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Effects of load-bearing exercise on skeletal structure and mechanics differ between outbred populations of mice

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ABSTRACT

Effects of load-bearing exercise on skeletal structure and mechanical properties can vary between inbred strains of mice. Here, we examine whether such variation also exists at the population level. An experiment was performed with two outbred mouse stocks that have been reproductively isolated for >120 generations (Hsd:ICR, Crl:CD1). Growing females from each stock were either treated with a treadmill-running regimen for 1 month or served as controls. Limb forces were recorded with a force plate and cage activity monitored to verify that they were similar between stocks. After the experiment, femoral cortical and trabecular bone structure were quantified with micro-CT in the mid-diaphysis and distal metaphysis, respectively, and diaphyseal structural strength was determined with mechanical testing. Among Hsd:ICR mice, running led to significant improvements in diaphyseal bone quantity, structural geometry, and mechanical properties, as well as enhanced trabecular morphology. In contrast, among Crl:CD1 mice, the same running regimen had little effect on cortical and trabecular structure and significantly reduced diaphyseal resistance to fracture. In neither stock was body mass, muscle mass, or cage activity level different between runners and controls. Given that most environmental variables were controlled in this study, the differential effects of exercise on Hsd:ICR and Crl:CD1 bones were likely due to genetic differences between stocks. These results suggest that the benefits of loading for bone may vary between human populations (e.g., ethnic groups), in which case exercise programs and technologies designed to promote bone health with mechanical signals may be more advantageous to certain populations than others.

Introduction

Studies of humans and animal models frequently demonstrate the potential for load-bearing exercise to augment skeletal structure and strength. In many cases, however, the skeletal benefits of exercise are found to vary between individuals, ranging from some individuals displaying substantial improvements to others remaining largely unaffected. Such studies underscore the fact that the responsiveness of an individual's bone to loading depends on a number of non-mechanical factors, particularly genetics [1–9], as well as age [10,11], sex [11–13], and others.

Experiments with inbred mouse strains provide compelling evidence for the role of an individual's genome in modulating bone mechanoresponsiveness [1–9]. For example, exogenous limb loading studies have shown that certain inbred strains require more mechanical deformation in their bone diaphyses to trigger osteogenesis [4], and once the osteogenic threshold is surpassed, they exhibit less bone formation per unit increase of deformation [1,4,6]. The limb bones of particular inbred strains are also less responsive to jumping and running exercise [2,9], as well as low-level mechanical vibration [3]. Mapping studies with inbred strain crosses have shown that numerous genomic regions harbor alleles that affect bone mechanotransduction [5,7,8], which heightens the potential for bone mechanoresponsiveness to vary from one individual to the next.

Variation in skeletal adaptability to loading may also exist at the population level, that is, between *groups of individuals* that differ genetically and by demographic or environmental variables. For example, several ethnically distinct human populations are known to exhibit marked differences in skeletal structure and strength [14,15]; inasmuch as such differences are due to inter-group divergence in allele frequency [16], it is reasonable to hypothesize that one way in which gene polymorphisms exert their influence on skeletal variation is by regulating bone mechanoresponsiveness [17,18]. The results of studies with inbred mice are of limited value in evaluating this hypothesis since each inbred strain is by definition genetically homogenous, unlike natural populations that are genetically variable.

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Outbred stocks of mice provide a useful model for experimentally establishing the degree to which genetically distinct populations differ in their skeletal response to loading. Outbred stocks by definition are closed populations of genetically variable animals that are bred to maintain maximum heterozygosity [19]. Large-scale genetic analyses have shown that the degree of genetic diversity within many stocks is similar to that which exists within ethnically distinct human populations [20], and that substantial genetic variation also exists between stocks, with certain ones differing from each other at least as much as human populations [20,21].

In this study, we compare the effects of load-bearing exercise on limb bone structure and mechanical properties between two commercially available outbred stocks, Hsd:ICR (ICR) and Crl:CD1 (CD1). These particular stocks were chosen for analysis because their ancestry is especially well documented [19,20] and their genetic diversity displays important similarities to human populations [20,21]. Ultimately, a more complete understanding of variation in skeletal adaptability at the population level will help clarify whether exercise interventions and therapeutic mechanical stimulation aimed at enhancing bone health in humans can be expected to be uniformly successful if applied broadly across different populations.

Materials and methods

Experimental design

Female ICR and CD1 mice were acquired from Harlan Sprague Dawley (Indianapolis, IN, USA) and Charles River Laboratories (Wilmington, MA, USA), respectively, at 3 weeks of age (n = 46/stock). Non-siblings were requested in order to maximize genetic diversity within samples. Young animals were employed because the osteogenic response to exercise is typically greatest during the growth period [10,11]. Mice were housed individually in standard cages and maintained on a 12:12-hr light–dark cycle with free access to water and food. At 4 weeks of age, 40 mice from each stock were divided into runners and sedentary controls (n = 20/stock/activity group). Runners were treated with 30 min of treadmill running 5 days/week for 4 weeks. Animals were exercised on a Columbus Instruments Exer-3/6 treadmill (Columbus, OH, USA) at a rate of 12 m/min. Exercise was administered with lights turned on when controls were typically sleeping. Controls were handled but did not run. Cage activity was quantified for all 80 animals at the end of weeks 2 and 3 of the exercise program. At 8 weeks of age, animals were euthanized by CO_2 inhalation, right quadriceps muscles were dissected and weighed, and right femora were extracted and wrapped in gauze soaked with 1× PBS and stored in a freezer (−20 °C). Hind limb forces generated in quadrupedal locomotion were measured in the 6 additional mice from each stock between 6 and 7 weeks of age. All experimental procedures were reviewed and approved by the IACUC of Stony Brook University.

Outbred stocks

The ICR and CD1 stocks both derive from a small group of non-inbred albino mice imported to the United States from Switzerland in 1926 [22]. Descendants were used to establish breeding colonies around the country, including one at Charles River Laboratories in 1959 named CD1 [23]. In 1983, Harlan Sprague Dawley acquired CD1 mice and started another colony named ICR [20]. Thus, ICR and CD1 mice have been reproductively isolated since 1983 for at least 120 generations, which would correspond in humans to at least 3400 years of genetic separation [24]. Genetic isolation has led to clear population stratification that is comparable to that of human groups [21]. For example, F_{ST}, a measure of genetic diversity between populations, is approximately 0.11 for ICR and CD1 stocks [20], whereas ethnically distinct but closely related human populations typically exhibit values less than 0.05 [25], meaning that allele frequency differentiation between ICR and CD1 mice is at least as great as that between many human groups. Average heterozygosity in both stocks is approximately 0.30 [20], which is well within the range of human populations [26].

Microcomputed tomography

Femora were scanned in distilled water at a 10-μm^3 voxel size (70 kVp, 114 μA, 150-ms integration time) using a μCT 40 scanner (Scanco Medical AG, Brüttisellen, Switzerland). Cortical bone was assessed in a 600-μm-long region of the diaphysis centered at half bone length. Trabecular bone was assessed in a 1100-μm-long region of the distal metaphysis starting 850 μm proximal to the growth plate. Micro-CT images were calibrated using hydroxyapatite phantoms. Volumes were segmented using a constrained 3D Gaussian filter to reduce noise (support = 1, sigma = 0.1 [diaphysis] and 0.5 [metaphysis]) and thresholded to extract the bone phase. Threshold values chosen for cortical and trabecular bone (593.1 and 428.1 mg HA/cm^3, respectively) were determined empirically to achieve maximal concordance between raw and thresholded images. Repeatability of this thresholding method is high [27]. Trabecular bone was isolated from the cortical shell using an automated algorithm [28]. Bone properties were computed using the internal imaging code supplied by the scanner manufacturer. Cortical bone traits included periosteal and endocortical areas (Ps.Ar, Ec.Ar; mm^2), cortical area (Ct.Ar; mm^2), average cortical thickness (Ct.Th; mm), maximum and minimum second moments of area (I_{max}, I_{min}; mm^4), polar moment of area (J; mm^4), intracortical porosity (Ct.Po; %), and tissue mineral density (TMD; mg HA/cm^3). Trabecular bone traits included bone volume fraction (BV/TV; %), trabecular number (Tb.N; 1/mm), trabecular thickness (Tb.Th; μm), and trabecular separation (Tb.Sp; μm). All traits were defined as recommended [29].

Mechanical testing

Femoral diaphyses were loaded in three-point bending to failure using an MTS 858 Mini Bionix II material testing machine (MTS System Corp., Cary, NC, USA) fitted with a 100-N force cell (SMT1-100N; Interface, Inc., Scottsdale, AZ, USA). Tests were conducted at room temperature. Bones were tested in the anteroposterior direction with the posterior surface in compression [30]. Loading velocity was 0.05 mm/s and data were recorded at 100 Hz. Load–displacement curves were used to calculate structural (whole-bone) mechanical properties, including ultimate force (F_u; N), yield force (F_y; N), and stiffness (S; N/mm). Yield was defined as the point where the load–displacement curve becomes non-linear. Mid-diaphyseal second moments of area about the bending axis (I_{ML}), as well as the distance from the bone area centroid to the surface in tension (c), were calculated from μCT images using BoneJ software [31]. Ultimate stress (σ_u; MPa) was calculated as $F_u \times Lc/4I_{ML}$, yield stress (σ_y; MPa) as $F_y \times Lc/4I_{ML}$, and Young's modulus (E; MPa) as $S \times (L^3/48I_{ML})$, where L equals the 6-mm span between the outer supports [32].

Cage activity

Habitual cage activity was monitored using a 16-chamber Opto-M3 system (Columbus Instruments), in which each cage was placed into a monitoring apparatus that casted a grid of infrared beams (1.27 cm^2) above the cage floor. No additional stimulus was provided by the monitoring system, and animals maintained free access to water and food. Each animal was monitored on two separate occasions during non-running days. Monitoring sessions lasted 24 hr, with measurements recorded in 1-hr intervals. Overall cage activity was quantified as the total number of times during a 24-hr period that a new infrared beam was broken. Repeated interruptions of individual beams caused by scratching, grooming, or other non-ambulatory movements were disregarded. For each animal, data collected during the two 24-hr sessions were averaged.