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32 wk old C3H/HeJ mice actively respond to mechanical loading

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Abstract

Numerous studies indicate that C3H/HeJ (C3H) mice are mildly responsive to mechanical loading compared to C57BL/6J (C57) mice. Guided by data indicating high baseline periosteal osteoblast activity in 16 wk C3H mice, we speculated that simply allowing the C3H mice to age until basal periosteal bone formation was equivalent to that of 16 wk C57 mice would restore mechanoresponsiveness in C3H mice. We tested this hypothesis by subjecting the right tibiae of 32 wk old C3H mice and 16 wk old C57 mice to low magnitude rest-inserted loading (peak strain: 1235 με) and then exposing the right tibiae of 32 wk C3H mice to low (1085 με) or moderate (1875 με) magnitude cyclic loading. The osteoblastic response to loading on the endocortical and periosteal surfaces was evaluated via dynamic histomorphometry. At 32 wk of age, C3H mice responded to low magnitude rest-inserted loading with significantly elevated periosteal mineralizing surface, mineral apposition rate and bone formation compared to unloaded contralateral bones. Surprisingly, the periosteal bone formation induced by low magnitude rest-inserted loading in C3H mice exceeded that induced in 16 wk C57 mice. At 32 wk of age, C3H mice also demonstrated an elevated response to increased magnitudes of cyclic loading. We conclude that a high level of basal osteoblast function in 16 wk C3H mice appears to overwhelm the ability of the tissue to respond to an otherwise anabolic mechanical loading stimulus. However, when basal surface osteoblast activity is equivalent to that of 16 wk C57 mice, C3H mice demonstrate a clear ability to respond to either rest-inserted or cyclic loading.

Keywords: Bone formation; Osteoblast activity; Mechanical loading; C3H/HeJ; C57BL/6J

Introduction

Beginning with the earliest stages of skeletal development through the attainment of peak bone mass and maintenance of homeostasis during adulthood, genetic and environmental factors interact to modulate skeletal morphology [1–3]. Given their relative ease of genetic manipulation, mice provide an effective model system to explore how specific genetic and environmental factors might influence these biologic processes. This is particularly due to the availability of transgenic and knockout mice that have defined numerous proteins and cytokines regulating skeletal phenotype, as well as availability of in-bred and cross-bred strains with distinct skeletal morphologies [4–11].

Within the realm of environmental influences, mechanical loading can serve as a powerful anabolic stimulus for bone following completion of skeletal development [12]. However, in human exercise trials augmentation of bone mass is modest and highly variable [13–16]. Observations that different genetic strains of mice vary greatly in their ability to respond to mechanical loading suggest that genetic variations in the ability of the skeleton to perceive and respond to mechanical loading may account for the equivocal results of exercise trials [17–23]. Consequently, it has been reasonably argued that a better understanding of the genetic contribution to bone's response to mechanical loading would enable tailored design of exercise interventions such that the beneficial effects of skeletal loading are realized across a diverse population [18].

The availability of various in-bred mouse strains facilitates a study of genetic interactions with mechanical loading. C3H/HeJ (C3H) mice in particular have been found to be less responsive to mechanical loading than other strains of mice (e.g., C57BL/

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6J (C57)). In a variety of studies, C3H mice have been found to be unresponsive to low magnitude loading, have consistently required greater magnitudes of mechanical stimulation to induce any anabolic response, and when induced, observed responses are muted compared to other strains of mice [17–19,24,25]. These studies have spawned a number of trait mapping efforts to identify specific genetic loci responsible for deficient mechanotransduction in C3H mice [26–31], as well as attempts to isolate specific alterations in downstream bone cell function underlying the muted tissue level responses [32–34].

Recently, our group reported in a preliminary study that inserting a 10 s rest period between each load cycle of a cyclic loading regimen significantly elevated bone's response to low magnitude mechanical loading in C57 mice [35]. The surprising anabolic effect of inserting a brief zero load interval between each load cycle was subsequently confirmed by our group and others [36–39]. At the tissue level, we have found that low magnitude rest-inserted loading is even capable of stimulating periosteal bone formation in senescent animals [40]. Given that rest-inserted loading is so stimulatory for surface osteoblast activity, we hypothesized that rest-inserted loading may provide a means of overcoming a genetic predisposition toward decreased mechanoresponsiveness in C3H mice. To test this hypothesis, we performed a series of *in vivo* experiments in which we exogenously loaded the tibiae of C3H and C57 mice. First, we assessed the response of 16 wk C3H mice (an age comparable to previous studies) to rest-inserted loading of a magnitude that is sufficient to induce prolific periosteal bone formation in 16 wk C57 mice. While the regimen did not alter periosteal bone formation rates compared to contralateral non-loaded tibiae, we noted high levels of basal periosteal osteoblast activity in the C3H mice. As it is difficult to imagine how an osteogenic mechanical loading regimen would further augment an already extremely high level of periosteal osteoblast activity (e.g., 55% periosteal mineralizing surface (Ps.MS, [41]) in 16 wk C3H mice [17]), we speculated that if C3H mice were aged until basal periosteal osteoblast activity was equivalently low to that found in 16 wk C57 mice, then the skeletal response to anabolic mechanical loading would be restored. To test this hypothesis, we eliminated the potentially confounding influence of high basal periosteal activity in the experiments by using C3H mice at an age (32 wk) where their basal periosteal bone formation was equivalent to that of 16 wk C57 mice. We first assessed whether low magnitude rest-inserted loading would elevate periosteal bone formation in C3H and C57 mice. We then examined whether 32 wk C3H mice would respond to two different magnitudes of repetitive cyclic loading to assess whether the observed response was due entirely to rest-inserted loading.

Materials and methods

Age-Matched Study

In this study, 16 wk C3H mice ($n=5$) underwent 50 cycles of loading with a 10 s rest interval inserted between each load cycle. Based upon a previous study reporting cross-sectional areas of 16 wk C3H mice [42], we estimated a priori loading regimen that would induce peak periosteal normal strains of 2200 με. This magnitude of waveform was 40% greater than a rest-inserted waveform that induced significant periosteal bone formation rate (Ps.BFR) in 16 wk C57 mice [43]. Post-hoc animal specific estimates of induced strains were determined using beam theory (as described in Calibration of induced normal strains).

Because we noted a high level of baseline osteoblastic activity in 16 wk C3H mice during this study, we evaluated basal osteoblast activity as C3H mice aged in order to equilibrate it to the baseline activity of 16 wk C57 mice. A C3H mouse was sacrificed every 2 wk following 16 wk of age and dynamic histomorphometry was used to determine Ps.BFR/BS at the tibia mid-diaphysis. We found that Ps.BFR/BS in C3H mice at 32 wk of age was equivalent to within 5% of that observed in 16 wk C57 mice in our previous studies [43]. C3H mice at 32 wk of age were therefore used in the following studies to examine if the C3H strain was capable of responding to low and moderate magnitude mechanical stimuli without the confounding influence of high basal surface osteoblast activity.

Rest-Inserted Study

The Rest-Inserted Study contrasted the response of 32 wk old C3H mice and 16 wk old C57 mice to rest-inserted loading protocols that induced equivalent peak periosteal normal strains. Groups of 32 wk old C3H mice ($n=8$) and 16 wk C57 mice ($n=8$) were exposed to 50 cycles/d of low magnitude loading with a 10 s unloaded rest interval inserted between each load cycle. Applied end loads were established a priori from calibration experiments such that peak periosteal normal strains of 1200 με would be equivalently induced across groups.

Cyclic Study

We next explored whether two strain magnitudes of repetitive cyclic loading (1 Hz, no rest-intervals) would stimulate bone formation in 32 wk old C3H mice. The loading protocols applied 50 cycles/d using end loads that, based on preliminary calibrations, were anticipated to induce peak periosteal normal strains of either 1200 με or 1900 με (termed Low and Moderate magnitudes, respectively).

In vivo mechanical loading

We mechanically loaded the right tibiae of female mice with a non-invasive device that applies cantilever bending to the tibia [44]. For each bout of loading, the mouse was anesthetized (2% isoflurane) and, proximal to the tibial crest, the right tibia was gripped between a brass block on the lateral side and a brass gripping cup on the medial side. An aluminum loading tine attached to a computer controlled linear actuator applied force to the lateral distal tibia, placing the tibia in cantilever bending. Digital signals controlled the strain rate (0.01/s), applied force and a 1 s load cycle (1-Hz trapezoidal waveform with dwell times at peak load decreased for increased magnitude loading such that strain rate was equivalent across protocols). Normal cage activity was allowed between loading sessions. Loading was applied 3 d/wk beginning on day 1, with contralateral tibiae serving as non-externally loaded controls. Calcein (15 mg/kg IP) was administered on days 10 and 19 and all mice were sacrificed on day 22. Experimental procedures were approved by the University of Washington Institutional Animal Care and Use Committee (IACUC).

Calibration of induced normal strains

The strain environment induced by our murine tibia loading device is non-uniform due to the irregular geometry of the tibia and the applied cantilever bending [44]. In this study, a further complication arose in that the cross-sectional tibia morphology of C3H and C57 mice differ substantially. As a result, it was not possible to simultaneously induce equivalent endocortical and periosteal normal strains in C3H and C57 mice with the same loading protocol. Given the primary importance of periosteal osteoblast function as a means of increasing bone's mechanical properties, we focused on inducing equivalent periosteal normal strains between C3H and C57 mice. A combination of *in situ* strain gage application and finite element modeling was used to quantify peak periosteal and endocortical normal strains at the tibia mid-diaphysis. One 16 wk C57 mouse and one 32 wk C3H mouse were utilized for calibration of induced