JOURNAL OF BONE AND MINERAL RESEARCH
Volume 19, Number 4, 2004
Published online on January 5, 2004; doi: 10.1359/JBMR.040110

Genetically Linked Site-Specificity of Disuse Osteoporosis

Stefan Judex,[1] Russell Garman,[1] Maria Squire,[1] Bhavin Busa,[1] Leah-Rae Donahue,[2] and Clinton Rubin[1]

ABSTRACT: The genetic influence on bone loss in response to mechanical unloading was investigated within diaphyseal and distal femoral regions in three genetically distinct strains of mice. One mouse strain failed to lose bone after removal of function, whereas osteopenia was evident in multiple regions of the remaining two strains but in different areas of the bone.

Introduction: It is well recognized that susceptibility to osteoporosis is, in large measure, determined by the genome, but whether this influence is systemic or site-specific is not yet known. Here, the extent to which genetic variations influence regional bone loss caused by disuse was studied in the femora of adult female mice from three inbred strains.
Materials and Methods: Adult C57BL/6J (B6), C3H/HeJ (C3H), and BALB/cByJ (BALB) mice were subjected to 15–21 days of disuse, achieved by hindlimb suspension, and six distinct anatomical regions of the femur were analyzed by high-resolution μCT.
Results and Conclusions: In B6 mice, the amount of disuse stimulated bone loss was relatively uniform across all regions, with 20% loss of trabecular bone and 10% loss of cortical bone. The degree of bone loss in BALB mice varied greatly, ranging from 59% in the metaphysis to 3% in the proximal diaphysis. In this strain, the nonuniformity of bone loss was directly related to the nonuniform distribution of baseline bone morphology ($r^2 = 0.94$). In direct contrast with BALB and B6, disuse failed to produce significant losses of bone in any of the analyzed regions of the C3H mice. Instead, these animals displayed a unique compensatory mechanism to disuse, where the large loss of calcified tissue from the endocortical surface (-24%) was compensated for by an expansion of the periosteal envelope (10%). These data indicate a strong, yet complex, genetic dependence of the site-specific regulation of bone remodeling in response to a powerful catabolic signal. Consequently, the skeletal region of interest and the genetic make-up of the individual may have to be considered interdependently when considering the pathogenesis of osteoporosis or the efficacy of an intervention to prevent or recover bone loss.
J Bone Miner Res 2004;19:607–613. Published online on January 5, 2004; doi: 10.1359/JBMR.040110

Key words: cortical bone, trabecular bone, microarchitecture, disuse, skeleton, mechanical stimuli, weight bearing, bed rest, space flight, genetic variations, osteoporosis

INTRODUCTION

THE SENSITIVITY OF the skeleton to changes in its mechanical environment is reflected by the rapid and site-specific loss of bone tissue after removal of function. Consequently, conditions such as bedrest, spinal cord injury, or space flight can be severely detrimental to the mass, architecture, and mechanical strength of the bone tissue, ultimately transforming specific sites of the skeleton into an osteoporotic state.[1]

Interestingly, the variability of bone loss between individuals when placed under conditions of disuse is highly variable, ranging from those who lose significant amounts of bone to those who remain largely unaffected. For instance, after 6 months of space flight, the decrease in trabecular BMD from the proximal tibia of astronauts ranges from 0% to 23%.[2,3] While several environmental factors (e.g., differences in diet and activity levels during or even before space flight) inevitably contribute to this variability, it is unlikely that such environmental factors are the predominant cause for the disparity between subjects because of the *similarity* of these imposed factors in this specific population. Alternatively, genetic variations between individuals that give rise to large differences in skeletal mass and morphology[4,5] may also account for the differences in the amount of bone loss. In fact, evidence from distinct strains of inbred mice suggests that genetic variations even influence the manner and magnitude of bone's sensitivity to altered demand—both for trabecular[6] and cortical bone.[7–10] Furthermore, the influence of genetics on rates of bone loss is not limited to disuse osteoporosis but extends to age-related and postmenopausal osteoporosis.[11]

Whereas these human and mouse studies indicate a strong genetic influence on bone's responsiveness to changes in its

The authors have no conflict of interest.

[1]Department of Biomedical Engineering, State University of New York at Stony Brook, Stony Brook, New York, USA; [2]Bone Biology Research Group, The Jackson Laboratory, Bar Harbor, Maine, USA.

Table 1. Mean Differences (±95% CIs of the Difference) in Trabecular Parameters of Bone Quantity and Microarchitecture Between Control and Disuse Animals of the Three Strains of Mice

	Index	*B6*	*C3H*	*BALB*
Metaphysis	BV/TV†	−26.0 ± 27.6%	−8.5 ± 19.8%	**−59.5 ± 28.1%***
	Tb.Th†	−4.6 ± 12.8%	**−13.0 ± 9.9%***	**−25.6 ± 10.9%***
	Conn.D†	−64.8 ± 89.9%	**22.6 ± 24.3%***	**−39.8 ± 31.1%***
	SMI†	0.6 ± 7.8%	−2.0 ± 14.8%	**79.9 ± 34.7%***
	DA	−1.6 ± 6.9%	2.0 ± 4.6%	−7.2 ± 5.8%
Epiphysis	BV/TV†	**−20.6 ± 13.4%***	4.6 ± 11.1%	**−26.7 ± 9.2%***
	Tb.Th†	**−13.1 ± 8.5%***	−1.1 ± 7.3%	**−17.2 ± 10.8%***
	Conn.D†	5.4 ± 10.9%	17.7 ± 24.8%	**59.3 ± 17.0%***
	SMI†	**36.9 ± 23.8%***	5.5 ± 16.7%	**182.6 ± 70.4%***
	DA	7.8 ± 8.6%	−0.1 ± 11.1%	−1.2 ± 10.3%

Differences in bold with asterisks indicate significant differences between control and disuse mice for each strain.
† Significant interactions between load and strain for a given index.

local and systemic environment, little is known about the extent by which genetic variations also influence the nature (cortical versus trabecular) and site-specificity (epiphyseal versus metaphyseal) of bone loss. If such as high degree of site-specificity exists, it raises the possibility that measurements are highly unique to that site,[12] thus confounding any overriding diagnostic interpretation for skeletal-wide remodeling.

Because of the strong site-specific effects of the genome on bone morphology observed in our accompanying paper, here we hypothesized that the *response* of bone to a catabolic stimulus would exhibit a similar interdependence on genetics and the specific anatomical location. Given that both genetically distinct human[13–15] and mouse[6] populations with high skeletal mass seem to be less susceptible to catabolic pressure, we further hypothesized that regions within a bone displaying a genetically defined large amount of bone will have a lower propensity to lose bone mineral.

MATERIALS AND METHODS

Experimental design

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee. Three strains of mice, C57BL/6J (B6), BALB/cByJ (BALB), and C3H/HeJ (C3H), all female and 4 months of age, were used in this study.[6] Animals served either as age-matched controls ($n = 8$, $n = 9$, and $n = 8$ for B6, BALB, and C3H, respectively) or mechanical disuse animals ($n = 8$, $n = 10$, and $n = 10$, respectively). Disuse in mice was induced by hind-limb elevation through tail suspension.[6,16] All mice were individually housed in standard cages (28 × 17 × 13 cm) and had access to standard rodent chow and water ad libitum. Control and experimental mice were killed after 15 (B6 and C3H) and 21 days (BALB). On death, right femurs were harvested and preserved in 70% EtOH.

μCT

Seven trabecular and cortical regions within the diaphyseal and distal regions of the femur, representing both cortical and trabecular bone, were analyzed by high-resolution μCT,[17] as described in the accompanying paper. Cortical bone was analyzed from the metaphysis and from three 30-μm-long regions of the diaphysis: the midshaft (defined at 50% of the total femoral length) and two diaphyseal regions, one proximal of the mid-diaphysis at 40% of the femoral length and one distal at 60%. Trabecular volumes of interest included the epiphysis and metaphysis. A local thresholding procedure was used that is described in detail in our accompanying paper. The same threshold was used for both disuse and control bones, thus removing any bias in determining the relative amount of bone loss induced by mechanical unloading.

Cortical bone area (Ct.Ar) as well as areas of the endocortical (Ec.En) and periosteal envelopes (Ps.En) were calculated as averages along the length of each volume of interest. For all trabecular regions, bone volume fraction (BV/TV), trabecular separation (Tb.Sp), trabecular thickness (Tb.Th), trabecular number (Tb.N), connectivity density (Conn.D), the geometrical degree of anisotropy (DA), and the structural model index (SMI) were determined.[18–20]

Statistics

Paired *t*-tests within groups established whether disuse resulted in significant weight loss. Two-tailed *t*-tests were used to determine whether the loss of weight bearing led to differences in bone morphometric indices between control and disuse mice in each of the three mouse strains. Two-way ANOVAs with "mouse strain" as one factor and the "level of weight bearing" as the second tested whether disuse produced differential effects across the strains (as indicated by statistically significant interactions between the two factors). Changes in bone morphological indices were presented as the difference in means between control and disuse mice and the 95% CI of the difference in means. For a given genetic strain, linear correlations were used to test for associations between the distribution of bone morphology (baseline bone quantity) and the distribution of bone loss across the evaluated sites within the femur. The significance level was set at 5%.

RESULTS

Body mass

B6, C3H, and BALB mice subjected to disuse lost 5.8% ($p > 0.05$), 6.4% ($p < 0.001$), and 3.9% ($p < 0.02$) of their body mass over the course of the experimental protocol,

respectively. The following comparisons between disuse and control mice are reported in the order of B6, C3H, and BALB mice, which have been previously labeled low, high, and medium BMD mouse strains, respectively.

Metaphyseal trabecular bone

Disuse failed to significantly alter any indices of metaphyseal trabecular bone quantity and architecture in B6 mice due to large interanimal variations (Table 1). Whereas trabecular bone volume (BV/TV) of the metaphyseal region in C3H mice was unaffected by disuse compared with controls, trabecular thickness was reduced by 13% ($p < 0.02$), and connectivity density was increased by 23% ($p < 0.05$). In contrast, in the metaphysis of BALB mice, disuse reduced BV/TV by 59% ($p < 0.001$), trabecular thickness by 26% ($p < 0.001$), trabecular number by 19% ($p < 0.002$), and connectivity density by 40% ($p < 0.02$; Fig. 1). Also, a change in the principal orientation of the trabeculae was indicated by a decrease of 7% ($p < 0.02$) in the degree of anisotropy (Table 1).

Epiphyseal trabecular bone

The epiphyseal region of B6 mice was highly responsive to mechanical unloading, marked by a 21% decrease in BV/TV ($p < 0.005$), a 13% decrease in trabecular thickness ($p < 0.006$), and a 37% increase in SMI ($p < 0.006$; Fig. 2). In C3H mice, however, tail suspension failed to affect the quantity and architecture of trabecular bone. In the epiphyseal region of BALB mice subjected to disuse, the amount of bone loss (BV/TV) relative to controls was approximately one-half of the reduction in bone volume in the metaphysis (-27%, $p < 0.0001$). Trabecular thickness (-17%, $p < 0.004$), connectivity density (59%, $p < 0.0001$), and SMI (183%, $p < 0.0001$) were also significantly altered (Table 1).

Metaphyseal cortical bone

Disuse caused significant erosion of the metaphyseal endocortical surface in all three strains of mice; the difference in the endocortical envelope between control and disuse mice was 9% in B6 mice ($p < 0.02$), 24% in C3H mice ($p < 0.002$), and 15% in BALB mice ($p < 0.02$). However, cortical bone area was lost only in B6 (-8%, $p < 0.008$) and BALB mice (-18%, $p < 0.001$); no change was observed in C3H mice because the expansion of the endocortical envelope in disuse mice was compensated for by an increase in the periosteal envelope (10%, $p < 0.02$; Fig. 3).

Diaphyseal cortical bone

The three diaphyseal regions (distal, mid-, proximal) were compared between control and disuse mice in each of the three genetically distinct strains. In B6 mice, control mice had significantly greater diaphyseal bone area than their disuse counterparts at all three regions. This difference was 11% ($p < 0.001$) averaged across the three regions and consistent throughout the regions. The decrement in bone volume was accompanied by expansion of the endocortical envelope and contraction of the periosteal envelope, although only the decrease in the periosteal envelope of the distal diaphysis was statistically significant (-4%, $p < 0.05$). In C3H mice, there were no differences in cortical bone area or periosteal and endocortical envelopes in any of the three diaphyseal regions analyzed except for the endocortical envelope at the mid-diaphysis, which was 18% greater ($p < 0.04$) in disuse mice. In BALB mice, disuse significantly decreased the amount of cortical bone in the mid-diaphysis (-11%, $p < 0.03$) as well as the distal diaphysis (-11%, $p < 0.02$), but not in the proximal diaphysis (Table 2).

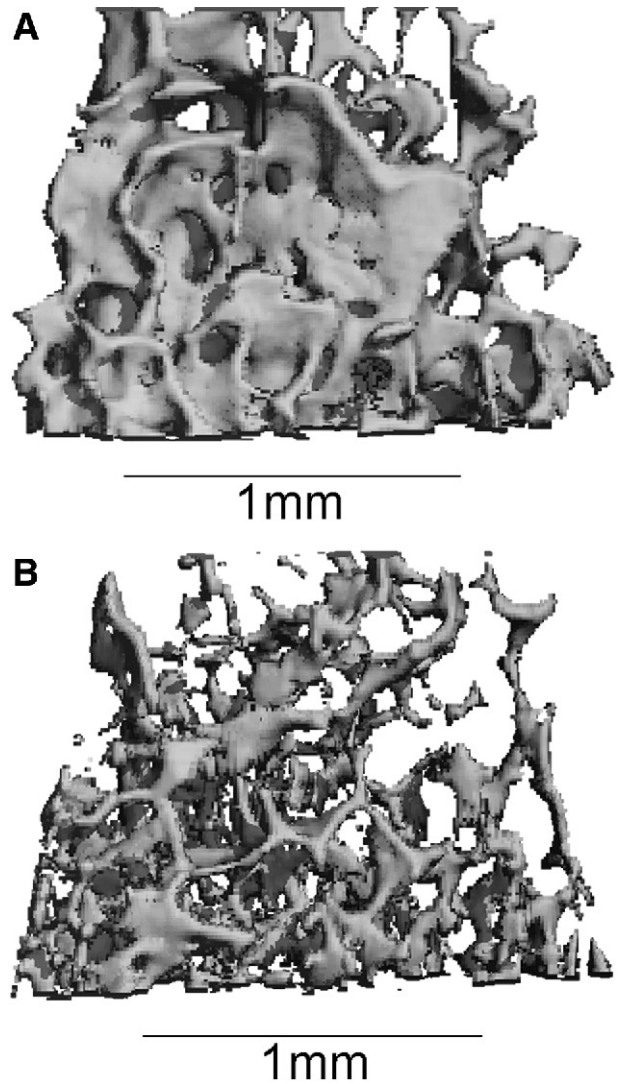

FIG. 1. 3D reconstructed μCT images of metaphyseal trabecular bone separated from their cortical shell. Mechanical disuse applied to BALB mice for 3 weeks induced a 59% difference in bone fractional volume between (A) control mice and (B) disuse mice.

Interactions between genetic variations and change in morphology

Two-way ANOVA tested more directly whether genetic variations between the three strains influenced altered bone morphology associated with the removal of the mechanical

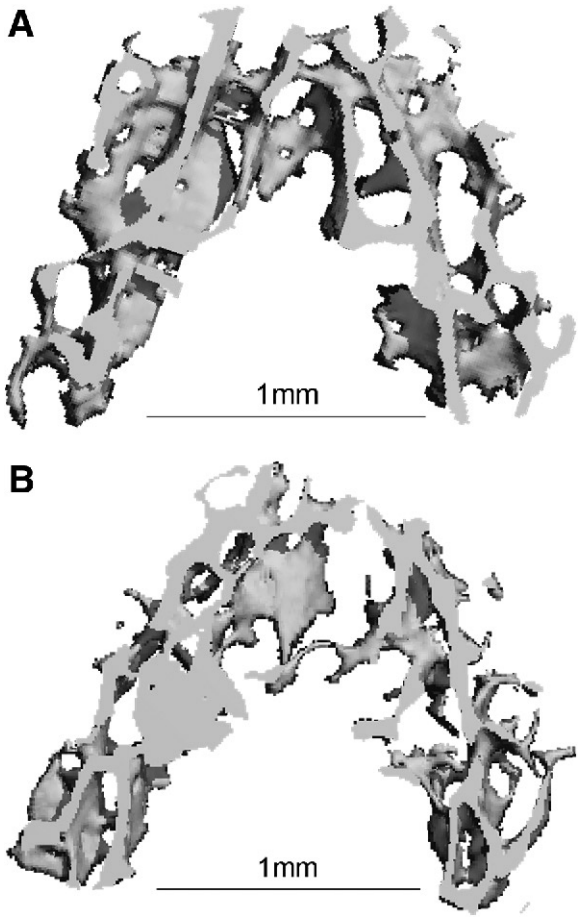

FIG. 2. Reconstructed trabecular bone from the epiphysis of a (A) control and (B) disuse B6 mouse. The loss of weight bearing caused decreased bone quantity and compromised architectural properties.

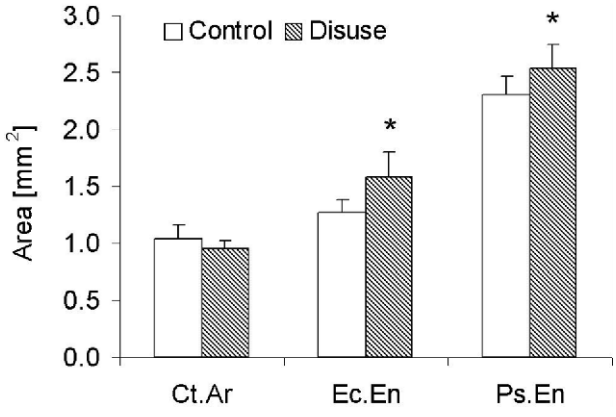

FIG. 3. Cortical bone area (Ct.Ar), endocortical envelope (Ec.En), and periosteal envelope (Ps.En) of the metaphyseal cortical shell in C3H mice in which erosion at the endocortical surface was compensated for by a larger periosteal envelope, preventing the loss of cortical bone area (mean ± SD). Asterisks denote significant differences ($p < 0.03$) between control and disuse groups.

environment. Significant interactions between the two factors were found for most indices, indicating that the site-specific magnitude of bone loss was indeed related to genetics (Table 1; Table 2).

Correlation between bone morphology and bone loss

For correlations between the magnitude of bone loss in the distinct femoral regions and "baseline" bone quantity, bone quantity was calculated as the relative difference in bone volume for control mice of a given strain to the average bone volume of the three strains at the specific site. In BALB mice, the strain that displayed the greatest femoral bone quantity, 94% ($p < 0.004$) of the variation in bone loss was accounted for by variations in existing bone quantity. No significant correlations between the difference in genetically determined bone quantity and bone loss were observed in B6 or C3H mice.

DISCUSSION

The influence of the genome on the manner and magnitude of the skeletal response to alterations in its mechanical environment was investigated by comparing the bone loss stimulated by disuse that occurred in three distinct strains of mice. Both B6 (low femoral bone quantity) and BALB mice (high femoral bone quantity) responded to the loss of weight bearing with significant reductions in bone quantity and architecture in diaphyseal as well epi- and metaphyseal regions of the distal femur, yet the degree of trabecular and cortical bone loss greatly depended on the specific region. In BALB mice, trabecular bone loss was twice as large in the metaphysis than in the epiphysis, similar to the 2-fold difference in cortical bone loss measured between the metaphysis and diaphysis. Tissue losses in B6 mice were more evenly distributed within the trabecular and cortical regions. The absence of altered tissue volume in high BMD C3H mice did not result from the absence of a response at the endocortical and periosteal envelopes but from a concomitant expansion of the two cortical envelopes. Whereas the large amount of bone loss in BALB mice led to the rejection of the hypothesis that high BMD skeletons lose less bone, an association between the amount of bone present at a specific site and the magnitude of the loss of bone tissue in this strain indicated the selective erosion of bone tissue from sites enjoying genetically determined greater bone morphology (relative to the other strains). Taken together, these data indicate that bone's plasticity to altered mechanical loading is strongly nonuniform within a bone and not only dependent on an individual's genotype but also on the specific site.

This study built on our accompanying paper that indicated a highly site-specific influence of genetics on bone morphology, thus stimulating the hypothesis that mechanosensitivity is also interdependently modulated by genetics and anatomical site. Prudence requires, however, to extrapolate any data collected from mice to human conditions with care because of obvious (e.g., size, metabolism) or more subtle differences (e.g., different type of bone, physeal closure occurs very late or never).[21] Despite mice being the model of choice in biomedical research in general and in skeletal genetics in specific, the effect of these differences on research data are still unknown, although most studies

Table 2. Relative Changes in Cortical Bone Area (Ct.Ar), the Area Enclosing the Endocortical Envelope (Ec.En), and the Area Enclosing the Periosteal Envelope (Ps.En) Induced by Disuse

	Index	*B6*	*C3H*	*BALB*
Metaphysis	Ct.Ar	**−8.5 ± 5.8%***	−8.1 ± 11.0%	**−18.5 ± 6.9%***
	Ec.En	**8.9 ± 6.5%***	**24.1 ± 13.7%***	**15.2 ± 13.2%***
	Ps.En	3.3 ± 4.1%	**9.7 ± 8.2%***	1.2 ± 8.1%
Proximal diaphysis	Ct.Ar†	**−11.0 ± 5.0%***	4.8 ± 9.9%	−3.2 ± 7.6%
	Ec.En	5.4 ± 6.9%	5.1 ± 12.9%	5.6 ± 12.6%
	Ps.En	−3.1 ± 4.7%	4.5 ± 8.0%	0.0 ± 8.2%
Mid-diaphysis	Ct.Ar†	**−10.9 ± 5.4%***	1.3 ± 8.9%	**−11.0 ± 9.0%***
	Ec.En	3.5 ± 5.3%	**17.8 ± 16.0%***	**15.4 ± 11.8%***
	Ps.En	−3.6 ± 4.6%	4.9 ± 7.5%	−1.3 ± 8.7%
Distal diaphysis	Ct.Ar†	**−10.0 ± 3.8%***	1.4 ± 7.6%	**−10.8 ± 8.1%***
	Ec.En	1.5 ± 6.3%	9.4 ± 17.1%	5.9 ± 14.8%
	Ps.En	**−4.5 ± 4.0%***	2.7 ± 8.1%	−4.6 ± 9.6%

Numbers represent the mean differences (± 95% CIs of the difference) in morphological parameters between control and disuse mice measured in the metaphysis and diaphysis.

Values in bold with asterisks indicate significant differences between control and disuse mice for each strain.

† Significant interactions between load and strain for a given index.

Table 3. Synopsis of Disuse Related Changes in Bone Quantity in Different Trabecular and Cortical Regions of the Three Strains of Mice

		B6	*C3H*	*BALB*
Metaphysis	BV/TV	↓ ↓ ↓	—	↓ ↓ ↓ ↓ ↓ ↓
Epiphysis	BV/TV	↓ ↓ ↓	—	↓ ↓ ↓
Metaphyseal cortex	Ct.Ar	↓	—	↓ ↓ ↓
	Ec.En	↑	↑ ↑ ↑	↑ ↑
	Ps.En	—	↑ ↑	—
Diaphysis	Ct.Ar	↓ ↓	—	↓
	Ec.En	—	—	—
	Ps.En	—	—	—

Differences are marked by arrows with one arrow denoting changes between 0% and 9%, two arrows corresponding to changes between 10% and 19%, etc.

—, differences that are below 10% and statistically not significant.

have observed similar effects in mice and humans both for the skeletal response to mechanical stimuli[22] as well as for gene–bone morphology interactions.[23] Furthermore, different experimental durations limited direct comparisons of *absolute* values between BALB mice and the other two strains that were subjected to 15 days rather than 21 days, yet these differential protocol lengths had likely a minimal effect on the primary aim of this study, which focused on *relative* spatial differences within a bone across strains.

Unlike the trabecular bone measured in the metaphyseal region, in which BALB mice showed a hypersensitivity to mechanical unloading, the diaphyseal region revealed that low bone quantity B6 mice were very responsive, showing a similar amount of bone loss to that observed in BALB's (despite being on a shorter protocol). In contrast, disuse failed to reduce bone volume at any cortical site of C3H animals, yet they lost three times as much bone from the endocortical surface of the metaphysis than B6 mice. A net loss of bone was avoided in these animals by stimulating a periosteal expansion, similarly to the expansion of the periosteal envelope during aging.[24] While the modulation of this compensatory mechanism is unclear at this point, preliminary μCT data from C3H mice of the same cohort (n = 4) indicate that the periosteal envelope does not change between 16 and 18 weeks of age. Thus, the genetically specific compensatory mechanism in C3H mice was most likely modulated by an expansion of the periosteal envelope rather than by inhibiting its contraction.

As described in the companion paper, bone quantity and microarchitecture at the six sites within the femur were also dependent on genotype and specific anatomical location, suggesting a possible relationship between baseline morphology and mechanosensitivity. In clinical studies of osteoporosis and aging, no consistent association between individuals with high or low BMD and high or low rates of bone loss has been established,[25–28] perhaps influenced by the timing of the baseline bone density measurements that did not coincide with the event of peak bone mass. Our data are consistent with the clinical studies indicating that, for a given skeleton, the propensity to resorb bone is not correlated with baseline bone mass (i.e., both low bone quantity B6 and high bone quantity BALB mice lose large amounts of bone). They suggest, however, that the manner by which bone is resorbed may be different between high and low bone quantity skeletons; thus, skeletons with large amounts of bone may enjoy a strategic advantage by removing tissue from sites where the genetic variations had placed a larger than normal amount of tissue. Extrapolated to humans, the data also suggest that the genes regulating bone morphology at a given site may overlap with the genes regulating mechanosensitivity only in some individuals (e.g., large bone mass, fast loser).

The wide spatial variations in bone's plasticity to disuse indicates that the genome enables the regulation of mechanosensitivity on a site-specific basis, with distinct genes or combination of genes modulating the response within each region within a bone. A strategy that enlists a distinct set of genes for each skeletal location would, however, add a great deal of complexity. Rigorous segregation studies will even-

tually elucidate the number of genes involved in the genetic manifestation of such a site-specific regulatory mechanism, but it is likely that gene–environment interactions also contributed to the observed site-specificity. Inevitably, the genetically regulated differences in baseline morphology within a bone and between mouse strains also produced distinct mechanical milieus within the femur when subject to load (or its removal). Our understanding of how bone senses the loss of functional weight-bearing is incomplete, but the loss of bone in disuse is related to the suppression of key regulatory signals that maintain homeostasis.[29] The distribution of these mechanical signals across a bone, independent of their identity (e.g., strain magnitude, strain gradients, or high-frequency strains), is nonuniform and related not only to differences in bone morphology between different intraosseous sites, but also to the imposed mechanical environment (e.g., as influenced by potential differences in muscle–bone interactions or body mass distribution between the strains). The nearly 5-fold greater endocortical envelope of the proximal metaphysis compared with the endocortical envelope of the mid-diaphysis in C3H mice reflects such a non-homogeneous distribution of bone tissue in addition to other morphological parameters, such as bone curvature or moments of inertia,[30] which will all affect the loading pattern at a given site. This hypothesis of strong gene–environment interactions is currently being tested by quantifying the distribution of candidate mechanical parameters within a bone through in vivo strain gauging and finite element modeling.[31]

In summary, this study suggests that genetic modulation of bone loss is strongly site-specific, hence greatly limiting our ability to categorize entire skeletons into varying degrees of responsivity based on their genotype. Although in this study a mechanical stimulus was used to generate catabolic pressure, it is likely that a similar interdependence applies to biochemical challenges (e.g., hormonal) that will ultimately transform the skeleton into an osteoporotic state. Thus, the optimal site of detection for osteoporosis may depend on an individual's genotype, and the definition of those genes controlling this site-specific relation may ultimately allow the identification of those at greatest risk to incur osteoporosis. In addition, preclinical evaluations of interventions designed to augment bone quantity and/or quality (or inhibit its demise) may have to consider the responsivity of the tissue, as well as region-specific responses.

ACKNOWLEDGMENTS

This research was supported, in part, by the National Space Biomedical Research Institute, the National Aeronautics and Space Administration, and the Alberta Heritage Foundation for Medical Research. Expert technical support from Sherry Wang was greatly appreciated.

REFERENCES

1. Houde JP, Schulz LA, Morgan WJ, Breen T, Warhold L, Crane GK, Baran DT 1995 Bone mineral density changes in the forearm after immobilization. Clin Orthop **317:**199–205.
2. Vico L, Collet P, Guignandon A, Lafage-Proust MH, Thomas T, Rehaillia M, Alexandre C 2000 Effects of long-term microgravity exposure on cancellous and cortical weight-bearing bones of cosmonauts. Lancet **355:**1607–1611.
3. Shackelford L, LeBlanc A, Feiveson A, Oganov V 1999 Bone loss in space: Shuttle/Mir experience and bed rest countermeasure program. Proceedings of the First Biennial Space Biomedical Investigator Workshop, Galveston, TX, USA, p. 17.
4. Eisman JA 1999 Genetics of osteoporosis. Endocr Rev **20:**788–804.
5. Kelly PJ, Hopper JL, Macaskill GT, Pocock NA, Sambrook PN, Eisman JA 1991 Genetic factors in bone turnover. J Clin Endocrinol Metab **72:**808–813.
6. Judex S, Donahue LR, Rubin C 2002 Genetic predisposition to low bone mass is paralleled by an enhanced sensitivity to signals anabolic to the skeleton. FASEB J **16:**1280–1282.
7. Akhter MP, Cullen DM, Pedersen EA, Kimmel DB, Recker RR 1998 Bone response to in vivo mechanical loading in two breeds of mice. Calcif Tissue Int **63:**442–449.
8. Akhter MP, Cullen DM, Recker RR 2002 Bone adaptation response to sham and bending stimuli in mice. J Clin Densitom **5:**207–216.
9. Kodama Y, Umemura Y, Nagasawa S, Beamer WG, Donahue LR, Rosen CR, Baylink DJ, Farley JR 2000 Exercise and mechanical loading increase periosteal bone formation and whole bone strength in C57BL/6J mice but not in C3H/Hej mice. Calcif Tissue Int **66:**298–306.
10. Kodama Y, Dimai HP, Wergedal J, Sheng M, Malpe R, Kutilek S, Beamer W, Donahue LR, Rosen C, Baylink DJ, Farley J 1999 Cortical tibial bone volume in two strains of mice: Effects of sciatic neurectomy and genetic regulation of bone response to mechanical loading. Bone **25:**183–190.
11. Brown MA, Haughton MA, Grant SF, Gunnell AS, Henderson NK, Eisman JA 2001 Genetic control of bone density and turnover: Role of the collagen 1alpha1, estrogen receptor, and vitamin D receptor genes. J Bone Miner Res **16:**758–764.
12. Gross TS, Rubin CT 1995 Uniformity of resorptive bone loss induced by disuse. J Orthop Res **13:**708–714.
13. Aloia JF, Vaswani A, Mikhail M, Badshah M, Flaster E 1999 Cancellous bone of the spine is greater in black women. Calcif Tissue Int **65:**29–33.
14. Bohannon AD 1999 Osteoporosis and African American women. J Womens Health Gend Based Med **8:**609–615.
15. Anderson JJ, Pollitzer WS 1994 Ethnic and genetic differences in susceptibility to osteoporotic fractures. Adv Nutr Res **9:**129–149.
16. Morey ER, Sabelman EE, Turner RT, Baylink DJ 1979 A new rat model simulating some aspects of space flight. Physiologist **22:** S23–S24.
17. Muller R 2002 The Zurich experience: One decade of three-dimensional high-resolution computed tomography. Top Magn Reson Imaging **13:**307–322.
18. Hildebrand T, Laib A, Muller R, Dequeker J, Ruegsegger P 1999 Direct three-dimensional morphometric analysis of human cancellous bone: Microstructural data from spine, femur, iliac crest, and calcaneus. J Bone Miner Res **14:**1167–1174.
19. Odgaard A 1997 Three-dimensional methods for quantification of cancellous bone architecture. Bone **20:**315–328.
20. Hildebrand T, Ruegsegger P 1997 Quantification of bone microarchitecture with the structure model index. Comput Methods Biomech Biomed Engin **1:**15–23.
21. Kilborn SH, Trudel G, Uhthoff H 2002 Review of growth plate closure compared with age at sexual maturity and lifespan in laboratory animals. Contemp Top Lab Anim Sci **41:**21–26.
22. Gross TS, Srinivasan S, Liu CC, Clemens TL, Bain SD 2002 Noninvasive loading of the murine tibia: An in vivo model for the study of mechanotransduction. J Bone Miner Res **17:**493–501.
23. Klein OF, Carlos AS, Vartanian KA, Chambers VK, Turner EJ, Phillips TJ, Belknap JK, Orwoll ES 2001 Confirmation and fine mapping of chromosomal regions influencing peak bone mass in mice. J Bone Miner Res **16:**1953–1961.
24. Bouxsein ML, Myburgh KH, van der Meulen MC, Lindenberger E, Marcus R 1994 Age-related differences in cross-sectional geometry of the forearm bones in healthy women. Calcif Tissue Int **54:**113–118.
25. Davis JW, Grove JS, Ross PD, Vogel JM, Wasnich RD 1992 Relationship between bone mass and rates of bone change at appendicular measurement sites. J Bone Miner Res **7:**719–725.
26. Bainbridge KE, Sowers MF, Crutchfield M, Lin X, Jannausch M, Harlow SD 2002 Natural history of bone loss over 6 years among

premenopausal and early postmenopausal women. Am J Epidemiol **156:**410–417.
27. Riis BJ, Hansen MA, Jensen AM, Overgaard K, Christiansen C 1996 Low bone mass and fast rate of bone loss at menopause: Equal risk factors for future fracture. A 15-year follow-up study. Bone **19:**9–12.
28. Qin L, Au SK, Leung PC, Lau MC, Woo J, Choy WY, Hung WY, Dambacher MA, Leung KS 2002 Baseline BMD and bone loss at distal radius measured by peripheral quantitative computed tomography in peri- and postmenopausal Hong Kong Chinese women. Osteoporos Int **13:**962–970.
29. Rubin C, Xu G, Judex S 2001 The anabolic activity of bone tissue, suppressed by disuse, is normalized by brief exposure to extremely low-magnitude mechanical stimuli. FASEB J **15:**2225–2229.
30. Jepsen KJ, Akkus OJ, Majeska RJ, Nadeau JH 2003 Hierarchical relationship between bone traits and mechanical properties in inbred mice. Mamm Genome **14:**97–104.
31. Karnik S, Choi E, Jacobson J, Busa B, Donahue LR, Rubin C, Judex S 2003 Tibial cortical strains induced by low-level vibrations are dependent on genetic make-up and frequency of the signal. J Bone Miner Res **18:**S219.

Address reprint requests to:
Stefan Judex, PhD
Department of Biomedical Engineering
Psychology A Building
3rd Floor
State University of New York at Stony Brook
Stony Brook, NY 11794-2580, USA
E-mail: stefan.judex@sunysb.edu

Received in original form May 11, 2003; in revised form October 24, 2003; accepted November 4, 2003.