

Bone, Muscle, and Physical Activity: Structural Equation Modeling of Relationships and Genetic Influence With Age

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ABSTRACT: Correlations among bone strength, muscle mass, and physical activity suggest that these traits may be modulated by each other and/or by common genetic and/or environmental mechanisms. This study used structural equation modeling (SEM) to explore the extent to which select genetic loci manifest their pleiotropic effects through functional adaptations commonly referred to as Wolff's law. Quantitative trait locus (QTL) analysis was used to identify regions of chromosomes that simultaneously influenced skeletal mechanics, muscle mass, and/or activity-related behaviors in young and aged B6×D2 second-generation (F₂) mice of both sexes. SEM was used to further study relationships among select QTLs, bone mechanics, muscle mass, and measures of activity. The SEM approach provided the means to numerically decouple the musculoskeletal effects of mechanical loading from the effects of other physiological processes involved in locomotion and physical activity. It was found that muscle mass was a better predictor of bone mechanics in young females, whereas mechanical loading was a better predictor of bone mechanics in older females. An activity-induced loading factor positively predicted the mechanical behavior of hindlimb bones in older males; contrarily, load-free locomotion (i.e., the remaining effects after removing the effects of loading) negatively predicted bone performance. QTLs on chromosomes 4, 7, and 9 seem to exert some of their influence on bone through actions consistent with Wolff's Law. Further exploration of these and other mechanisms through which genes function will aid in development of individualized interventions able to exploit the numerous complex pathways contributing to skeletal health.

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INTRODUCTION

THE DEVELOPMENT AND maintenance of adequate bone strength is a complex process that involves both direct and indirect genetic and environmental pathways. Previous studies have shown that increases in loading frequency, strain, and strain rate, above maintenance thresholds, increase bone formation,^(1,2) whereas decreases in strain magnitude result in increased bone resorption or bone loss.⁽³⁾ Variations in peak bone mass and rates of bone loss are therefore thought to be influenced by variations in muscle mass or muscle strength,^(4–6) as well as the frequency and duration of activity.^(7,8)

The highest routinely applied loads experienced by bone come from the muscles used to maintain posture and produce movement. In general, the moment arms (i.e., distance to the center of joint rotation) through which muscles act are much shorter than those through which

environmental forces act; thus, proportionately greater muscle forces are required to balance the external forces imposed on the body.⁽⁹⁾ Frost⁽¹⁰⁾ suggested that age-related changes in bone mass follow the age-related increases in muscle strength occurring during growth, the plateau in strength seen in young adults, and the decline after 30–40 yr of age. During the plateau, the skeleton has adapted to stronger young-adult muscles. With age, decreases in muscle mass, strength, and activity level produce corresponding declines in strain magnitude that eventually fall below a threshold required for bone maintenance, and bone loss ensues. Along these same lines of reasoning, interindividual variations in muscle mass and strength or the frequency and duration of activity may produce correlated variations in peak bone mass and rates of bone loss. Because peak bone mass has been shown to predict fracture risk, insight into the mechanisms through which genes influence both bone acquisition and the rate of bone loss could lead to new methods for preventing osteoporosis and its attendant increased risk of fracture late in life.

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The continuous distribution of skeletal phenotypes such as BMD or whole bone breaking strength indicates that they are complex traits regulated through a myriad of interconnected pathways that originate through gene expression. Several such genes may affect bone through actions on muscle and behavior. Twin studies in humans⁽¹¹⁾ and studies using inbred mice^(12–15) have confirmed that bone properties are highly heritable. Previous studies have also shown that muscle mass is significantly correlated with BMD in humans⁽⁴⁾ and in mice⁽⁵⁾ and that physical activity influences skeletal quality.^(7,8) Kaye and Kusy⁽⁷⁾ studied these relationships across several mouse strains and suggested that activity as well as muscle size and strength are genetically influenced and that part of the genetic influence on bone size and strength is modulated through activity and muscle size.

The study reported here used F₂ mice derived from C57BL/6J (B6) and DBA/2J (D2) inbred strains. B6 mice have been shown to be more active compared with D2 mice.^(16–19) This is exemplified in the findings of Lerman et al.,⁽¹⁹⁾ who showed that B6 mice voluntarily ran the greatest exercise wheel distance, duration, and speed compared with several inbred mouse strains including D2 mice.⁽¹⁹⁾ The genetic influence on skeletal differences observed in B6 and D2 mouse strains could therefore be at least partially modulated by genetically induced differences in activity level, as well as a differential skeletal response to loading and environmental factors. A pleiotropic approach to the study of musculoskeletal relationships is not a novel concept and has received more attention in recent years. For a recent review, the reader is referred to Karasik and Kiel,⁽²⁰⁾ who point out that the sources of covariation between muscle and bone could occur during patterning in embryonic life, allometric growth, and the homeostatic period of adult life.⁽²¹⁾

This report explores simultaneous relationships among measures of muscle strength (implied by muscle mass), skeletal integrity, physical activity-related behaviors, and genetic loci affecting these traits in F₂ progeny of B6 and D2 inbred mice. Muscle mass, several skeletal phenotypes, and activity measures from F₂ mice were used in quantitative trait locus (QTL) analyses. Chromosomal regions that were identified from the QTL analyses to have pleiotropic effects across at least two of the three domains (bone, muscle, or activity) were selected for follow-up analyses using structural equation modeling (SEM). Our aim was to learn the extent to which the gene or genes functioning within select pleiotropic loci contribute to bone strength through their actions on behavior and/or muscles, which is conveyed to bone through functional adaptations commonly referred to as Wolff's Law.

MATERIALS AND METHODS

Overview

Activity-related behavioral measures, muscle mass, and skeletal phenotypes were assessed in young adult ($n = 191$ males and 185 females) and aged ($n = 187$ males and 185 females) F₂ progeny of DBA/2J (D2) and C57BL/6J (B6)

inbred mouse strains. These measures were used for QTL analysis and SEM. The same measures were also obtained from a second population of 23 B×D recombinant inbred (RI) mouse strains with 10 male and 10 female mice per strain. RI data were used as a means of comparing our activity measures to various measures of activity found in the literature.

Animals

Breeding and maintenance of progenitor and RI strains, and F₂ animals derived from the progenitor strains, were conducted in the barrier facility maintained by The Center for Developmental and Health Genetics at The Pennsylvania State University. Mice were weaned into like-sex sibling groups at ~23 days of age with four animals per cage. They were fed a diet of autoclaved Purina Mouse Chow 5010 (content: 1.0% calcium, 0.67% phosphorus, 0.22% magnesium, and 4.4 IU/g vitamin D) ad libitum designed to be equivalent, after autoclaving, to Purina 5001 (content: 0.95% calcium, 0.67% phosphorus, 0.21% magnesium, and 4.5 IU/g vitamin D). The barrier facility was maintained under positive pressure with a temperature- and humidity-controlled environment and a 12-h light/dark cycle.

Animal activity measures

Several assessments of activity-related behaviors were recorded on each animal. Measurements began at ~130 days of age for the F₂ young animals, 150 days of age for the RI young animals (± 2 wk), and 430 days of age for the F₂ aged animals. Measurements of each activity were made on each F₂ animal at 1-mo increments on three separate occasions. The mean of the three measurements was used for subsequent analyses in the F₂ cohorts. For RI animals, the mean of each RI strain was used for comparison with relevant activity data found in the literature.

Activity was measured in a 40 × 40 × 15-cm-deep, black opaque plastic arena marked into four quadrants with a 15-mm hole cut in the center of each quadrant. Animals were consistently tested, starting early in the light phase of the 12/12 light/dark cycle, typically 8:00–9:00 a.m. The test room was dimly illuminated with the test equipment illuminated using a red light. Animals were individually placed into a clear plastic cylinder in the center of the arena. After 10 s, the cylinder was lifted, and each mouse was observed for 3 min. The number of quadrants entered and the number of times the animal reared onto its hind limbs were recorded. Hind limb rearings were not distinguished from wall-leanings (i.e., a rearing that touched the wall was counted as a rear). We interpreted the number of quadrants entered as a measure of general locomotor activity and hind limb rearing as more similar to resistance-type exercise or training.

Genotyping

F₂ mice were genotyped in-house using 96 microsatellite markers distributed throughout the genome, with an average spacing of 15–20 cM. Marker analyses were conducted on purified DNA samples procured from tail snips

using an automated, fluorescence-based detection system described in detail in Vandenbergh et al.⁽²²⁾

Tissue harvesting and muscle mass measurement

Animal weights were recorded before death by cervical dislocation at ~200 days of age for the young cohort and 500 days of age in the aged cohort. Nose-to-anus length was recorded immediately after death. The gastrocnemius, soleus, tibialis anterior, and extensor digitorum longus muscles were carefully removed from the right hind limb and weighed to the nearest hundredth of a milligram. The right hind limb of each animal was harvested, and the tibia and femur were cleaned, wrapped in saline soaked gauze, and stored at -20°C for future mechanical testing.

Skeletal assessment

At the time of testing, the tibia and femur were thawed at ambient temperature. The midshaft of the right tibia and femur were tested to failure in three-point bending using a Materials Testing System (MTS) MiniBionix testing apparatus with a support span of either 10 (tibia) or 8 mm (femur) and a deformation rate of 1 mm/min. Tibias were oriented so that the nosepiece was anteriorly directed with respect to the tibial shaft. This was necessary to prevent the tibia from rolling during testing. Femurs were loaded from anterior to posterior. Each tibia and femur were loaded to failure while recording load and actuator displacement at 20 Hz, and a load-deformation curve was generated using MATLAB scientific software (version 6.5, release 13; MathWorks). Yield load, yield deformation, energy absorbed at yield (area under the load-deflection curve), failure load, failure deformation, energy absorbed at failure, and stiffness (initial slope of the load-deflection curve) were determined.

Data analyses

Sex and age group differences and descriptive statistics: Before analysis, all phenotypic data were screened for normality and, when necessary, transformed using either a natural log or square root transformation. Group differences were determined from a two-way analysis of variance (ANOVA) and covariance (ANCOVA) using a multivariate general linear model in SPSS 16.0 statistical software. The main effects of sex and age and the interaction of sex and age were determined for body size measures from ANOVA; for all other measures, an ANCOVA was performed with body weight and body length as covariates. Within each sex separately, pairwise comparisons for age differences were performed, and body size-adjusted means and SEs were obtained.

Assessment of activity-related behavioral measures: To verify the suitability of our behavioral measures as measures of activity, we compared the within-strain means for quadrants entered and hind limb rears, from our B×D RI animals, with other measures of activity performed on the same B×D RI strains in previous studies (<http://www.genenetwork.org/>). The activity-related behaviors, as measured in our B×D RI strains, were significantly correlated to other more commonly used measures of activity

performed in previous studies. The number of quadrants entered was significantly correlated with distance traveled ($r = 0.62$, $p < 0.01$ ⁽²³⁾; $r = 0.74$, $p < 0.0005$ ⁽²⁴⁾; $r = 0.64$, $p < 0.01$ ⁽²⁵⁾; and $r = 0.67$, $p < 0.005$ ⁽²⁶⁾), and hind limb rears was significantly correlated with rearings ($r = 0.65$, $p < 0.005$).⁽²⁴⁾

QTL analysis

Genome-wide QTL analyses were performed on the F₂ cohort to locate chromosomal regions influencing the continuously distributed phenotypic measures, as previously reported for muscle and bone from individual age groups.^(15, 27–30) QTL analyses were conducted using QTL Cartographer software to perform interval mapping.⁽³¹⁾ LOD scores of 4.3 or greater were considered significant, whereas scores between 2.8 and 4.3 were considered suggestive.⁽³²⁾ Analyses were performed on pooled data from the 200- and 500-day-old male and female cohorts unadjusted for age or sex and also on pooled data for each sex separately. In each case, analyses were conducted using both raw, unadjusted data and then data adjusted for body weight and length through multiple regression.⁽³³⁾ QTLs identified as having significant pleiotropic effects on phenotypic measures from at least two of the three domains (activity, muscle, or bone) were selected to be included in subsequent structural equation models.

SEM specification and testing

SEM was performed using Amos 7.0 statistical software, and parameter estimates were obtained using a full information maximum likelihood algorithm. A model was developed to study the hypothesized relationships among skeletal quality, muscle mass, and activity-related behavior (Fig. 1). The model included latent variables representing a bone factor (indicated by unadjusted measures of yield load, ultimate load, and stiffness), a muscle factor (indicated by unadjusted measures of gastrocnemius, soleus, extensor digitorum longus, and tibialis anterior muscle masses), locomotion-free loading (indicated by quadrants entered residualized from hind limb rearing), and loading-free locomotion (indicated by rearing residualized from quadrants entered). Quadrants entered and hind limb rearing were highly correlated ($r = 0.73$; $p < 0.001$).

Whereas both quadrants entered and hind limb rearing are indicators of the animals' behavioral activities, we reasoned that the two types of activity could have different effects on the musculoskeletal system, particularly if the more intense skeletal loading associated with hind limb rearing could be effectively separated from the other physiological effects of routine ambulation. We therefore removed the correlation between hind limb rearing and quadrants entered by regressing them on each other to isolate unique aspects of rearing that are separate from quadrants entered and unique aspects of quadrants entered that are separate from rearing. In the first case, the unique aspects should include enhanced skeletal loading without the repetitive low-load component associated with normal ambulation, a model deemed to be more representative of resistance type exercise. The latter case includes routine

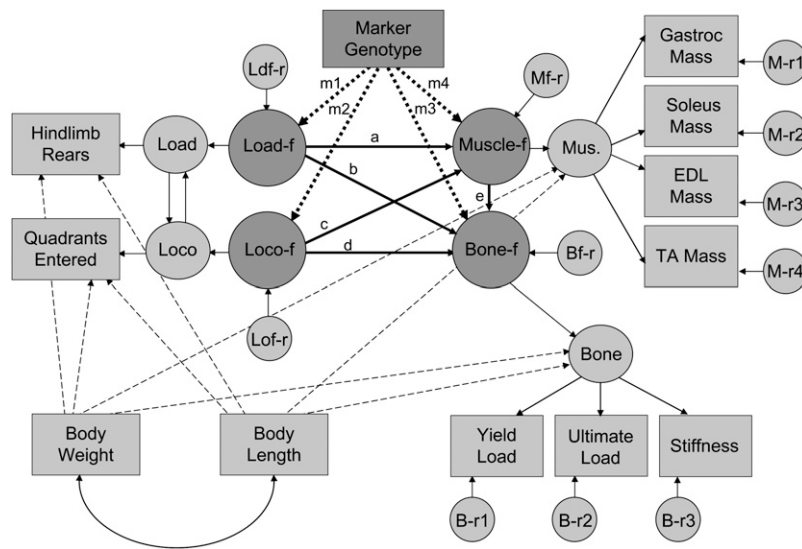


FIG. 1. Full path diagram for the tibia and femur SEM model. Model A (without genetic marker) does not include paths m1–m4. Model B (with genetic marker) includes all paths. Rectangles, measured variables; large circles, latent factors; small circles, residual variance.

ambulatory motions devoid of high skeletal loads, which is more representative of aerobic type activity. Regressing one on the other allowed the separate effects of loading and locomotion to be independently analyzed.

In contrast to the QTL analyses, unadjusted musculoskeletal and activity measures were used in the SEM model; however, body weight and body length were included in the model as independent predictors to account for the known association of body size with musculoskeletal measures. The body size adjustment was made at an intermediate level between the phenotypic measures and the main structural factors (Fig. 1).

Two different SEM models were evaluated. Model A examined structural paths without accounting for the genotype, whereas model B included animal genotype at selected loci. As shown in Fig. 1, the main structural paths connecting the primary factors after the body size adjustments are labeled a–e. The primary relationships of interest in model A included the structural paths for loading and locomotion on the muscle factor (paths labeled a and c, respectively), loading and locomotion on the bone factor (paths labeled b and d, respectively), and the muscle factor on the bone factor (path labeled e). Model B tested additional structural paths, m1–m4, by adding the genotype the genetic marker closest to select QTL identified as having potentially pleiotropic effects across at least two of the three domains of activity, muscle, and bone. Models A and B were evaluated using skeletal measures from femur and tibia separately.

Paths a–e and m1–m4, as well as the influence of body weight and length on the muscle and bone factors and the quadrants entered and hind limb rearing measurements, were estimated. Standardized (SRW) and unstandardized regression weights (URW), SEs, and critical ratios ($CR=URW/SE$) for the tibia and femur models were determined. The critical ratio was tested against a t -distribution to determine whether the path coefficient was significantly different than zero.

RESULTS

General trends in measured phenotypes

Female mice had smaller muscles, were less active (fewer quadrants entered and rearings), and had bones that were weaker than their male counterparts. Body weight increased, and hind limb rearing decreased as a function of age in both sexes (Table 1). When controlling for body weight and length, muscle mass as well as yield and ultimate load in the tibia and femur decreased with age in both males and females. The stiffness of both bones increased as consequence of age.

Structural equation model A: pooled data

Table 2 presents the standardized regression weights from tibial and femoral models run on pooled data and data from the four individual cohorts. Models run on combined data from all groups revealed significant positive associations of both body weight and length with the muscle factor, but only body weight was significantly associated with the bone factor. Body weight was negatively associated with quadrants entered indicating that larger animals were less active. Hind limb rearing was not significantly associated with either body weight or length. When controlling for body weight and length, locomotion-free loading was positively associated with tibial bone mechanics ($\beta = 0.215$) and muscle mass ($\beta = 0.255$), and muscle was positively associated with tibial bone mechanics ($\beta = 0.365$).

Structural equation model A: sex- and age-dependent analyses

As with the pooled data set, body weight and length were significant predictors of the muscle factor in all age and sex subgroups except older males (Table 2). Body weight was also a significant predictor of the tibial factor in older females and young males and the femoral factor in young and

TABLE 1. Descriptive Statistics: Means and SEs for Young and Old Female and Male Cohorts

Phenotypic measure	Group differences	Females		Males	
		Young (n = 185)	Old (n = 185)	Young (n = 191)	Old (n = 187)
Body weight (g)	a s i	28.19 (0.44)	36.30 (0.44)	39.30 (0.43)	47.20 (0.44)
Body length (cm)	a s	9.75 (0.03)	10.33 (0.03)	10.36 (0.03)	10.98 (0.03)
Body mass index (kg/m ²)	a s i	2.95 (0.03)	3.35 (0.03)	3.66 (0.03)	3.90 (0.03)
Cohort means adjusted for size covariates (body weight and length)					
Hind limb rears (#)	a s i	11.09 (0.56)	9.81 (0.56)	14.43 (0.64)	11.49 (0.68)
Quadrants entered (#)	s i	20.05 (0.86)	23.32 (0.85)	23.83 (0.91)	23.99 (0.96)
Gastrocnemius (mg)	a s i	118.7 (0.90)	111.3 (0.91)	153.4 (1.21)	135.0 (1.28)
Soleus (mg)	a s	8.31 (0.12)	7.68 (0.12)	10.62 (0.14)	9.60 (0.15)
Extensor digitorum longus (mg)	a s	9.15 (0.10)	9.07 (0.10)	11.79 (0.12)	10.82 (0.13)
Tibialis anterior (mg)	a s	42.74 (0.31)	40.99 (0.31)	54.14 (0.42)	51.12 (0.44)
Tibia yield load (N)	a i	11.94 (0.17)	11.04 (0.17)	14.00 (0.24)	11.16 (0.25)
Tibia ultimate load (N)	a s	13.66 (0.18)	12.01 (0.18)	15.94 (0.24)	13.65 (0.25)
Tibia stiffness (N/mm)	a s i	56.55 (0.90)	62.07 (0.90)	70.58 (1.40)	81.80 (1.48)
Femur yield load (N)	a s i	15.80 (0.26)	15.23 (0.26)	16.49 (0.24)	14.40 (0.26)
Femur ultimate load (N)	a s i	19.62 (0.30)	18.98 (0.30)	20.53 (0.26)	18.21 (0.27)
Femur stiffness (N/mm)	a s i	110.3 (2.7)	154.0 (2.7)	108.6 (2.3)	139.2 (2.4)

Group Differences ($p \leq 0.05$) from ANOVA/ANCOVA as a function of age (a), sex (s), and sex by age interactions (i). Shading indicates significant within-sex age differences ($p \leq 0.05$).

older males, whereas body length was a significant predictor of femoral bone mechanics in older females. Body weight and length were not associated with hind limb rearing or quadrants entered in females, but body weight significantly predicted these activity measures in young and older males.

The associations between loading and the muscle factor that were noted for the pooled data set were not evident in models run separately for each sex and age; however, loading was positively associated with the tibial factor in older females and young males and the femoral factor in young females and older males. A positive association of muscle mass with bone mechanics was identified in all cohorts except older female tibia and older male femur. Perhaps the most striking result, found in both the tibial and femoral models for older males, was that locomotion was negatively associated with bone mechanics.

QTL analyses

QTL analyses of pooled data from the 200-day and 500-day F_2 cohorts revealed QTL for phenotypic measures

from at least two of the three domains of activity, muscle, and bone on chromosomes 4, 7, and 9. On Chr 4, hind limb rears and tibial yield and ultimate load co-located near marker D4Mit255 at 48.5 cM; quadrants entered and femur ultimate load co-located near marker D4Mit204 at 61.9 cM (Fig. 2). Muscle and skeletal measures for the femur mapped near marker D7Mit69 at 24.5 cM on Chr 7 (Fig. 3). Unadjusted muscle and skeletal measures for females mapped to Chr 9 between markers D9Mit196 at 48 cM and D9Mit212 at 61 cM. Although present, corresponding peaks for size adjusted data were much less impressive and not significant (Fig. 4).

Structural equation model B

Tables 3 and 4 present standardized regression weights from the tibial models incorporating genotype at markers D4Mit255 and D9Mit212 and the femoral models incorporating genotype at markers D4Mit204 and D7Mit69. Within the tibial model, marker D4Mit255 was positively linked to loading in young females, locomotion in young and older females, and bone mechanics in older females.

TABLE 2. Tibia and Femur Model A (Without Genetic Marker)

SEM model paths: model A				Tibia model					Femur model				
				All Groups	Females		Males		All Groups	Females		Males	
					Young	Older	Young	Older		Young	Older	Young	Older
Bone	←	Loading	(b)	0.215	0.127	0.246	0.211	0.129	0.049	0.230	-0.066	-0.010	0.267
Bone	←	Locomotion	(d)	-0.055	0.000	0.191	-0.131	-0.152	-0.061	-0.020	0.055	0.028	-0.163
Muscle	←	Loading	(a)	0.255	0.004	0.039	-0.075	0.037	0.258	0.008	0.041	-0.076	0.038
Muscle	←	Locomotion	(c)	0.059	0.107	0.066	0.134	-0.007	0.059	0.104	0.065	0.135	-0.005
Bone	←	Muscle	(e)	0.365	0.274	0.110	0.267	0.260	0.073	0.224	0.232	0.246	0.114
Muscle	←	Length		0.123	0.303	0.228	0.379	0.163	0.122	0.301	0.230	0.379	0.161
Muscle	←	Weight		0.611	0.295	0.380	0.303	0.340	0.612	0.300	0.378	0.303	0.339
Bone	←	Length		-0.080	0.036	0.153	-0.014	0.143	-0.051	0.178	0.251	0.130	-0.056
Bone	←	Weight		0.293	0.112	0.192	0.254	-0.006	0.260	0.125	0.044	0.360	0.289
Quadrants	←	Length		0.157	0.122	-0.057	0.046	0.165	0.157	0.123	-0.056	0.044	0.163
Rears	←	Length		0.017	0.057	0.044	0.026	0.043	0.018	0.058	0.047	0.024	0.041
Quadrants	←	Weight		-0.162	-0.153	-0.047	-0.268	-0.342	-0.162	-0.154	-0.047	-0.266	-0.341
Rears	←	Weight		-0.048	-0.047	-0.081	-0.195	-0.228	-0.048	-0.048	-0.082	-0.193	-0.227
Loading	↔	Locomotion		0.360	0.370	0.395	0.297	0.316	0.360	0.370	0.395	0.297	0.316

Standardized regression weights for pooled data and young and old female and male cohorts. Shading indicates paths that were significant at $p \leq 0.05$; bold italics indicates paths that were suggestive at $p \leq 0.10$. Paths a–e are indicated on Fig. 1, and were allowed to be free across groups.

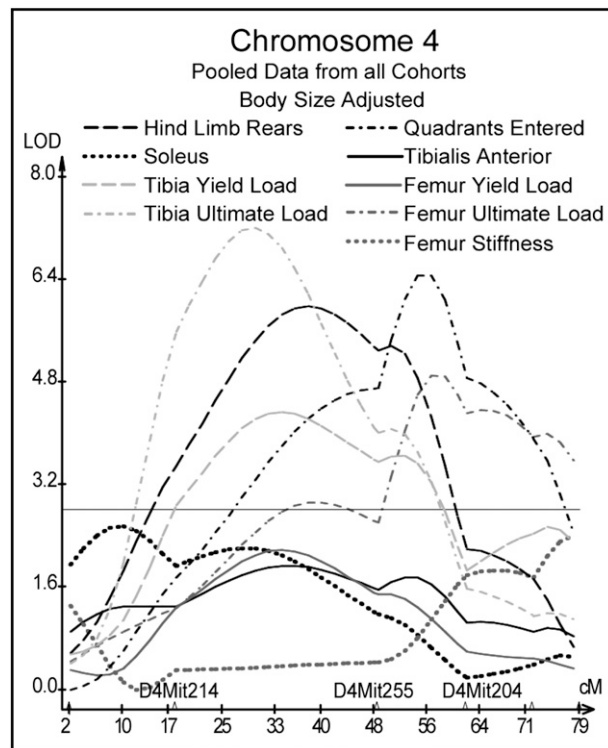


FIG. 2. Interval mapping results on Chr 4 for body size-adjusted residuals for activity, muscle, tibia, and femur measures pooled from all cohorts. LOD scores are plotted against centimorgan position along the chromosome. The horizontal line indicates the suggestive LOD score threshold of 2.8.

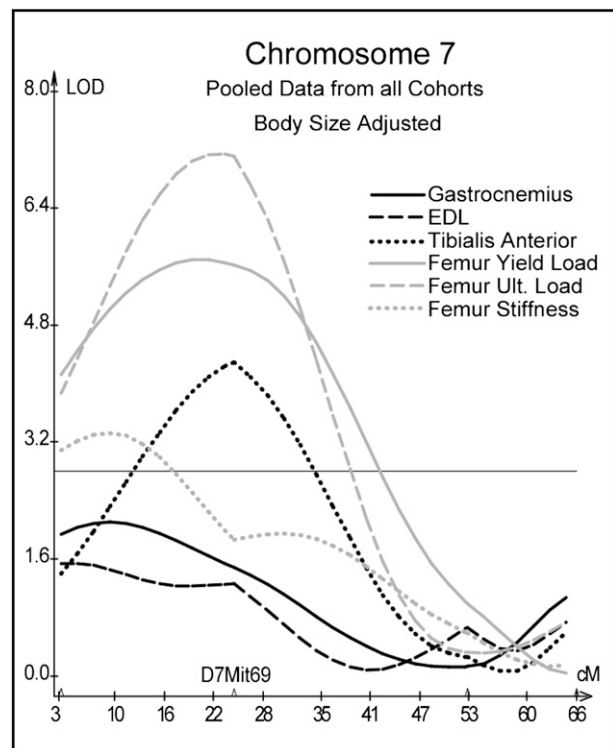


FIG. 3. Interval mapping results on Chr 7 for body size-adjusted residuals for muscle and femur measures pooled from all cohorts. See Fig. 2 legend.

This marker was negatively linked to muscle and positively linked to bone mechanics in young males (Table 3). Within the femoral models, marker D4Mit204 was positively linked to locomotion in young and older females and bone mechanics in older females and young and older males

(Table 4). The markers on Chr 7 and 9 were significantly linked to both muscle and bone mechanics in some of the subgroups. Marker D7Mit69 was positively linked to both muscle and bone mechanics in older females and young males and bone mechanics in older males (Table 4). Marker D9Mit212 was negatively linked to muscle and

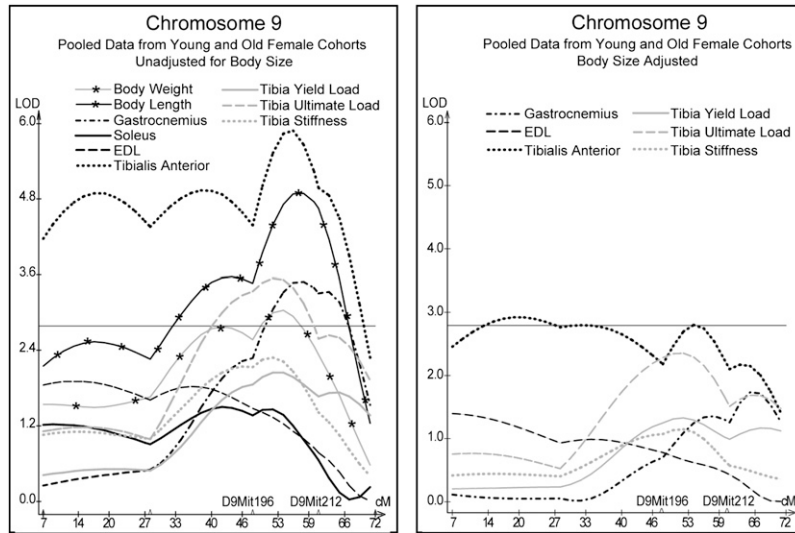


FIG. 4. Interval mapping results on Chr 9 for unadjusted and body size-adjusted residuals for muscle and tibia measures pooled from young and old female cohorts. See Fig. 2 legend.

TABLE 3. Tibia Model B (With Genetic Marker)

SEM for tibia model B paths			Marker: D4Mit255				Marker: D9Mit212			
			Females		Males		Females		Males	
			Young	Old	Young	Old	Young	Old	Young	Old
Loading	←	Marker	0.191	0.131	0.069	0.048	-0.059	0.049	0.050	0.050
Locomotion	←	Marker	0.201	0.204	0.012	0.017	0.013	0.003	0.001	-0.051
Muscle	←	Marker	-0.131	-0.139	-0.221	-0.122	-0.189	-0.023	-0.093	-0.389
Bone	←	Marker	0.078	0.173	0.308	0.132	-0.189	-0.146	-0.048	0.041
Bone	←	Loading	0.106	0.220	0.187	0.111	0.109	0.262	0.215	0.122
Bone	←	Locomotion	-0.022	0.142	-0.141	-0.152	0.013	0.190	-0.132	-0.148
Muscle	←	Loading	0.037	0.055	-0.048	0.048	-0.012	0.042	-0.069	0.091
Muscle	←	Locomotion	0.139	0.104	0.136	-0.007	0.112	0.066	0.132	-0.048
Bone	←	Muscle	0.283	0.135	0.341	0.282	0.238	0.111	0.262	0.276

Standardized regression weights with genetic marker D4Mit255 (left) and marker D9Mit212 (right) for young and old female and male data. Shading indicates paths that were significant at $p \leq 0.05$; bold italics indicates paths that were suggestive at $p \leq 0.10$.

TABLE 4. Femur Model B (With Genetic Marker)

SEM for femur model B paths			Marker: D4Mit204				Marker: D7Mit69			
			Females		Males		Females		Males	
			Young	Old	Young	Old	Young	Old	Young	Old
Loading	←	Marker	0.100	0.038	0.041	0.042	0.032	-0.031	-0.077	-0.069
Locomotion	←	Marker	0.182	0.199	0.083	0.075	-0.049	0.084	-0.014	0.030
Muscle	←	Marker	-0.121	-0.087	-0.054	-0.097	0.028	0.342	0.246	0.142
Bone	←	Marker	0.088	0.164	0.215	0.172	0.146	0.274	0.289	0.238
Bone	←	Loading	0.218	-0.070	-0.010	0.258	0.219	-0.035	0.051	0.310
Bone	←	Locomotion	-0.045	0.006	-0.005	-0.176	-0.007	0.019	0.046	-0.174
Muscle	←	Loading	0.021	0.040	-0.073	0.042	0.006	0.076	-0.033	0.062
Muscle	←	Locomotion	0.132	0.093	0.146	0.005	0.107	0.013	0.139	-0.014
Bone	←	Muscle	0.232	0.245	0.259	0.127	0.220	0.145	0.182	0.081

Standardized regression weights with genetic marker D4Mit204 (left) and marker D7Mit69 (right) for young and old female and male data. Shading indicates paths that were significant at $p \leq 0.05$; bold italics indicates paths that were suggestive at $p \leq 0.10$.

bone mechanics in young females, bone mechanics in older females, and muscle in older males (Table 3).

DISCUSSION

Structural equation model A

Mechanical loading, from both aerobic and resistance exercise, has been shown in both human^(34,35) and animal⁽³⁶⁾ studies to produce an osteogenic response. We therefore expected that higher levels of activity would be associated with stronger bone and indeed we found that both hind limb rearing and quadrants entered were positively correlated with all three tibial measures. SEM modeling using residualized scores for locomotion-free loading and loading-free locomotion confirmed a positive association between loading and bone mechanics in both sexes, but also revealed an interesting sex-dependent association between locomotion and bone mechanics that was positive in females and negative in males.

These findings suggest a differential response of bone to locomotion and loading as a function of sex and age. The positive effects of loading on bone are well documented; the negative effects of locomotion are more difficult to interpret. One possible explanation could be that a greater level of general locomotion (quadrants entered) results in a higher level of energy expenditure, thereby reducing non-lean mass (fat). Supporting this argument is the observed negative association between body weight and activity measures in males. More active animals had lower body weight, presumably because of enhanced energy use with attendant decreases in fat mass. Previous studies have shown within-subject reductions in bone mass as a consequence of weight loss,^(37–38) but the extent to which body weight induces alterations in skeletal loading and bone mass across individuals is unknown.

A study by Stewart et al.⁽³⁹⁾ examined the relationships of physical activity level, fitness, and body composition on BMD in older men and women. In women, body weight, fat mass, abdominal total fat, and lower and upper body strength were all highly correlated with BMD at the spine, hip, and total skeleton. In men, abdominal total fat was positively correlated with lumbar spine BMD, lower body strength was positively correlated with total hip BMD, and, similar to our findings in mice, daily physical activity was negatively correlated with lumbar spine BMD. The authors suggested that this finding could be explained by a trend of lower energy expenditure in more obese men.

A positive association between loading and bone mechanics was evident in the tibia of young females but only reached significance in the older female cohort. With age, rearing decreased in both sexes, although only significant in males, as did muscle mass after correcting for body size. Human studies have shown sex differences in the decline of muscle force and cross-sectional area with a significant decline in women around menopause and men at age 75.⁽⁴⁰⁾ A decrease in muscle strength with age was suggested by Frost⁽¹⁰⁾ to place bones previously adapted to stronger young-adult muscles in a state of partial and gradual-onset disuse. In this study, the decrease in muscle mass and

activity level may have precipitated these types of adaptations, thereby strengthening the relationship between loading and bone mechanics in older females. The decrease in rearing and muscle mass combined with a significant increase in quadrants entered could have contributed to the strengthened relationship of locomotion with bone mechanics in older females.

QTL analyses

Correlated phenotypes of muscle mass, bone mechanics, and activity often mapped in close proximity to each other indicating potential pleiotropic genetic effects. However, co-localization of correlated phenotypes to the same broad chromosomal region is not definitive evidence that all are under the control of any single gene or even a subset of genes. The broad marker spacing used in this QTL analyses is a limiting factor in teasing out pleiotropic effects and in the identification of potential genes of interest. The chromosomal regions identified here could include many genes and the exact locations of the gene or genes influencing the quantitative traits examined are unknown. High-resolution mapping will be required to distinguish between true pleiotropy and linkage of multiple QTLs. Nevertheless, anonymity of the gene or genes at work does not prevent exploration of the phenotypic relationships that they modulate.

Genetic influence of chromosome 4: markers D4Mit255 and D4Mit204 (model B)

A QTL for activity measures was identified near marker D4Mit255 (48.5 cM) in this study. Henderson et al.⁽⁴¹⁾ previously reported a QTL on Chr 4 at 52 cM that influenced general locomotor activity levels in 70-day-old male and female F₂ mice derived from inbred mice that were bidirectionally selected for open field activity. Results from the tibial model suggest that a gene or genes near marker D4Mit255 on Chr 4 has an influence on activity in females throughout young adulthood into old age, but the associated loading and locomotion does not produce a measurable effect on bone until old age, consistent with findings from model A.

Marker D4Mit255 was linked to bone mechanics in young males, with a similar trend in older males, but was not linked to loading or locomotion, suggesting that genes in this vicinity do not exert their effects on bone through mechanisms related to Wolff's Law. Interestingly, the adjacent marker, D4Mit204 at 61.9 cM, was significantly linked to tibial bone mechanics (results not shown) and femoral bone mechanics in both young and older males and femoral mechanics in older females.

Genetic influence of chromosome 7: marker D7Mit69 (model B)

Both muscle mass and femoral measures peaked near marker D7Mit69 on Chr 7. Body weight also mapped to this site. The *D2* allele had an increasing effect on the muscle and skeletal measures, whereas the *B6* allele had an increasing additive effect on body weight. When adjusting muscle and skeletal measures for body size, the LOD

scores increased. For example, the LOD score increased from 4.3 to 7.2 for femoral ultimate load and from 0.3 to 4.3 for tibialis anterior muscle mass. Removing the variance associated with body size increased the significance of the skeletal QTL by decreasing the residual "error" variance relative to the true phenotypic value.⁽³³⁾

Marker D7Mit69 was significantly linked to both muscle and femoral bone mechanics in older females. There was also the suggestion of a positive linkage between the muscle factor and bone mechanics in model B and a definite linkage when the marker is omitted (model A). Thus, it seems that this site affects female bone mechanics through muscle action but also through other as yet undefined pathways. In males, the results suggest that the influence of this QTL on muscle size was much stronger early in growth and development and acted on bone through muscle as evidenced by the significant path from the muscle factor to the bone factor in young males. With age, muscle mass could change more rapidly than bone mechanics irrespective of genotype, thereby reducing the association between the marker and muscle while maintaining a significant linkage between the marker and bone.

Whereas QTL analyses are limited in the precision with which a locus is mapped, identifying potential candidate genes within the region of a QTL is often the first step taken to elucidate the underlying mechanisms and genes responsible for the association. There are two candidate genes near marker D7Mit69 (23 cM) worth mentioning. Myogenic differentiation factor 1 (MyoD1) is located at 23.5 cM (MGI database 7-15-08) on Chr 7. MyoD1 is a muscle regulatory factor with the ability to convert fibroblasts to muscle cell lineages.⁽⁴²⁾ Variations in muscle cell differentiation could be contributing to variations in muscle mass and the corresponding covariation with bone strength. A second candidate gene, IGF1 receptor (Igf1r), is located at 33 cM on Chr 7. IGF1 is associated with increased bone strength and muscle volume and strength,⁽⁴³⁾ and variations in the Igf1 receptor at the locus on Chr 7 could be contributing to the covariance of muscle and bone.

Genetic influence of chromosome 9: marker D9Mit212 (model B)

Similar to the site on Chr 7, body weight and body length also co-located with muscle mass and skeletal phenotypes; however, on Chr 9, the B6 genotype contributed the increasing allele for all phenotypes. In this case, the most convincing QTL for muscle and skeletal measures on Chr 9 were from female data that were unadjusted for body size. When muscle and skeletal measures were corrected for body size, the LOD scores lost significance. Despite the negative impact of size correction in the QTL analysis, marker D9Mit212 remained significantly linked to the muscle and bone factors in the SEM analysis even when controlling for body weight and length. Similar to the results for marker D7Mit69 (in males), this QTL could be influencing muscle size and strength during growth and development resulting in larger and or stronger bones; with age, muscle mass varies because of other factors,

whereas bone mechanics maintains its linkage with marker D9Mit212.

Conclusions

The complex system responsible for bone adaptation, and ultimately fracture resistance, extends beyond the skeletal system to encompass other physiologic processes. Principal among these may be components related to muscle mass and force generation as well as components dictating locomotion and activity-related behaviors, because these contribute substantially to the loads borne by the skeleton.

QTL results indicate that a gene or genes on Chr 4 has pleiotropic effects on muscle mass, activity, and bone mechanics and genes on Chrs 7 and 9 affect muscle mass and bone mechanics. Follow-up SEM showed that the effects of frequency and magnitude of loading on the skeleton were both sex and age dependent. Muscle mass was the best predictor of bone mechanics in young female mice, whereas loading was a better predictor in older females, suggesting that skeletal loading through resistance type activities is closely linked to skeletal health in older females. Both loading and muscle mass predicted bone mechanics in males. Interestingly, locomotion was negatively associated with bone mechanics in older males, suggesting again that more resistance type activities may be better able to maintain bone health in older individuals. Further exploration of these and other mechanisms through which genes function will aid in the development of individualized interventions able to exploit the numerous complex pathways contributing to skeletal health.

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