Do different locomotor modes during growth modulate trabecular architecture in the murine hind limb?

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Synopsis Vertebrate morphologists often implicate functional adaptations of bone to mechanical milieu when comparing animals with distinct behavioral repertoires. Functional morphologists frequently use comparative osteology and locomotor behavior to construct correlative form–function relationships. While some experimental work has investigated functional adaptations of bone elicited by specific locomotor behaviors, these studies usually manipulate repertoires by introducing artificial situations (e.g., treadmills) or creating differences in the level of activity (i.e., exercise), either of which can compromise extrapolations to free-ranging animals. Here, we present trabecular bone morphology and microarchitecture from an inbred mouse model in which components of naturalistic locomotor repertoires were accentuated. Using inbred mice, we control for genetic variability, further isolating the osteogenic responses to these behaviors. Single female (BALB/cByJ) mice (n = 10 per group) were housed for 8 weeks beginning at 30 days postbirth in custom-designed cages that accentuated either linear quadrupedalism or turning. Concurrently, mice in a control group were housed singly in open cages. The distal femoral metaphysis was scanned by micro-computed tomography at the end of the 8-week experiment protocol. The experimental groups, particularly the “linear” group, differed significantly from the control group (simulated “free-ranging” condition) in several variables: bone volume fraction (“linear” 42% less than controls; “turning” 24% less than controls), trabecular number (“linear” 12% less than controls; “turning” 9% less than controls), connectivity density (“linear” 43% less than controls; “turning” 35% less than controls), and a characterization of trabecular surfaces (“linear” 15% greater than controls; “turning” 11% greater than controls). No differences in the degree of anisotropy were observed among groups, and generally, “linear” and “turning” groups did not differ significantly from one another in any measures of trabecular microarchitecture. Considering the distinct differences in locomotor behaviors between the “linear” quadrupedalism and “turning” groups, these data suggest that comparisons at the distal femoral metaphysis of trabecular microarchitecture or orientation between different groups of animals may be somewhat limited in accurately reconstructing the loading conditions associated with different locomotor modes.

Introduction

Behavioral input in the osteogenic response of limb bones is of primary interest to functional morphologists studying the vertebrate skeleton. While heredity and developmental history contribute to variability in the musculoskeletal system of vertebrates, deformation of bones resulting from dynamic loads that are generated during locomotor modes is a source of variability as well (Martin et al. 1998; Judex et al. 2008). This latter signal—an osteogenic response to deformations during locomotion—is of particular interest to functional morphologists since, when used with appropriate caution, it can be used to infer locomotor activities and the loading patterns that characterize them. Much effort has been devoted towards documenting the biomechanics of vertebrate locomotor activities in order to appreciate how organisms load their limbs during locomotion (Alexander 2003; Biewener 2003). Partly due to a lack of appropriate models, relatively less attention has been devoted towards documenting osteogenic responses at an organismal level during naturalistic locomotor activities. Such information, however, would be extremely valuable to functional morphologists, particularly those studying extinct taxa.

Mouse models provide unique opportunities to study changes in modeling and remodeling responses within limb bones that arise from locomotor behaviors (e.g., Middleton et al. 2008). This is particularly true for inbred mouse models because mice within any given inbred strain are considered genetically identical. A variety of inbred strains are available, representing...
a range of sensitivities in osteogenic potential (Wergedal et al. 2005). These models have been used successfully to separate genetic from environmental variables both during periods of increased (Robling et al. 2007) and decreased (Judex et al. 2004) mechanical loading. The small body size and rapid development of mice are favorable characteristics when designing experimental animal models of the osteogenic response to locomotor behaviors.

The mechanical basis of trabecular architecture has been an area of interest ever since Wolff (1892) introduced his trajectorial hypothesis and Roux (1881) injected the notion of functional adaptation into trabecular architecture (see reviews in Roesler 1981; Huiskes 2000). The introduction of micro-computed tomography, or microCT (Layton et al. 1981; Huiskes 2000). The introduction of micro-computed tomography, or microCT (Layton et al. 1981; Feldkamp et al. 1989; Kuhn et al. 1990; Rüegsegger et al. 1996), as a nondestructive means of visualizing intact trabecular bone, undoubtedly accelerated the emergence of comparative studies. The assessment of internal characteristics of bony elements (e.g., femoral trabecular architecture) between organisms characterized by different locomotor modes has been a relatively recent pursuit (Rafferty 1998; Fajardo and Müller 2001; MacLatchy and Müller 2002; Ryan and Ketcham 2002a; Ryan and van Rietbergen 2005; Maga et al. 2006; Fajardo et al. 2007; Volpato et al. 2008). Comparative studies, such as these, have proposed associations between degree of anisotropy (DA) in trabecular architecture and locomotor stereotypy.

Relatively fewer studies (Pontzer et al. 2006; Carlson and Judex 2007) have manipulated locomotor behaviors experimentally to assess direct alterations in bone architecture. Pontzer et al. (2006) used a Radon transformation-based analysis (i.e., combined trabecular strut orientation, size, number, and localized mineral density) to investigate trabecular orientation in the distal femur of guinea fowl. Distinct trabecular orientations that they observed were attributed to the difference between running on an inclined treadmill (more flexed-knee posture) versus running on a level treadmill (more extended-knee posture). Carlson and Judex (2007) reported a modest but significant difference in shape of the femoral midshaft between inbred female BALB/cByJ mice that were encouraged to perform turns during quadrupedal locomotion compared with individuals encouraged either to travel in a linear direction or permitted unrestricted movement. While Carlson and Judex (2007) did not measure external forces or deformations of bone matrix, the turning group exhibited slightly increased mediolateral (ML) rigidity coupled with slightly decreased anteroposterior (AP) rigidity relative to the other two groups. When these differences were combined as a ratio (i.e., a measure of the shape of the femoral midshaft), they reported significant differences between groups. The distinct shape of the femoral midshaft of the turning group is supported by other studies of turning behavior (Walter 2003; Demes et al. 2006). Mice performing 90° turns were observed to display greater limb abduction during turns than during linear locomotion (Walter 2003). Increased limb abduction during linear locomotion (Carlson 2005b) and turning (Demes et al. 2006) have been associated with increased ML external forces in primates, and presumably occurs in mice too.

Here, we demonstrate another opportunity that the use of inbred mouse models may offer to issues of interest among functional morphologists. Specifically, we test the hypothesis that trabecular architecture in the distal femoral metaphysis adjusts itself during skeletal growth to reflect different habitual locomotor behaviors. To this end, we compare two experimental groups—one in which linear locomotion is emphasized and one in which turning is emphasized—to a group of (control) mice that are permitted to freely roam an open cage. We predict that control mice will exhibit a lesser DA in their trabecular structure relative to the experimental groups because of greater variability in the locomotion of the former. We further ask the question whether differences in locomotor behavior (i.e., linear versus turning versus “free-ranging” locomotion) will modulate other measures of trabecular microarchitecture.

**Methods and materials**

The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Stony Brook University. Thirty female BALB/cByJ mice (The Jackson Laboratory, Bar Harbor, ME, USA) were assigned randomly to one of two experimental groups, or to a control group (n = 10 per group). Mice in experimental groups were housed singly in one of two experimental enclosures (Fig. 1) for the duration of the 57-day experimental period [see Carlson and Judex (2007) for additional details]. One experimental enclosure was designed to emphasize linear locomotion (subsequently called the linear group), while the other was designed to accentuate turning during locomotion (subsequently called the turning group). Mice in the control group were housed singly in standard laboratory mouse cages with wire tops. Food pellets (standard mouse chow) were available to all mice ad libitum. Water was freely available...
Trabecular bone response to murid locomotion

Fig. 1 Custom-designed enclosures for “turning” (left) and “linear” (right) treatment groups.

through a tube protruding downward into the cage. Use of a short vertically oriented sipper tube effectively restricted climbing by the experimental groups. Climbing also was discouraged in the experimental groups by using a flat plexiglass cage top rather than a standard wire top. For experimental groups, pellets were placed on the end of the cage floor opposite the water source in order to stimulate use of the intervening tunnel. The control group had access to food pellets placed onto the top of the wire cage. Mice in the control group could access food pellets without climbing, but climbing was not discouraged. When climbing was observed, it usually approximated suspension by forelimbs followed by some “brachiation” using predominantly forelimbs, or on occasion involved all four limbs similar to “inverted quadrupedalism” in primates (Hunt et al. 1996).

Mice were introduced to enclosures ~30 days after birth and subject to 12:12 (h) light:dark cycles over the duration of the protocol period. Individual body mass and food intake were monitored weekly to ensure acceptable health, stress, and activity levels during the protocol period. Weekly water consumption was not monitored.

Daily behavioral assessments were performed over the course of the protocol period [see Carlson and Judex (2007) for additional details]. Positional behavior (Prost 1965) was documented approximately twice per day for each subject (n = 92 observations per individual). Behavioral observations for any given day usually were performed at least 8 h apart, but always at least 5 h apart (mean difference in daily observation times = 10.8 ± 2.3 h). Daily observation times were varied in order to prevent oversampling particular periods of activity or sleep of subjects. Behavioral observations were recorded using an instantaneous focal-sampling strategy (Altmann 1974).

This consisted of carefully recording the behavior of a focal animal at a prearranged time. During a behavioral assessment, mice served sequentially as instantaneous focal animals until the behavior of each subject was recorded. Behavioral categories were constructed from standard positional behavioral modes used previously for quantifying primate behavioral repertoires (Hunt et al. 1996; Carlson 2005a; Carlson et al. 2006). Behavioral modes were devised to be self-descriptive and to cover the range of all observed behaviors. Behavioral modes can be divided into two broad categories: locomotor behaviors (i.e., walk, run, jump, climb) and postural behaviors (i.e., lie, sit, stand using three or four limbs, stand using both forelimbs only, stand using both hind limbs only). Percentage locomotor behavior was used as a proxy measure for activity level (Table 1).

To assess whether twice daily behavioral observations were representative of underlying activity patterns, additional behavioral observations using an instantaneous focal sampling strategy with 1-min intervals (Altmann 1974) were applied for 1 h to randomly selected individuals from each experimental group. These observations also provided an opportunity to assess use of the tunnel by experimental groups. A tendency towards higher frequency of tunnel use in the linear group was equalized by a longer distance the turning group traveled through the tunnel in one pass (mean linear travel distance per hour = 2183.85 cm; mean turning travel distance per hour = 1953.6 cm) (Carlson and Judex 2007). Thus, equivalent tunnel use by subjects from the experimental groups was assumed. In other words, a difference in the level of activity (i.e., an exercise effect) was unlikely.

At day 57, subsequent to being placed in enclosures, mice were sacrificed and limbs were disarticulated. Left femora were submerged in a solution of 70% ethanol and 30% distilled water and scanned in a microCT system (Scanco μCT 40: Scanco Medical AG, Bassersdorf, Switzerland). All specimens were scanned longitudinally with a 10 μ isotropic spatial resolution. Reconstructed images were filtered with a constrained 3D Gaussian filter to reduce noise (σ = 0.5 and support = 1) and segmented using a global threshold of 31.8% of the maximum gray value for the acquisition of all volumes of interest (VOIs).

Several comparative primate studies of trabecular architecture have focused on proximal regions of the femur (Fajardo and Müller 2001; MacLatchy and Müller 2002; Ryan and Ketcham 2002a; Ryan and Ketcham 2005; Fajardo et al. 2007) because kinematic differences in the hind limb associated with various habitual locomotor modes likely create differences in hip joint loading. In our initial analysis of femoral
Trabecular architecture with an inbred mouse model, we selected the distal femoral metaphysis because of an abundance of trabecular bone at this location (unlike the proximal femur), and because it is a standard anatomical region for comparing responses of trabecular architecture to adjustments in loading conditions (Judex et al. 2004). The knee (i.e., a hinge joint), however, has a more restricted range of movement relative to the hip (i.e., a ball-and-socket joint), which could constrain group differences in loading variability at the distal femur relative to the proximal femur. Analysis of trabecular architecture at additional anatomical locations is either ongoing (e.g., proximal femur and proximal tibia) or planned (i.e., proximal and distal humerus).

Trabecular VOIs were extracted from 1200 μm-long regions of the distal femoral metaphysis (Fig. 2) using a custom-written script routine in Image Processing Language (Lublinsky et al. 2007). Measures of trabecular bone microarchitecture (Parfitt et al. 1983, 1987; Benn 1994; Hildebrand and Rüegsegger 1997) were calculated including: total volume (TV), bone volume (BV), bone volume fraction (BV/TV), number of trabeculae (Tb.N), average trabecular thickness (Tb.Th), average trabecular separation (Tb.Sp), connectivity density (Conn.D), structural model index (SMI), and DA.

Table 1 Mean values of characteristics of the two experimental groups and of controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Linear, n = 10</th>
<th>Turning, n = 10</th>
<th>Control, n = 10</th>
<th>P-value</th>
<th>Post hoc tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locomotor behavior (%)</td>
<td>14.2 (3.7)</td>
<td>14.0 (8.8)</td>
<td>12.9 (4.2)</td>
<td>NS</td>
<td>–</td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>22.0 (1.3)</td>
<td>22.7 (1.2)</td>
<td>22.9 (1.6)</td>
<td>NS</td>
<td>–</td>
</tr>
<tr>
<td>Femur length (mm)</td>
<td>12.7 (0.3)</td>
<td>12.6 (0.1)</td>
<td>12.5 (0.4)</td>
<td>NS</td>
<td>–</td>
</tr>
<tr>
<td>TV (mm³)</td>
<td>1.205 (0.137)</td>
<td>1.230 (0.087)</td>
<td>1.205 (0.137)</td>
<td>0.876</td>
<td>C≈L (+0.06%)</td>
</tr>
<tr>
<td>BV (mm³)</td>
<td>0.088 (0.029)</td>
<td>0.101 (0.021)</td>
<td>0.124 (0.032)</td>
<td>0.025</td>
<td>C&gt;&gt; L (−41%)</td>
</tr>
<tr>
<td>BV/TV (%)</td>
<td>7.2 (1.9)</td>
<td>8.2 (1.5)</td>
<td>10.2 (2.1)</td>
<td>0.005</td>
<td>C&gt;&gt; L (−42%)</td>
</tr>
<tr>
<td>Tb.N (1/mm)</td>
<td>4.100 (0.484)</td>
<td>4.184 (0.213)</td>
<td>4.574 (0.332)</td>
<td>0.015</td>
<td>C&gt;&gt;L (−12%)</td>
</tr>
<tr>
<td>Tb.Th (mm)</td>
<td>0.038 (0.002)</td>
<td>0.040 (0.002)</td>
<td>0.041 (0.003)</td>
<td>0.092</td>
<td>C≈L (−8%)</td>
</tr>
<tr>
<td>Tb.Sp (mm)</td>
<td>0.256 (0.034)</td>
<td>0.249 (0.013)</td>
<td>0.232 (0.016)</td>
<td>0.075</td>
<td>C≈L (+9%)</td>
</tr>
<tr>
<td>Conn.D (1/mm³)b</td>
<td>77.425 (36.599)</td>
<td>82.285 (10.584)</td>
<td>110.946 (16.380)</td>
<td>0.008</td>
<td>C&gt;&gt;L (−43%)</td>
</tr>
<tr>
<td>SMI</td>
<td>2.550 (0.314)</td>
<td>2.430 (0.240)</td>
<td>2.159 (0.212)</td>
<td>0.007</td>
<td>C&lt;&lt;L (+15%)</td>
</tr>
<tr>
<td>DA</td>
<td>1.428 (0.074)</td>
<td>1.476 (0.095)</td>
<td>1.480 (0.077)</td>
<td>0.316</td>
<td>C≈L (−4%)</td>
</tr>
</tbody>
</table>

Cells report group means on top and 1 SD in parentheses.

*Corresponds to P<0.05; **Corresponds to P<0.01; values in parentheses on the far right are percentage difference in means of linear (L) and turning (T) groups versus the control (C) group. See text for abbreviation definitions.

*Locomotor behavior (%) reports the percentage of 92 observations per individual in which the individual was engaged in a dynamic behavioral mode (Carlson and Judex 2007).

*bFailed a Levene test of homogeneity of group variances. Since unequal variances can call an F-statistic into question, additional robust tests for the equality of means were performed (i.e., Welch and Brown-Forsythe tests). In each case, groups mean connectivity differed significantly (Welch test statistic = 10.959, P = 0.001; Brown-Forsythe test statistic = 5.724, P = 0.015). Thus, ANOVA post hoc analysis was performed using a Tamhane’s T2 procedure. Post hoc testing for all other variables was performed using Fisher’s LSD.
(Tb.Sp = 1.767, TV = −1.055), further indicating normality of the data. We used one-way ANOVA to examine the extent of differences among groups.

Equality of group variances is assessed by a Levene test of homogeneity of variances. When groups exhibit unequal variances for a variable (i.e., Conn.D), we conducted additional one-way Robust tests for the equality of means (i.e., Welch and Brown-Forsythe tests). If these tests also indicated a significant difference between group means, we pursued further analysis of the ANOVA using Tamhane’s T2 post hoc analyses. For evaluation of significant differences in group means when variances were not different between groups, we used Fisher’s least significant difference (LSD) post hoc analyses. Statistical significance was established at $P < 0.05$. All analyses were performed using SPSS 15.0 for Windows (SPSS, Inc., Chicago, IL, USA).

**Results**

Previous comparisons demonstrated that mean percentage locomotor behavior, body mass, and mechanical length of the femur do not differ significantly among groups (Carlson and Judex 2007). At the termination of the 8-week protocol, trabecular bone in the distal femoral metaphysis of the linear group exhibited 41% lower BV ($P = 0.008$), 42% lower BV/TV ($P = 0.002$), 12% lower Tb.N ($P = 0.007$), and 43% lower Conn.D ($P = 0.004$) than the control group (Table 1, Fig. 3). The linear group exhibited a 15% higher SMI ($P = 0.002$) than did the control group (Table 1, Fig. 3), meaning the linear group exhibited significantly more rod-like trabeculae relative to more plate-like trabeculae in the control group. Trabecular VOIs from the analogous location of the turning group exhibited 24% lower BV/TV ($P = 0.024$), 9% lower Tb.N ($P = 0.022$), and 35% lower Conn.D ($P = 0.012$) than the control group (Table 1, Fig. 3). The turning group exhibited an 11% higher SMI ($P = 0.027$) than did the control group (Table 1, Fig. 3), meaning the turning group exhibited significantly more rod-like trabeculae relative to more plate-like trabeculae in the control group.

Groups differed by <10% in DA ($P = 0.316$), average TV ($P = 0.876$), Tb.Th ($P = 0.092$), and Tb.Sp ($P = 0.075$) (Table 1). Thus, groups did not differ in the uniformity of orientation of trabecular struts (Fig. 3). Linear and turning groups did not differ significantly in any trabecular microarchitecture or strut-orientation variables, although it is interesting that averages for the turning group were consistently intermediate between control and linear groups.

**Discussion and conclusions**

In order to demonstrate the opportunities that inbred mouse models may offer to functional morphologists, we sought to assess functional adaptations of bone in the murine hind limb in response to distinct locomotor behaviors. Specifically, we tested the hypothesis that trabecular architecture in the distal femoral metaphysis adjusts itself during skeletal growth in ways that reflect different frequencies of linear locomotion versus turning versus “free-ranging” locomotor behavior. The only significant differences among groups in trabecular architecture were observed between the two experimental groups, particularly the linear group, versus the control group. Contrary to our expectation, DA did not differ between any groups, despite their stark differences in locomotor repertoires. The absence of significant differences in trabecular architectures between the experimental groups implies that trabecular architecture of the distal femoral metaphysis may not distinguish the mechanical loading regimes associated with turning (Eilam 1994; Walter 2003; Jindrich et al. 2006, 2007; Demes et al. 2006) from those associated with linear locomotion (Clarke and Still 1999; Clarke et al. 2001; Zumwalt et al. 2006). While bone deformations or external forces were not quantified in the present study, previously reported differences in shape at the femoral midshaft diaphysis of the same mice (Carlson and Judex 2007) provide strong evidence for distinct femoral midshaft loading
conditions during linear locomotion versus turning activities.

The absence of differentiation in trabecular architectures between the experimental groups is complicated by an incomplete understanding of how the distal femur is loaded during turning behavior. Even though mice in the linear group performed turning behavior at a much reduced frequency, they still engaged in turns when entering or exiting tunnels. While this minimal frequency was not enough to obscure significant differences in shape of their femoral diaphyses (Carlson and Judex 2007), it may have been large enough to surpass a specific threshold required for initiation (or enhancement) of remodeling and modeling events in trabecular regions elsewhere in the femur (i.e., the distal femoral metaphysis). Alternatively, ML loading of the hind limb at the distal femoral metaphysis may be rather similar across groups regardless of turning frequency because, as a hinge joint, the knee intuitively would

Fig. 3 Boxplots for BV/TV (A), Tb.N (B), Conn.D (C), SMI (D), and DA (E). Box hinges represent 25th and 75th percentiles, while the horizontal line inside a box represents the median. Whiskers represent minimum and maximum observed values that are not statistical outliers; outliers are represented by circles.
seem structurally restricted in ML movements. Trabecular architecture in the distal femoral metaphysis could be more attuned to vertical (compressive) forces transmitted through the knee joint, which may not have distinguished groups (e.g., average body mass did not differ between groups). While mice in the present study were still growing at the termination of the experimental protocol, it is unclear if an insensitivity to functional loading may have become entrenched at some point leading up to their cessation of growth. Biewener et al. (1996) suggested that following the cessation of growth, insensitivity in adjustments to trabecular orientation despite altered functional loading may have arisen in the calcanei of tenotomized potorooys. Ultimately, detailed data on the loading conditions imposed by turning behavior versus linear locomotion in these mice will be critical for interpreting the observed lack of differences in trabecular architectures at the distal femoral metaphyses of the two experimental groups.

It may be worthwhile to consider the observed pattern of trabecular architectural adjustment in the three groups in the context of potentially reduced activity levels. Future behavioral assessments will be expanded to investigate this possibility. If controls were more physically active than the experimental groups in the present study, the previously observed differences in shape of the femoral midshaft between turning and control groups (Carlson and Judex 2007) becomes even more intriguing. Perhaps loading regimes at the femoral midshaft diaphysis (e.g., bending strain magnitudes) differed between linear locomotion and turning (i.e., direction change) to a greater extent than currently appreciated. Alternatively, trabecular bone in the control group, as assessed by BV, BV/TV, Tb.N, Conn.D, and SMI properties, may not have benefited from potentially increased activity levels, but from a more diverse range of locomotor activities when compared with linear and turning mice.

Less trabecular bone in the distal femoral metaphysis of experimental groups also could indicate differences in the circulating levels of stress hormones among the groups. In rodents, elevated levels of serum glucocorticoids (e.g., corticosterone), which are associated with augmented stress, can increase bone resorption, suppress bone formation, and lead to altered trabecular BV, trabecular number, and trabecular thickness (Dalle Carbonare et al. 2001; Lane et al. 2006). Linear mice had significantly less trabecular bone with fewer struts relative to control mice, while turning mice had fewer struts relative to control mice. Mice in the experimental groups, however, did not differ significantly from control mice in trabecular thickness. Thus, differences between experimental and control mice and the architectural patterns of trabecular bone associated with elevated stress levels are not entirely consistent. Furthermore, experimental mice did not exhibit other classic symptoms of elevated stress over the duration of the behavior protocol (e.g., poor grooming or weight loss). Further studies will be necessary to clarify whether different circulating levels of stress hormones may have impacted group comparisons.

The lack of significant differences between trabecular architectures of the experimental groups, in which substantially different modes of locomotion were emphasized (i.e., linear locomotion versus turning), suggests that trabecular architecture, at least within the distal femoral metaphysis, may be of limited value in reconstructing habitual locomotor modes of vertebrates. As a hinge joint, loading of the knee may be more restricted than loading of more proximal joints of the femur (i.e., the hip), and anatomy of the soft tissues (e.g., associated ligamentous structures) would seem to support such a characterization. Several comparative studies of trabecular architecture from more proximal femoral locations, particularly in primates exhibiting broad differences in locomotion and hip joint loading (e.g., leapers versus nonleapers, suspensory versus quadrupedal), attributed differences in trabecular architecture and orientation (i.e., DA) of the femoral head and neck (MacLatchy and Müller 2002; Ryan and Ketcham 2002a; Ryan and Ketcham 2005) to distinct hip joint loading during these habitual modes of locomotion, while other studies reported no support for such relationships in the femoral head (Fajardo et al. 2007). It is unclear why groups of mice did not differ in trabecular anisotropy in the distal femoral metaphysis, but guinea fowl running on an inclined treadmill versus a level treadmill did (Pontzer et al. 2006). The difference may be due in part to methodological differences since Pontzer et al. (2006) measured the orientation of peak trabecular density (i.e., combining trabecular strut orientation, size, number, and localized mineral density) rather than measuring trabecular orientation in multiple planes (i.e., a plane orthogonal to the analyzed plane). Other possibilities include the nature of the signal (treadmill versus modified cages), a taxon-specific signal, or differences between the locomotor behaviors of interest (inclined locomotion versus turning). Alternatively, the guinea fowl had a treatment period of 10 min/day, 6 days/week, 45-day (Pontzer et al. 2006) rather than a treatment period of 24 h/day, 7 day/week, 57-day (Carlson and Judex 2007). Given the lack of consensus on how well trabecular architecture of the femur reflects habitual
differences in locomotion, the inferential value of femoral trabecular architecture should be approached cautiously when reconstructing habitual locomotor behavior of extinct vertebrates (Macchiarelli et al. 1999; Ryan and Ketcham 2002b). At the very least, relationships between habitual mode of locomotion and trabecular architecture should be viewed with some caution. Additional experimental verification of effects of locomotor behaviors on trabecular architecture from other femoral locations, from other skeletal elements, and from additional vertebrate taxa are needed.

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