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Genetic variations that regulate bone morphology in the male mouse skeleton do not define its susceptibility to mechanical unloading

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Abstract

Genetics can substantially influence bone morphology and may define the skeleton's response to mechanical unloading. Recent data indicated that disuse produces different site-specific responses in the skeleton of genetically distinct adult *female* C3H/HeJ (C3H) and BALB/cByJ (BALB) mice; while disuse BALB mice had significantly less bone than age-matched controls in the distal and diaphyseal femur, the removal of weight bearing had a much smaller influence in C3H. Using adult *male* mice from these two inbred strains, the hypothesis was tested that interactions between genetic variations and anatomic location define bone morphology and its susceptibility to unloading. Four-month-old male BALB and C3H mice were either subjected to 21 days of hindlimb unloading or served as controls. Multiple cortical and trabecular regions within the distal and diaphyseal femur were analyzed by micro-computed tomography. C3H controls had significantly greater diaphyseal and metaphyseal cortical bone area (45% and 32%) and greater metaphyseal trabecular bone volume fraction (67%) than BALB controls, but epiphyseal trabecular bone volume fraction was similar between the two strains. Despite these substantial, site-specific differences in bone morphology, disuse induced similar changes in bone morphology in these two strains. Compared to controls, disuse BALB and C3H had significantly less metaphyseal (17% and 19%) and epiphyseal (10% and 13%) trabecular bone, while diaphyseal and metaphyseal cortical bone geometry was unaffected. These data indicate that the genetic variations that caused spatially nonuniform differences in trabecular and cortical bone morphology between the two strains had little influence on the susceptibility of a specific site to unloading. Cross-gender comparisons with previous data from female BALB and C3H mice further suggest strong interactions by which gender, genotype, and anatomical location define the response of the skeleton to the removal of weight bearing.

Keywords: Trabecular bone; Cortical bone; μCT scanning; Hindlimb unloading; Gender

Introduction

Peak bone mineral density (BMD) as well as the degree by which the skeleton responds to aging, menopause, or mechanical unloading, define an individual's susceptibility to osteoporosis [1–4]. Identification of the factors that may control these two primary predictors of osteoporosis, including their potentially complex interactions, will be critical for the development and optimization of diagnostics and treatment interventions. While the majority of variations in BMD are modulated by genetic make-up [5–10], the relative contribution of genetics to the attainment of BMD may vary between anatomic sites [11] and can also be influenced by gender [11,12]. Given the dominance of genetics in the pool of factors that influence peak BMD, it is not surprising that the skeleton's sensitivity to disuse is also influenced by the genome. This relation may be reflected in the up to 10-fold differences in individual rates of bone loss both on Earth and in space. For instance, losses of trabecular BMD in patients confined to bed rest vary from 0.3% to 2.3% after 17 weeks [13] and the magnitude of bone wasting in astronauts returning to earth after 6-month space missions ranges from 0.4% to 23.4% for tibial trabecular BMD and 0–4% for tibial

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cortical bone [14,15]. These data highlight the influence of genetics on bone morphology and its susceptibility to the loss of weight bearing, but critical interactions by which genetics, gender, and anatomical location affect these two processes are difficult to identify in heterogeneous human populations.

Inbred strains of mice have been used successfully to describe the influence of genetics and gender on skeletal morphology [16–19] and its propensity to develop osteopenia [20–23]. The degree of site specificity by which genetic variations define cortical and trabecular bone morphology is high, with large differences between adjacent regions even within a single bone [18]. Consistent with observations in humans, genetic variations also affect bone's mechanosensitivity in mice [20,21,24–26]. For example, trabecular and cortical bone of female BALB mice is highly sensitive to mechanical unloading in a site-specific manner [20] while the skeleton of female C3H mice appears to be much less responsive [20,24]. Investigations into the influence of gender on bone's response to disuse have yielded equivocal results with many studies unable to observe gender effects [22,23,27]. While some of the discrepancies may lie with the different age of the animals, it is entirely possible that gender effects are co-regulated by genetics and anatomical location and, therefore, may have escaped detection in some studies.

Here, using males from two mouse strains in which we previously determined site-specific differences in bone morphology and a dramatically different sensitivity to the loss of functional weight bearing in females, we asked the following questions: (1) Are genetic effects on bone quantity and architecture site-specific within a single bone of the male skeleton? (2) Are (site-specific) alterations in bone morphology induced by disuse related to indices of bone morphology within and/or across strains? (3) By cross-comparing to previous data from the female skeleton, are interrelations evident between gender, genetics, and anatomical location in defining bone morphology and its susceptibility to disuse?

Materials and methods

Experimental design

Sixteen-week-old male BALB/cByJ (BALB) and C3H/HeJ (C3H) mice (Jackson Laboratory, Bar Harbor, ME) were randomly assigned to age-matched control (n = 11 BALB and n = 10 C3H) and disuse (n = 11 BALB and n = 10 C3H) groups. Skeletal maturity in (female) mice, as indicated by the attainment of peak bone mass, is typically achieved by 4 months of age [17]. To document potential changes in bone morphology during the 3-week experimental protocol, a second set of male BALB (n = 9) and C3H (n = 8) mice served as baseline controls. These mice were obtained from the same supplier, raised under identical conditions, and sacrificed at 16 weeks of age.

Disuse mice were subjected to 21 days of hindlimb unloading using a model modified from the established Morey–Holton rat tail suspension model [28]. All mice were individually housed in standard cages at 24°C (±1°C) and allowed free access to standard rodent chow and tap water. Weights for all animals were recorded at the beginning of the study and monitored throughout the experiment. At sacrifice, right femurs were harvested and preserved in 70% ethanol at 4°C. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the State University of New York at Stony Brook.

Femur length

High precision calipers (Mitutoyo Inc., Japan) were used to measure femoral length of all baseline and age-matched control mice of both strains. Each femur was measured three times and mean lengths were recorded.

Microcomputed tomography

Indices of cortical and trabecular bone morphology of the distal and diaphyseal femur were assessed by microcomputed tomography (μCT 40, Scanco Medical, SUI). A 1500-μm region containing the distal femoral metaphysis, a 450-μm region containing the distal femoral epiphysis, and three 224-μm regions of the femoral diaphysis were scanned at a resolution of 12 μm. The metaphyseal region of interest started 410-μm proximal of the physeal–metaphyseal junction and extended 1500 μm proximally, which maximized the amount of metaphyseal trabecular bone present across all specimens. Similarly, the region of interest chosen for the epiphysis maximized the trabecular bone volume present and encompassed trabecular bone from the junction of the medial and lateral femoral condyles to a slice 450-μm proximal. The three regions of diaphyseal cortical bone were chosen to obtain representative segments of cortical bone in which trabecular bone was absent, and included proximal, mid, and distal diaphyseal sections centered at 60%, 50%, and 40% of the femur length, respectively.

A Gaussian filter removed noise from the μCT images [29]. Local thresholding, rather than global thresholding [30], which failed to correctly reconstruct trabecular and cortical bone morphology across all sites, separated bone from non-bone [18]. Segmentation thresholds were determined empirically from multiple slices within a given bone in both strains of control and disuse mice [18] to achieve realistic bone thickness without compromising trabecular connectedness. Threshold reproducibility for this method is high [18].

Trabecular bone analyses

Morphometric parameters including bone volume fraction (BV/TV), connectivity density, (Conn.D), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp) were determined in all mice for the metaphyseal and epiphyseal region of the distal femur. To measure

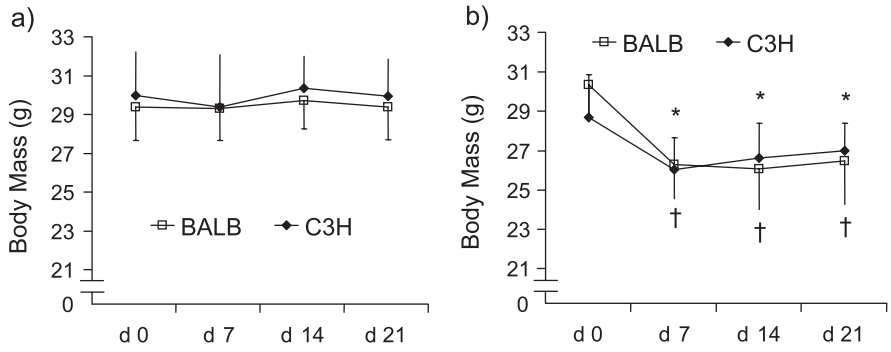

Fig. 1. Body mass (mean ± SD) at the start of the experiment and after 7, 14, and 21 days of the experimental protocol in (a) control BALB and C3H mice and (b) disuse BALB and C3H mice. † = significant difference between BALB control and disuse. * = significant difference between C3H control and disuse.

differences in growth plate closure between baseline and age-matched controls, sagittal CT-slices were rendered to visualize the physeal growth plate in the distal femur. Bone bridges spanning the growth plate [31] were averaged across 20 bone slices in each sample.

Cortical bone analyses

Cortical, periosteal, and endocortical areas (Ct.Ar, Ps.Ar, and Ec.Ar) were determined for the metaphyseal and the three diaphyseal cortices. Customized software routines (MATLAB, The MathWorks Inc., MA) calculated principal and polar moments of inertia (I_{max}, I_{min}, and I_p, respectively) and section moduli (Z_{max} and Z_{min}) [32–34] at the midpoint of each region.

Statistics

Comparisons of body mass between age-matched control and disuse mice at a given time point included unpaired *t* tests (two-tailed) while initial and final body mass within each group of mice were compared with paired *t* tests (two-tailed). Unpaired *t* tests (two-tailed) also tested for differences in bone morphology between age-matched control mice across the two strains and between age-matched control and disuse mice within each strain. Differences between baseline control (16 weeks) and age-matched control (19 weeks) mice within each strain were evaluated by *t* tests as this comparison served primarily to evaluate differences in the maturity level between these two groups. For this analysis, bone morphological parameters of BALB baseline controls were corrected for their 10% ($P < 0.01$) greater body mass (via linear regressions for each anatomical region). Age-matched, rather than baseline, controls of the two strains were used to delineate genetic effects on bone morphology in the young adult (4 months) skeleton because these mice were siblings of the disuse animals and their sample size was larger. All data were presented as mean ± SD, and differences were considered significant at a level of 0.05.

Results

Body mass

At the start of the hindlimb unloading protocol, there were no significant differences in body mass between age-matched control and disuse mice for either BALB or C3H (Fig. 1). Body mass of age-matched control BALB and C3H animals did not fluctuate significantly throughout the duration of the

Table 1
Morphological indices of metaphyseal and epiphyseal trabecular bone in age-matched control and disuse BALB (n = 11 each) and C3H (n = 10 each) mice (mean ± SD)

		BALB		C3H	
		Age-matched control	Disuse	Age-matched control	Disuse
Metaphysis	BV/TV [%]	9.7 ± 2.0†	8.0 ± 1.7*	16.2 ± 3.0†	13.1 ± 2.8*
	Tb.Th [μm]	35.6 ± 2.3†	35.6 ± 2.7	54.8 ± 2.4†	51.7 ± 3.8
	Tb.N [1/mm]	4.8 ± 0.3†	4.5 ± 0.4	4.4 ± 0.4†	4.0 ± 0.4*
	Tb.Sp [mm]	0.21 ± 0.01	0.22 ± 0.02	0.22 ± 0.02	0.24 ± 0.02*
	Conn.D [1/mm^3]	144 ± 43†	100 ± 42*	101 ± 26†	79 ± 26
Epiphysis	BV/TV [%]	29.5 ± 2.3	26.4 ± 2.2*	29.0 ± 2.5	25.0 ± 2.7*
	Tb.Th [μm]	49.1 ± 2.8†	48.3 ± 2.7	67.5 ± 2.6†	62.7 ± 3.6
	Tb.N [1/mm]	7.1 ± 0.4†	6.7 ± 0.2*	6.0 ± 0.4†	5.5 ± 0.4*
	Tb.Sp [mm]	0.14 ± 0.01†	0.15 ± 0.00*	0.17 ± 0.01†	0.19 ± 0.01*
	Conn.D [1/mm^3]	249 ± 36†	246 ± 17	92 ± 17†	96 ± 11

* Significant difference between age-matched control and disuse mice within a given strain.
† Significant difference between age-matched control BALB and age-matched control C3H mice.

21-day experiment. In disuse BALB and C3H mice, body mass decreased sharply at the beginning of the experiment (13% and 9%, $P < 0.001$, after the first experimental week). Despite a gradual gain of body mass during the second half of the experiment, final body mass of disuse BALB and C3H was significantly smaller than their initial values body mass after 21 days of unloading (13% and 6%, $P < 0.01$ each). Sixteen-week-old baseline control C3H mice had similar body mass compared to age-matched controls but baseline control BALB mice were 10% heavier ($P < 0.01$ each) than age-matched controls.

Genetic effects on bone quantity and architecture

Genetic differences between the two strains were associated with substantial but site-specific differences in trabecular bone morphology. Male BALB controls had 40% smaller ($P < 0.01$) metaphyseal trabecular BV/TV than C3H controls (Table 1; Fig. 2), but epiphyseal BV/TV did not significantly differ between the two strains. The two strains were also characterized by a significantly different ($P < 0.02$ each) trabecular microarchitecture in the metaphysis and epiphysis; BALB had lower Tb.Th (35% for metaphysis, 28% for epiphysis), higher Tb.N (8%, 20%), and higher Conn.D (44%, 168%) than C3H (Table 1).

Strain-specific differences in bone geometry were also evident for cortical bone but the genetic effect across the four cortical regions was spatially much more uniform than for the trabecular regions (Table 2). Cortical bone area (Ct.Ar) was lower ($P < 0.01$ each) in the metaphysis (25%) and in each of the three diaphyseal regions (31%; morphological parameters were averaged across the three diaphyseal regions as the influence of genetics on bone morphology was uniform in the diaphysis) of BALB when compared to C3H. The smaller cortical bone area in BALB was associated with smaller Ps.Ar (5% in metaphysis, $P < 0.05$ and 14% in diaphysis, $P < 0.001$) and larger Ec.Ar (7% and 37%, $P < 0.05$ each). Differences in cortical bone geometry between BALB and C3H caused significantly smaller ($P < 0.01$) second moments of area and section moduli in the metaphysis and diaphysis; these differences ranged from 18% to 37% (Table 2).

Effect of age on bone quantity and architecture

Comparisons between baseline and age-matched control mice were used to assess potential alterations in bone morphology and the level of maturity during the 3-week experimental period. In both strains of mice, there were no significant differences between baseline and age-matched

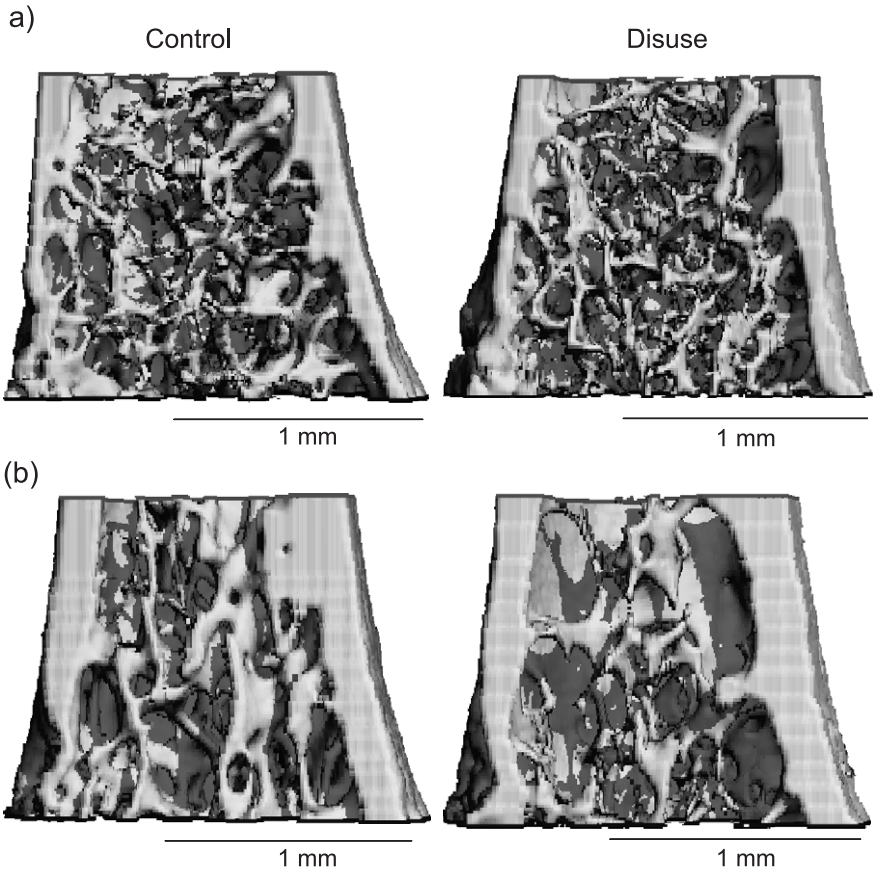

Fig. 2. Micro-computed tomography (12 μm) images of a control (left panel) and disuse (right panel) (a) BALB and (b) C3H mouse depicting differences in trabecular bone quantity and architecture in the metaphysis.

Table 2
Indices of metaphyseal and diaphyseal cortical bone area (Ct.Ar), periosteal (Ps.Ar) and endocortical area (Ec.Ar), principal and polar moments of inertia (I_{max}, I_{min}, and I_p), and section moduli (Z_{max} and Z_{min}) in age-matched control and disuse BALB and C3H mice (mean ± SD)

		BALB		C3H	
		Age-matched control	Disuse	Age-matched control	Disuse
Metaphysis	Ct.Ar (mm^2)	0.84 ± 0.04†	0.83 ± 0.05	1.12 ± 0.06†	1.12 ± 0.04
	Ps.Ar (mm^2)	2.82 ± 0.14†	2.85 ± 0.23	2.98 ± 0.16†	2.99 ± 0.21
	Ec.Ar (mm^2)	1.98 ± 0.12†	2.02 ± 0.19	1.86 ± 0.13†	1.87 ± 0.20
	I_p (mm^4)	0.77 ± 0.07†	0.74 ± 0.10	0.98 ± 0.11†	1.00 ± 0.10
	I_{max} (mm^4)	0.54 ± 0.04†	0.51 ± 0.07	0.62 ± 0.14†	0.69 ± 0.07
	I_{min} (mm^4)	0.22 ± 0.03†	0.23 ± 0.03	0.35 ± 0.14†	0.31 ± 0.03
	Z_{max} (mm^3)	0.54 ± 0.04†	0.49 ± 0.06	0.65 ± 0.06†	0.68 ± 0.06
	Z_{min} (mm^3)	0.14 ± 0.02†	0.15 ± 0.02	0.20 ± 0.02†	0.20 ± 0.02
Diaphysis	Ct.Ar (mm^2)	0.95 ± 0.05†	0.96 ± 0.07	1.38 ± 0.09†	1.38 ± 0.08
	Ps.Ar (mm^2)	1.57 ± 0.09†	1.61 ± 0.11	1.83 ± 0.13†	1.84 ± 0.15
	Ec.Ar (mm^2)	0.62 ± 0.06†	0.64 ± 0.06	0.45 ± 0.06†	0.46 ± 0.08
	I_p (mm^4)	0.43 ± 0.05†	0.44 ± 0.06	0.61 ± 0.06†	0.60 ± 0.07
	I_{max} (mm^4)	0.32 ± 0.04†	0.32 ± 0.04	0.43 ± 0.05†	0.42 ± 0.05
	I_{min} (mm^4)	0.12 ± 0.01†	0.12 ± 0.02	0.18 ± 0.02†	0.18 ± 0.03
	Z_{max} (mm^3)	0.48 ± 0.05†	0.48 ± 0.05	0.62 ± 0.06†	0.60 ± 0.06
	Z_{min} (mm^3)	0.11 ± 0.01†	0.11 ± 0.01	0.17 ± 0.01†	0.16 ± 0.02

There were no significant differences between control and disuse mice within each strain. * = Significant difference between control and disuse mice within each strain.
† Significant difference between control BALB and C3H mice.

controls for femoral length (15.7 ± 0.3 mm each for BALB, 15.6 ± 0.5 mm vs. 15.8 ± 0.2 mm for C3H) or number of bone bridges in the physes (2.0 ± 0.5 vs. 1.9 ± 0.5 for BALB, 1.9 ± 0.3 vs. 2.2 ± 0.3 for C3H). Similarly, no differences in femoral morphology between baseline and age-matched control BALB and C3H were detected for any of the cortical and trabecular regions after correcting for the higher body mass in the added BALB baseline control group.

Disuse effects on bone quantity and architecture

Hindlimb unloading elicited a similar response in the two femoral trabecular regions of both strains (Table 1, Fig. 3). In the metaphysis, BALB and C3H disuse mice had significantly less trabecular bone volume (BV/TV) than their age-matched controls (17% and 19%, $P < 0.05$ each). Architecturally, disuse resulted in lower metaphyseal Conn.D (30%, $P < 0.03$) in BALB mice and lower metaphyseal Tb.N (8%, $P < 0.05$) and higher Tb.Sp (11%, $P < 0.05$) in C3H mice. Similar to the metaphysis, epiphyseal BV/TV was significantly lower in both disuse BALB (10%) and disuse C3H (13%) as compared to their age-matched controls ($P < 0.01$ each) (Table 1). Epiphyseal trabecular bone architecture was also significantly affected by the loss of weight bearing in BALB and C3H, as indicated by a 6% lower ($P < 0.03$) Tb.N and 7% greater ($P < 0.01$) Tb.Sp in disuse BALB and a 7% lower ($P < 0.05$) Tb.N, 7% lower ($P < 0.01$) TbTh, and 9% greater ($P < 0.05$) Tb.Sp in C3H. In contrast to trabecular bone, exposure to 21 days of hindlimb unloading did not significantly alter cortical morphology in the metaphysis or in any of the three diaphyseal regions in either strain of mice (Table 2). Cortical cross-sectional moments of area and section moduli were also similar between disuse and control mice from both strains of male mice (Table 2).

Across the two regions that responded to the removal of weight bearing (trabecular bone of the metaphysis and epiphysis) in both strains, no consistent relation between baseline bone morphology and its susceptibility to disuse were observed; epiphyseal BV/TV was greater than metaphyseal BV/TV in each strain but disuse induced differences in bone quantity were not significantly different between the two regions (Fig. 3). Across strains, C3H controls had greater metaphyseal and similar epiphyseal BV/TV compared to BALB but the relative loss of bone (as indicated by differences between disuse and controls) was not significantly different within each region (Fig. 3).

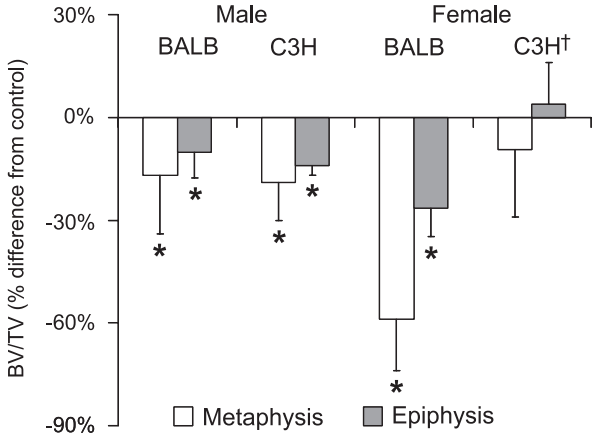

Fig. 3. Mean difference in bone volume fraction between control and disuse mice of both strains plotted for the two trabecular regions in which disuse effects were observed. Across strains and gender, the metaphysis was consistently more affected by disuse than the epiphysis, indicating a site-specific response. A gender-dependent response was evident primarily in the metaphysis of BALB mice while the genetic influence of unloading was much greater in females than in males. * = significant difference between control and disuse. † = Female C3H were on a 6-day shorter protocol.

Discussion

The site-specific influence of genetic variations on bone morphology and its susceptibility to disuse osteopenia was investigated in male mice from two inbred strains. Genetic effects on bone morphology were evident in the diaphyseal and distal femur and, across the six trabecular and cortical regions, exhibited spatial nonuniformity. Consistent with data from astronauts [15], disuse targeted preferentially trabecular regions in both strains in which the deterioration in bone quantity and architecture was uniform across regions and similar between genetic strains. Considering this similarity in the response of these two inbred strains of mice, our results indicate that the genetic polymorphisms that site-specifically alter bone morphology are unlikely to be directly involved in determining bone's response to the loss of functional weight bearing. Caution is required when extrapolating data from an animal model to humans, but if such an extrapolation is appropriate, our data are may also imply that some individuals with vast differences in genetically regulated BMD may lose bone at similar rates and sites during periods of inactivity.

A recent hindlimb suspension study with 17-week-old male C3H mice [21] yielded, in part, different results from ours. We observed that 21 days of unloading resulted in a significant reduction in trabecular BV/TV in the distal femoral metaphysis of C3H mice, while Amblard et al. found no significant change (+3.5%) in this parameter over the same experimental period. The reasons for this discrepancy are unclear at this point but may be related to differences in the volume and position of the selected metaphyseal region, the method used to differentiate cancellous from cortical bone, or a slightly different suspension apparatus. Epigenetic factors such as diet and the amount of weight loss in the suspended mice may also have contributed to the disparate results. In both studies, bone length or morphology did not change during the experimental protocol in the 4-month-old mice. Similarly, suspended mice initially lost weight and then experienced gradual increases in weight throughout the duration of the experiment. The final weights of mice in their study returned to presuspension levels, yet the weights of our suspended mice did not return to initial levels, perhaps indicating interactive effects of weight loss [35,36] and disuse on bone morphology. In aggregate, data from this study are consistent with preliminary data from a third group [37] but further studies will be necessary to delineate the specific factor(s) accounting for the discrepancies between the two studies.

Comparisons between the current data on disuse effects in male BALB and C3H mice to our previous data from female BALB and C3H mice [20] (using identical methodology) revealed interactions between gender and genetics. Following hindlimb unloading, female disuse BALB had significantly less metaphyseal and epiphyseal BV/TV in the distal femur than controls (60% and 28%, respectively), whereas in males, these differences (16% and 10%, respectively) were much smaller in these two regions. A different gender effect was observed in C3H mice, in which disuse altered metaphyseal and epiphyseal BV/TV in males (19% and 14%, respectively) but not in females (Fig. 3). Further, the nature of the relation between gender and bone's sensitivity to disuse was independent of weight loss yet dependent on the anatomical location and bone tissue type. In C3H mice, for example, cortical bone exhibited disuse-related morphological differences only in the metaphysis of females, whereas the sensitivity of metaphyseal and epiphyseal trabecular bone to hindlimb unloading was greater in males. Taken together, these data reveal complex interactions by which gender, genotype, and anatomical location may determine bone's response to the loss of mechanical stimuli.

Previous investigations into the influence of gender on disuse-related changes in bone morphology yielded equivocal results. While no gender effects were observed after 2 weeks of unloading in trabecular and cortical bone of the proximal tibia in 6-month-old Fisher 344 rats [27], or in the diaphyseal femur of 52-day-old heterogeneous and inbred (BALB/cJ, C57Bl/6J, C57Bl/6J, DBA/2J) strains [23,38], another study using 52-day-old C57Bl/6J mice found gender-specificity by which the endocortical and periosteal surface of the diaphyseal femur responded to disuse [22]. While it is unclear how other factors such as age or experimental duration may have influenced the sensitivity of the skeleton to gender differences, these studies are largely consistent with our hypothesis that gender effects may be site-specific and co-dependent on genetics.

Although not evaluated in this study, altered levels of gonadal hormones may have affected our results. Circulating levels of estrogen [39–41] and testosterone [42,43] as well as their corresponding receptors may modify bone's adaptive response to altered mechanical loading. Further, estrogen may be more critical in regulating mechanosensitivity in females than in males [44]. Hindlimb suspension in male rodents decreased circulating testosterone levels, which have been associated with decreased BMD [42,43]. Ligation of the testes to prevent them from descending into the abdomen may [45], or may not [46], eliminate decreased testosterone levels. In this, as in many other recent hindlimb unloading studies [21,27], testes were not ligated nor were serum levels of gonadal hormones measured. It is clear, however, that the future identification of the precise roles that estrogen and testosterone play in defining bone's sensitivity to unloading will provide important clues about the physiologic mechanism underlying gender-specific responses in bone.

In summary, we described the influence of genetic variations on bone morphology and its sensitivity to the loss of functional loading in male mice. Genetic variations between the two strains of mice were linked to site-specific differences in bone morphology but did not cause a differential response to the loss of functional

weight bearing. Compared to previous observations in female mice, the relation between genetic make-up and disuse-related differences in morphology was modified by gender and dependent on the specific anatomic site. The lack of supportive data on gender differences in the human response to space flight [15,47,48] is likely associated with small sample sizes and high variability across the heterogeneous astronaut population. If our current data are, in fact, applicable clinically, future strategies toward the optimal prophylaxis, diagnosis, and treatment of osteoporosis may have to consider gender and genetic make-up of an individual and recognize that the specific anatomical region most prone to disuse-induced osteopenia will depend on these interactions.

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