s of rest may represent a suboptimal rest interval [33], a third group of animals ($n = 7$) was subject to 50 cycles/day of high-magnitude loading but with a 20-s rest interval inserted

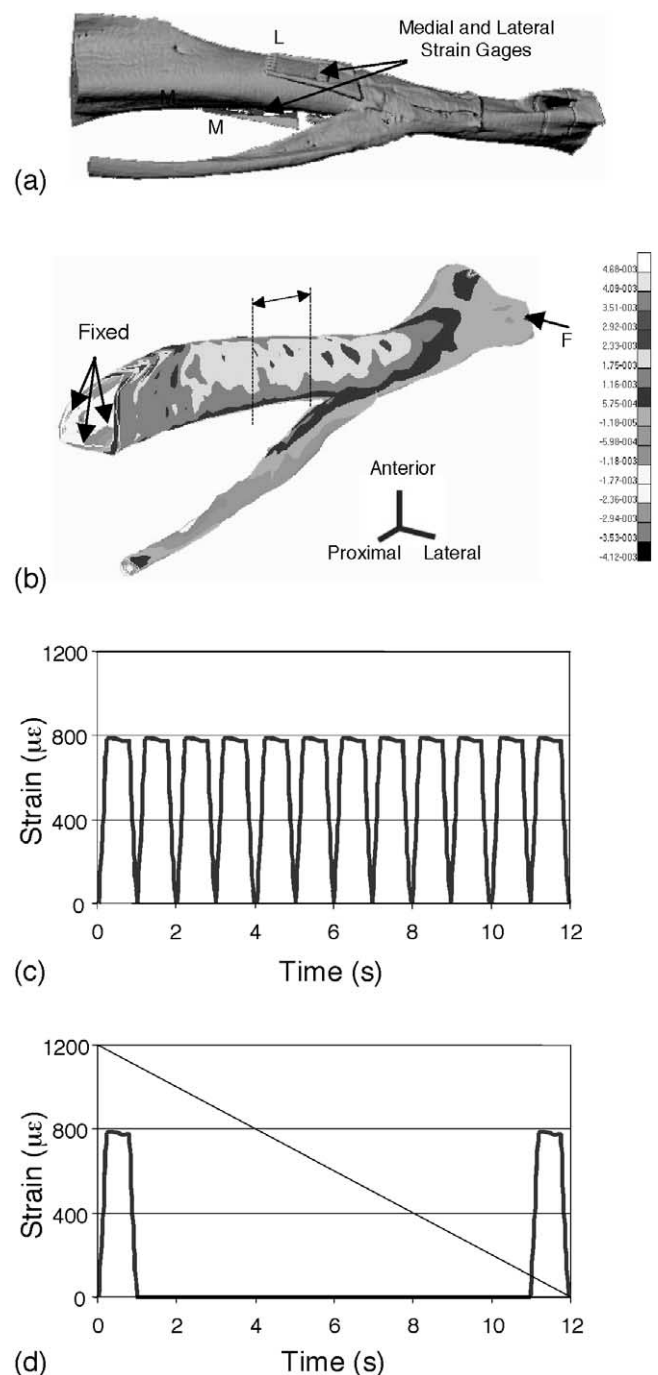


Fig. 1. Calibration of in vivo strains via strain gauging and finite element analysis. 3-D reconstruction of the tibia–fibula structure from transaxial μCT images with medial and lateral gauges noted (a). Longitudinal strains induced in an FE model of the structure with boundary conditions noted (b). Peak strains induced about the mid-shaft (spanning black arrows) are equivalent due to interaction of increasing bending moments (from distal to proximal) and moments of inertia and, as a result, bone responses are equivalent in sections obtained over this span. Strain gauge (lateral cortex) recordings for the first 12 s of low-magnitude cyclic loading (c, Low; first 12 load cycles displayed) and low-magnitude rest-inserted loading (d, Low–10 s Rest; first 2 load cycles displayed). The loading waveforms are identical except that a 10-s unloaded rest interval is inserted between each load cycle for the Low–10 s Rest protocol (d).

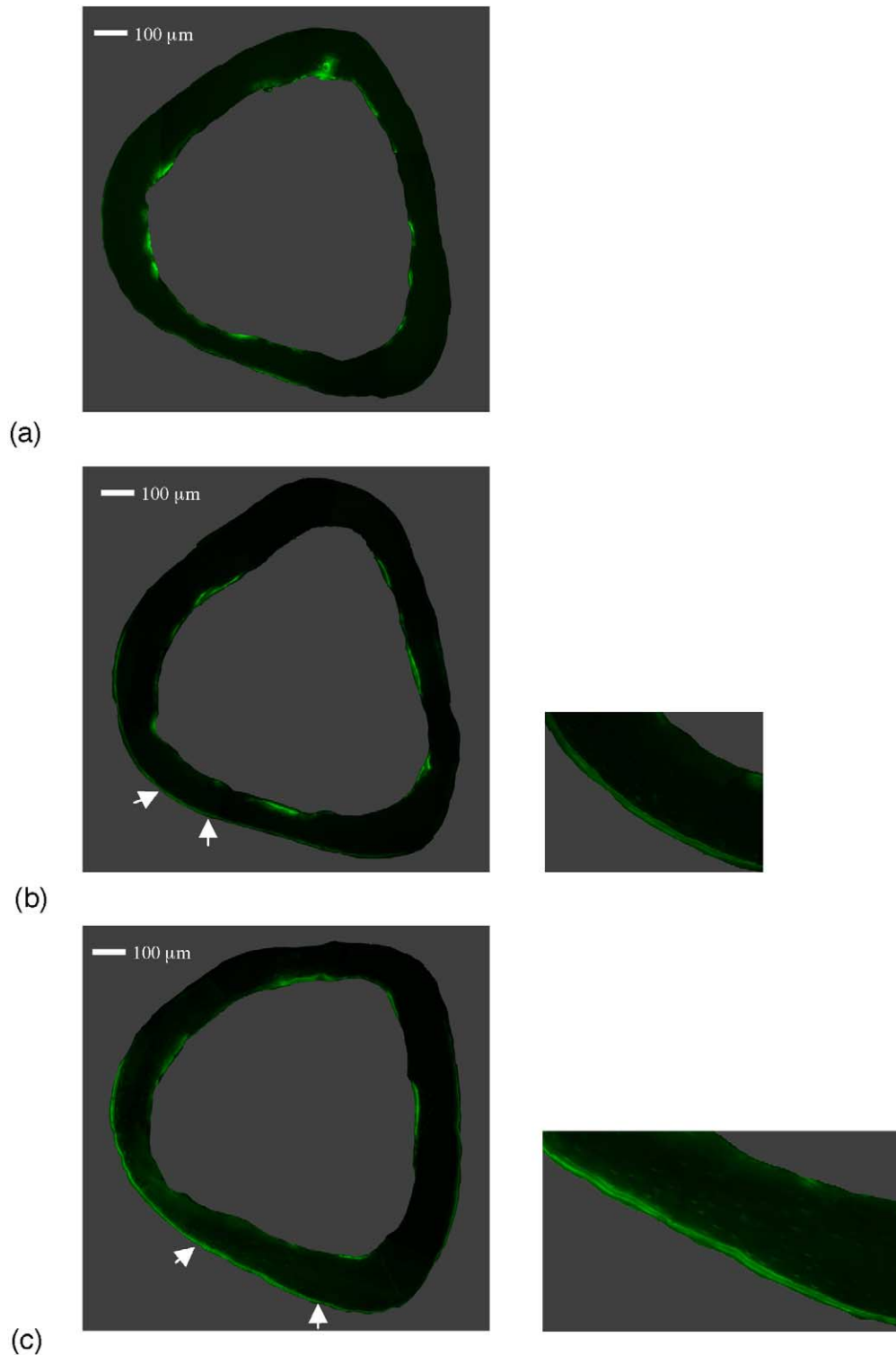


Fig. 2. Composite digital images of tibia mid-shaft sections under fluorescent excitation from intact contralateral tibiae (a) and from experimentally loaded tibiae of animals receiving low-magnitude cyclic loading (b, Low) and low-magnitude rest-inserted loading (c, Low–10 s Rest). In these aged mice, intact bones display minimal periosteal labeling (a; scale bar noted). Low-magnitude cyclic loading only minimally influences periosteal labeling (b, inset is a 2.5× magnified image (original magnification) of a small portion of the cortex indicated by white arrows, where double labeling is present). Low-magnitude rest-inserted loading induces substantial double labeling with a greater separation or thickness between labels (c, inset at 2.5× original magnification).

between each load cycle (High–20 s Rest); and (d) the last group of animals ($n = 7$) was subject to high-magnitude, 10-s rest-inserted loading but over 250 cycles/day (High–10

s Rest, 250 cycles/day). All animals received calcein labeling (ip) on days 3 and 12 and animals were killed on day 15. Static and dynamic histomorphometry analyses were per-

Table 1
Peak strains induced in experimental groups

Groups	Endocortical strain ($\mu\epsilon$)	Periosteal strain ($\mu\epsilon$)
Low	834 \pm 40	1237 \pm 61
Low–10 s Rest	854 \pm 40	1262 \pm 75
High	1660 \pm 130*	2398 \pm 101*
High–10 s Rest	1466 \pm 75*	2242 \pm 134*
High–20 s Rest	1883 \pm 90* [†]	2568 \pm 140*
High–10 s Rest, 250 cycles/day	1880 \pm 100* [†]	2688 \pm 195*

Note. Peak longitudinal normal strains (mean \pm SE) induced at the periosteal and endocortical surfaces of experimental groups. Peak strains significantly different from the respective low-magnitude groups (* $P < 0.001$) and from the High–10 s Rest group ([†], $P = 0.01$).

formed and animal-specific peak longitudinal normal strains were determined as previously described. Animals receiving the low-magnitude rest-inserted loading protocol (Low–10 s rest) from our first experiment were included as an additional comparative group.

Statistical analysis

Nonparametric statistics were utilized to determine significant differences ($P \leq 0.05$) between groups and within groups to control for small groups sizes and nonnormal data distributions. A one-tailed Wilcoxon paired test was performed to detect significant differences between dynamic histomorphometry parameters in loaded (right) versus intact bones (left). A two-tailed Kruskal–Wallis H test was performed to detect differences between groups with Mann–Whitney tests used as a post hoc follow-up.

Results

Initiating bone formation in the aged skeleton via low-magnitude rest-inserted loading

At the endocortical surface in intact contralateral tibiae (left), none of the dynamic histomorphometry parameters (MS, MAR, BFR) was significantly different across groups. Peak endocortical surface strains induced by low-magnitude and low-magnitude rest-inserted loading were not significantly different (Table 1). Neither low-magnitude (Low) nor low-magnitude rest-inserted loading (Low–10 s Rest) significantly influenced any dynamic histomorphometry parameters at the endocortical surface of experimentally loaded tibiae compared to intact tibiae (Table 2). In experimentally loaded (right) tibiae, none of the dynamic histomorphometry parameters was significantly different across groups.

At the periosteal surface in intact contralateral tibiae (left), none of the dynamic histomorphometry parameters was significantly different across groups. The peak periosteal surface strains induced by low-magnitude and low-

magnitude rest-inserted loading were not significantly different (Table 1). Fifty cycles of low-magnitude loading (Low) significantly increased only MS (by 90%, $P = 0.02$) but not MAR or BFR in experimentally loaded tibiae compared to intact tibiae (Figs. 2 and 3). In contrast, inserting a 10-s unloaded rest interval between each of 50 low-magnitude load cycles (Low–10 s Rest) increased MS (over 200%), MAR (nearly 300%), and BFR (400%) compared to that observed in intact tibiae ($P < 0.01$ for MS, MAR, and BFR). Histological sections displayed distinct double calcein labels indicative of lamellar bone formation with no evidence for woven bone (Fig. 2). In experimentally loaded tibiae, while MS was not significantly different between groups, low-magnitude rest-inserted loading (Low–10 s Rest) increased both MAR ($P = 0.02$) and BFR ($P = 0.04$) over 200% compared to low-magnitude cyclic loading (Low). In contrast to the ability of low-magnitude rest-inserted loading to enhance MS, MAR, and BFR, the static histomorphometry parameters (i.e., Ct.Ar, Ps.Ar, Ec.Ar, Ct.Th) were not significantly altered compared with both intact control and low-magnitude loading (data not shown).

Enhancing bone formation in the aged skeleton via high-magnitude rest-inserted loading

At the endocortical surface in intact contralateral tibiae (left), none of the dynamic histomorphometry parameters (MS, MAR, BFR) was significantly different across groups (Table 3). Peak endocortical surface strain induced by high-magnitude loading was significantly enhanced compared to the low-magnitude loading ($P < 0.001$; Table 1). Within the high-magnitude loading groups, while peak endocortical strains induced in groups receiving rest-inserted versus cyclic loading was not significantly different, strains induced in the High–20 s Rest and High–10 s Rest, 250 cycles/day groups were significantly different from the High–10 s Rest group. In terms of bone responses to these loading protocols, 50 cycles of high-magnitude loading (High) significantly increased MS ($P = 0.01$), MAR ($P = 0.04$), and BFR ($P < 0.01$) at the endocortical surface of experimentally loaded tibiae compared to intact tibiae. Inserting 10 s

Table 2
Initiating bone responses at the endocortical surface via low-magnitude loading with and without rest insertion

		Ec.MS (%)	Ec.MAR ($\mu\text{m}/\text{day}$)	Ec.BFR ($\mu\text{m}^3/\mu\text{m}^2/\text{day}$)
Low	Intact	22.45 \pm 4.19	0.83 \pm 0.17	0.23 \pm 0.08
	Load	25.06 \pm 5.34	0.79 \pm 0.10	0.20 \pm 0.04
Low–10s Rest	Intact	24.17 \pm 5.17	0.67 \pm 0.12	0.20 \pm 0.06
	Load	21.28 \pm 3.53	0.68 \pm 0.10	0.15 \pm 0.03

Note. Dynamic histomorphometry parameters at the endocortical surface (mean \pm SE) from animals subject to low-magnitude (Low) and low-magnitude rest-inserted loading (Low–10 s Rest). None of the parameters (in either group) was significantly different between experimentally loaded and intact bones.

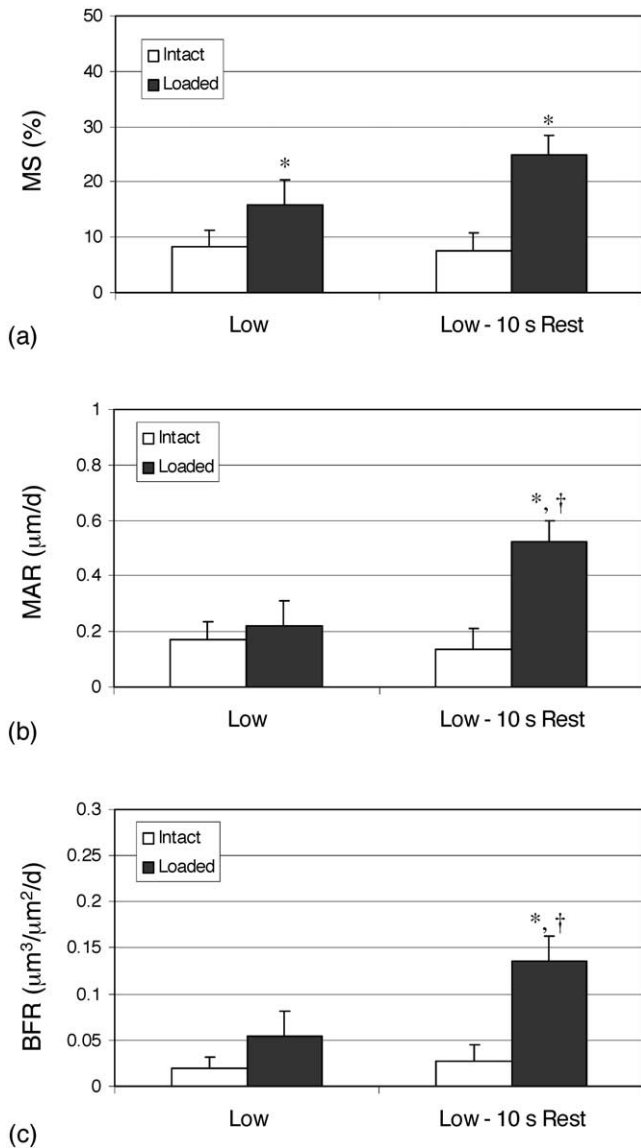


Fig. 3. Initiating bone formation at the periosteal surface of the aged-skeleton via low-magnitude rest-inserted loading. Low-magnitude loading (Low) significantly (*) influenced MS (a) but not MAR (b) and BFR (c) compared to intact contralateral controls (mean \pm SE). Low-magnitude rest-inserted loading significantly (*) enhanced MS, MAR, and BFR compared to intact contralateral controls. Last, low-magnitude rest-inserted loading significantly (†) enhanced both MAR and BFR (but not MS) compared to low-magnitude cyclic loading.

(High–10 s Rest) and 20 s (High–20 s Rest) of rest between each of 50 high-magnitude load cycles significantly increased MAR ($P \leq 0.04$) and BFR ($P = 0.03$) compared to intact tibiae. Subjecting experimental tibiae to 250 cycles/day of high-magnitude rest-inserted loading (High–10 s Rest, 250 cycles/day) significantly increased MS, MAR, and BFR compared with intact tibiae ($P \leq 0.01$). In experimentally loaded tibiae, none of the histomorphometry parameters (MS, MAR, BFR) was significantly different across these groups and when compared with low-magnitude rest-inserted loading.

At the periosteal surface in intact contralateral tibiae, none of the histomorphometry parameters was significantly different across groups (Fig. 4). While peak periosteal surface strains induced by high-magnitude loading were significantly enhanced compared to the low-magnitude loading ($P < 0.001$; Table 1), strains induced within the high-magnitude loading groups were not significantly different. In terms of bone responses, doubling-induced strain magnitudes (High) increased MS (over 100%), MAR (over 200%), and BFR (over 300%) in experimental compared with intact tibiae ($P < 0.01$ for MS, MAR, and BFR). Inserting 10 s of rest (High–10 s Rest) or 20 s of rest (High–20 s Rest) between each of 50 high-magnitude load cycles or extending the number of 10-s rest-inserted load cycles five-fold (High–10 s Rest, 250 cycles/day) significantly increased MS, MAR, and BFR at the periosteal surface of experimentally loaded tibiae compared with intact tibiae ($P \leq 0.02$). In experimentally loaded bones, none of the histomorphometry parameters (MS, MAR, BFR) was significantly different across these groups and when compared with low-magnitude rest-inserted loading. Last, none of the static histomorphometry parameters (i.e., Ct.Ar, Ps.Ar, Ct.Th) was significantly different within groups (i.e., loaded vs intact) and between groups (data not shown).

Discussion

In this study, we hypothesized that inserting rest between load cycles will initiate and enhance bone formation in the aged skeleton. We tested our hypothesis in the aged murine model by examining whether (a) insertion of rest between low-magnitude load cycles initiates bone formation in the aged skeleton and (b) bone formation response in the aged skeleton is further enhanced when strain magnitude, rest interval, and load cycle number were increased. In terms of primary outcomes, low-magnitude cyclic loading did not initiate periosteal bone formation in the aged skeleton. In contrast, inserting 10 s of rest between each low-magnitude load cycle enabled initiation of significant periosteal bone formation. However, attempts to further enhance bone formation responses by subjecting the aged skeleton to high-magnitude loading (strain magnitude doubled) and inserting 10 s rest, inserting 20 s of rest, and utilizing five-fold the number of high-magnitude rest-inserted (10 s) loading cycles were all ineffective and reflected a marked blunting of the osteogenic response to otherwise potent mechanical stimuli.

In these experiments, we subjected aged female C57BL/6 mice (average age: 21.5 months) to low- and high-magnitude loading protocols. In C57BL/6 mice, cortical bone morphology and material properties attain their peak by 6 months of age [34], and by 28 months exhibit changes in bone morphology and structure typical of aging-related osteopenia [22]. Importantly, the osteogenic response to mechanical stimuli is suppressed beyond 17

Table 3
Enhancing aged bone response at the endocortical surface via high-magnitude loading with and without rest insertion

		Ec.MS (%)	Ec.MAR ($\mu\text{m}/\text{day}$)	Ec.BFR ($\mu\text{m}^3/\mu\text{m}^2/\text{day}$)
High	Intact	19.09 \pm 4.90	0.63 \pm 0.12	0.15 \pm 0.04
	Load	29.97 \pm 5.22	0.87 \pm 0.13	0.28 \pm 0.06
High–10 s Rest	Intact	22.27 \pm 6.45	0.66 \pm 0.11	0.19 \pm 0.07
	Load	29.87 \pm 5.89	0.87 \pm 0.11	0.29 \pm 0.08
High–20 s Rest	Intact	22.05 \pm 4.98	0.62 \pm 0.13	0.16 \pm 0.05
	Load	38.47 \pm 5.80	1.09 \pm 0.09	0.42 \pm 0.07
High–10 s Rest, 250 cycles/day	Intact	09.46 \pm 3.59	0.45 \pm 0.12	0.05 \pm 0.03
	Load	28.08 \pm 4.21	0.92 \pm 0.14	0.25 \pm 0.05

Note. Dynamic histomorphometry parameters at the endocortical surface (mean \pm SE) from animals subject to high-magnitude loading with and without rest insertion between load cycles. Responses significantly different ($P \leq 0.05$) between loaded and intact bones are shown in boldface.

months of age; 50% mortality is attained by 24 months and in general, mice at 24 months are considered senescent [35,36]. While recognizing fundamental differences between rodents and higher mammals, we believe the animals (21.5 months, C57BL/6 mice) included in these experiments are a reasonable model for the attenuated responsiveness of the aged skeleton to mechanical stimuli [13,14]. In these aged animals, we subjected the tibia to exogenous mechanical loading and induced strains representative of “low-” and “high-” magnitude functional loading events. While functional strains induced within the murine tibia are unknown, our loading protocols are within the range of strain frequency (i.e., 1 Hz) and magnitudes (i.e., 1200, 2400 $\mu\epsilon$) recorded in long bones of animals and humans during mild (e.g., walking) and strenuous (e.g., sprinting or jumping) functional activity [37,38]. As well, the current protocols are within the strains recently recorded in mouse ulnae during functional activity [39]. Given the dynamic strain similarity between vertebrates [40], it is likely that the protocol-specific strains (Low, 1200 $\mu\epsilon$; High, 2400 $\mu\epsilon$) are at the low and high end of the range induced in the murine tibia during functional activity (e.g., from cage ambulation to jumping off a ledge).

In these aged animals, we observed that low-magnitude cyclic loading, while sufficient to enhance periosteal mineralizing surface, was ineffective in initiating bone formation (at both endocortical and periosteal surfaces). This result is similar to previous reports suggesting that mild or moderate types of activity, while potentially sufficient to mitigate bone loss, are ineffective in initiating de novo bone formation or enhancing bone mass in the aged skeleton [12,41]. Nevertheless, the aged skeleton retains the ability to respond if exposed to a stimulus perceived as anabolic, considering that doubling-induced strain magnitudes initiated bone formation. Interestingly, low-magnitude rest-inserted loading was also similarly effective and sufficient to significantly enhance periosteal bone formation in aged animals. However, both low-magnitude rest-inserted and high-magnitude loading were not sufficient to enhance bone areal properties within 2 weeks. Whether this inability reflects an inadequate rate of bone formation, the short term protocols

(2 weeks), and/or a “balance” between increased bone formation and enhanced (coupled) osteoclastic resorption is unclear. In the last context, the lack of difference in endocortical areas between loaded and intact bones suggests that the background levels of osteoclastic activity in these aged animals are minimally influenced by rest-inserted (and high-magnitude) loading. However, the influence of loading upon osteoclastic activity in aged animals may be more pronounced and manifest when current short-term (2-week) loading protocols are extended over longer durations.

Similar to previous observations [28,29], rest-inserted loading altered a low-magnitude, impotent regimen into an “above-threshold” stimulus that induced an osteogenic response in aged animals. This result, at first, appears counterintuitive considering that insertion of an unloaded rest interval between loading events represents a “passive” alteration of an impotent loading stimulus. However, insertion of rest could allow inertial effects associated with load-induced interstitial fluid flow to be overcome and thereby enhance fluid-flow-related stimulation of bone cells [27]. As well, our recent preliminary studies indicate that rest insertion between load cycles promotes enhanced signaling coordination within the network of mechanosensory osteocytic cells [42]. Either of these mechanisms associated with rest insertion could enable transformation of previously impotent mechanical loading events into anabolic stimuli. Further, while these mechanisms may operate synergistically, additional underlying mechanisms are also likely (e.g., modulation of accommodation [29,43], metabolic fatigue [44,45]).

Regardless, the osteogenic potential of rest-inserted loading, particularly at low strain magnitudes, appears to hold promise for implementation in the frail elderly. However, the rates of bone formation induced in aged animals by low-magnitude rest-inserted loading (1200 $\mu\epsilon$, 50 cycles/day, 10 s rest) was nearly 2.5-fold less than that elicited by a similar protocol in young adult C57BL/6 mice (21.5 months: 0.14 ± 0.03 , vs 4 months: $0.33 \pm 0.06 \mu\text{m}^3/\mu\text{m}^2/\text{day}$, $P < 0.01$) [28]. As well, in contrast to previous observations in adolescent mice [29], the induced rates of bone formation were not sufficient to influence bone areal prop-

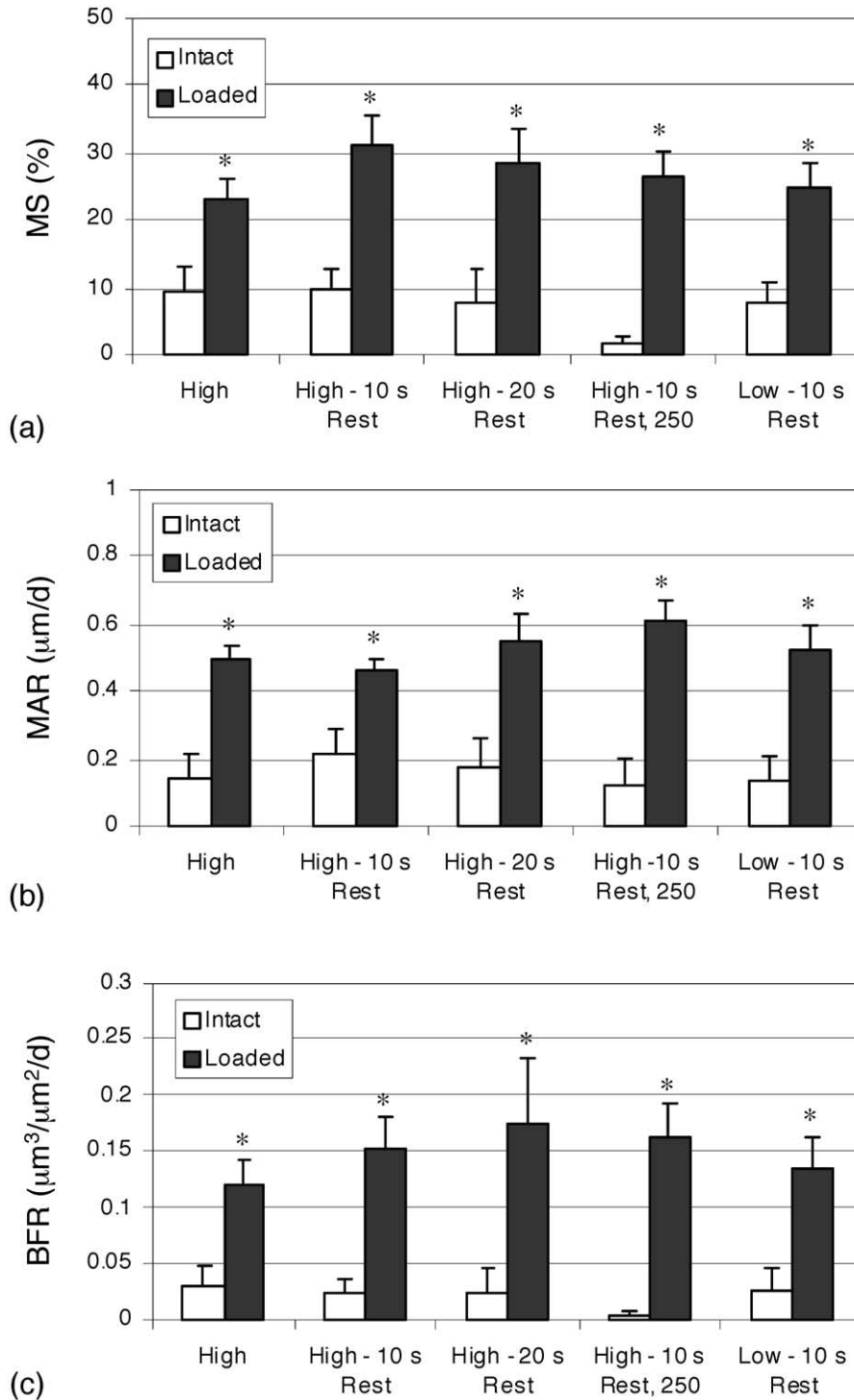


Fig. 4. Enhancing aged bone response at the periosteal surface by doubling strain magnitude, inserting a rest interval, and utilizing five-fold the number of rest-inserted loading cycles. High-magnitude cyclic loading, high-magnitude rest-inserted loading with 10 s and 20 s of rest inserted between 50 cycles, high-magnitude rest-inserted loading with 250 cycles/day, and low-magnitude 10 s rest-inserted loading enhanced MS (a), MAR (b), and BFR (c) significantly compared to intact contralateral bones (*mean \pm SE). However, in experimentally loaded tibiae (and in intact tibiae), MS, MAR, and BFR were not significantly different across groups.

erties (e.g., Ct.Ar, Ps.Ar, Ct.Th), albeit over 2-week protocols. For example, if the response induced (e.g., BFR $\sim 0.15 \mu\text{m}^3/\mu\text{m}^2/\text{day}$) were sustained over 8 weeks, bone areal properties could be enhanced by nearly 5%. However, even

in young adults, it is known that rates of bone formation decelerate over time as bone tissue adapts to exogenous loading [46]. While measures such as BFR only provide an indirect estimate of long-term outcomes, the preceding ob-

servations, especially in the context of the aged skeleton, suggest that robust increases in BFR are likely required in the short term to impact bone mass over the longer term.

We therefore examined whether doubling strain magnitude, inserting rest, and increasing the number of rest-inserted load cycles could further enhance bone formation response in aged animals. Attempts at further enhancing BFR by doubling strain magnitudes and inserting rest between these high-magnitude load cycles were ineffective in the aged skeleton (i.e., High and High-Rest groups not significantly different from Low-10 s Rest). This is especially surprising considering that increasing strain magnitudes by as little as 25% (and inserting rest between load cycles) resulted in substantial additional increases in the quantity of bone formation in young adult animals [28]. The similar BFR induced by protocols with 10 and 20 s of rest inserted between high-magnitude load cycles, as well as previous observations with rest-inserted stimuli [33,44], suggest that the above result was unlikely to be due lack of optimization of rest intervals. Last, the strategy of inserting rest, doubling strain magnitude, and utilizing five-fold the number of rest-inserted load cycles, potent in the young adult skeleton (nearly doubles BFR for five-fold increase in load cycle) [47], was ineffective in additionally enhancing bone formation in aged animals (i.e., High-10 s Rest, 250 cycles/day, and Low-10 s Rest, 50 cycles/day, not significantly different). We conclude that maximal bone formation in this aged model was attained via low-magnitude rest-inserted loading and the natural degradative processes of aging overwhelm attempts to further optimize and enhance this response.

Previous reports [28,47], as well as observations in this study, suggest that rest-inserted loading (particularly at low magnitudes) enhances rates of bone formation by primarily increasing mineral apposition rates compared to cyclic protocols. While measures such as MS and MAR only provide indirect information regarding osteoblastic activity, it appears that rest-inserted loading derives its potency by enhancing the biosynthetic activity of teams of osteoblasts (as opposed to expanding the surface over which osteoblasts are involved in biosynthetic activity). In this context, the blunting of the osteogenic response to otherwise potent loading signal alterations (i.e., doubling strain magnitude, inserting rest, five-fold number of load cycles) more likely occurred via age-related potential for deficits in osteoblastic function [19,21] and propensity to undergo apoptosis [18,20]. As well, age-related declines in biomolecules involved in mechanotransduction (e.g., IGF-1, TGF- β) may exacerbate or underlie these osteoblastic deficits [23,24]. However, the ability of low-magnitude rest-inserted loading to initiate bone formation in aged animals holds promise as a minimally invasive strategy to enhance structural properties in the aged skeleton. For example, if rest-inserted loading was to be supplemented with low-dose pharmacological agents (e.g., IGF-1, PTH) [48,49] that selectively counteract osteoblastic deficits, bone formation could thereby be initiated, enhanced, and sustained sufficiently to augment aged bone mass in a structurally significant fashion. In support of this

proposal, our preliminary data in senescent mice show that low-magnitude rest-inserted loading, when supplemented with IGF-1 even at low doses, synergistically enhances bone formation [50].

In summary, similar to previous reports [11,12], low-magnitude cyclic loading was not sufficient to initiate bone formation in the aged skeleton. Inserting 10 s of rest between each of these low-magnitude load cycles transformed this impotent regimen and initiated significant periosteal bone formation (five-fold vs control and over two-fold vs low-magnitude loading). However, attempts at further enhancing the bone formation response to rest-inserted loading by doubling strain magnitude, inserting rest-intervals, and subjecting animals to five-fold the number of high-magnitude rest-inserted loading cycles were all ineffective in the aged skeleton. This age-related suppression of the osteogenic response is similar to previous reports in the context of cyclic mechanical stimuli [13,14]. Notwithstanding these observations, the strategy of rest-insertion does permit use of low-magnitude and mild loading events to, at the very least, initiate *de novo* bone formation activity in the aged skeleton. If these mild regimens were supplemented with cytokines that explicitly counter age-related osteoblastic deficits, such a combination could present a potent and readily implemented strategy to enhance and sustain bone formation activity in the elderly.

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