Response of the Axial Skeleton to Bipedal Loading Behaviors in an Experimental Animal Model

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ABSTRACT

Many derived aspects of modern human axial skeletal morphology reflect our reliance on obligate bipedal locomotion. Insight into the adaptive significance of features, particularly in the spine, has been gained through experimental studies that induce bipedal standing or walking in quadrupedal mammals. Using an experimental animal model (Rattus norvegicus), the present study builds on earlier work by incorporating additional metrics of the cranium, employing quantitative methods established in the paleoanthropological literature, and exploring how variation in mechanical loading regimes impacts axial anatomy. Rats were assigned to one of five experimental groups, including “fully loaded bipedal walking,” “partially loaded bipedal walking,” “standing bipedally,” “quadrupedal walking,” and “no exercise control,” and engaged in the behavior over 12-weeks. From μCT data obtained at the beginning and end of the experiment, we measured foramen magnum position and orientation, lumbar vertebral body wedging, cranial surface area of the lumbar and first sacral vertebral bodies, and sacral mediolateral width. Results demonstrate that bipedal rodents generally have more anteriorly positioned foramina magna, more dorsally wedged lumbar vertebrae, greater articular surface areas of lumbar and first sacral vertebral bodies, and sacra that exhibit greater mediolateral widths, compared to quadrupedal rodents. We further document variation among bipedal loading behavior groups (e.g., bipedal standing vs. walking). Our experimental animal model reveals how loading behaviors and adaptations may be specifically linked, and implicates a potential role for developmental plasticity in the evolutionary acquisition of bipedal adaptations in the hominin lineage. Anat Rec, 303:150–166, 2020. © 2018 American Association for Anatomy

Key words: bipedalism; human evolution; experimental animal model; developmental plasticity; foramen magnum; lumbar vertebrae; sacrum

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Modern humans employ one of the most unusual forms of locomotion found among extant terrestrial vertebrates—obligate striding bipedalism. Accordingly, it has long been appreciated that many aspects of the postcranial skeleton of modern humans, clearly derived relative to other great apes (Pan, Gorilla, and Pongo), are likely related to our unique reliance on bipedal stance and locomotion. Few regions of the human body reflect bipedal adaptations so clearly as the axial skeleton (the head and vertebral column). In the cranium, modern humans exhibit a more anteriorly positioned and anteroinferiorly inclined foramen magnum than other great apes, the former trait reflecting the head’s position atop a vertically oriented neck (e.g., Broca, 1872; Topinard, 1880; Bolk, 1909; Dart, 1925; Broom, 1938; Aiello and Dean, 2002; Russo and Kirk, 2013, 2017; Neaux et al., 2017). In the spine, modern human lumbar vertebral bodies (in particular the lower lumbar vertebrae) are characterized by dorsal wedging to facilitate a lordotic lower back, which provides sagittal balance of the upper body over the hind limbs and positions the sacrum behind the center of mass; in contrast, nonhuman ape lumbar vertebrae lack wedging or are ventrally wedged, consistent with a non-lordotic (or slightly kyphotic) lower back (Latimer and Ward, 1993; Sanders, 1998; Aiello and Dean, 2002; Whitcome et al., 2007). Compared to nonhuman apes, modern humans also have lumbar vertebrae showing a gradual increase in zygapophyseal interfacet spacing and coronal orientation (moving cranial to caudal) to resist compressive and ventral shearing forces, postzygapophyses that project beyond the body’s inferior centrum, relatively larger articular joint surfaces (e.g., body, zygapophyseal) to dissipate increasing axial compressive forces and facilitate transmission of body weight to the lower limbs, and, a mediolaterally wider sacrum with more marked insertions on the upper lateral angles for the lumbar sacral and sacroiliac ligaments that partly serve to resist excessive rotation (i.e., anterior rotation of the promontory) or translation (Schultze, 1953; Davis, 1961; Robinson, 1972; Stern and Susman, 1983; Abitbol, 1987a, 1987b; Jungers, 1988; Lovejoy, 1988; Abitbol, 1989; Jungers, 1991; Latimer and Ward, 1993; Shapiro, 1993a, 1993b; Sanders, 1998; Aiello and Dean, 2002; Lovejoy, 2005; Whitcome et al., 2007; Kapanjdi, 2008). Thus, the axial skeleton is a region of special interest to researchers investigating the evolutionary process of “bipedalization” in our lineage, from the hominin-panin last common ancestor to members of the genus Homo that exhibit modern human-like body proportions (e.g., Homo ergaster; Bramble and Lieberman, 2004; Collard and Wood, 2015).

While discoveries and inferences of fossils are undoubtedly the main drivers behind our understanding of the evolution of human bipedalism, the identification of the specific mechanical factors that lead to the skeletal features under consideration is an important complementary line of research (Preuschoft, 2004). Previous workers have demonstrated that the ontogenetic appearance and trajectories of key modern human axial skeletal features linked to bipedalism coincide with the stages of adopted bipedal walking in children, attesting to the importance of developmental mechanical loading in shaping the resultant adult phenotype. For example, the lumbar lordosis and associated sacral dorsoventral tilting, is present in modern humans (and absent in nonhuman apes) and confers sagittal plane balance of the upright torso over the hind limbs during striding bipedalism (Abitbol, 1987a). The lumbo-sacral angle is precocious in humans who begin to walk relatively early, delayed in formation in humans who begin to walk relatively late, and minimal in humans who never walk due to pathologic conditions (Abitbol, 1987a). Further, although an incipient lordotic spine appears early in fetal development (Reichmann and Lewin, 1971) and shows a relationship with increasing body weight/height and age, a stronger correlation exists with the onset and progression of bipedal walking (Abitbol, 1987a). These studies provide insight into how phenotypic differences can be produced by mechanical forces placed on the spine during development, and additionally highlight the utility of employing an ontogenetic framework for enhancing our understanding of the acquisition of key anatomical features, such as lumbar lordosis, in hominin phylogeny.

A number of workers have artificially induced bipedal stance and locomotion (or exploited situations wherein bipedal movements were necessary due to naturally occurring pathologies) in quadrupedal animal models to further understand the adaptive significance of features in the axial skeleton, as well as to investigate the etiology of musculoskeletal disorders, associated with standing and walking on two legs (e.g., Colton, 1929; Slijper, 1942b, 1942b; Pratt, 1943; Goff and Landmesser, 1957; Sakamoto, 1959; Sato, 1959; Ushikubo, 1959; Yamada et al., 1960; Moss, 1961; Adachi, 1963, 1964; Nathan et al., 1964; Riesenfeld, 1966; Kay and Condon, 1987; Cassidy et al., 1988; Preuschoft et al., 1988; Nakatsukasa et al., 1995; Bailey et al., 2001; Nakatsukasa et al., 2004). For example, bipedal standing and/or walking in monkeys (baboons and macaques) (e.g., Nathan et al., 1964; Yamada et al., 1961) and rodents (rats and mice) (e.g., Riesenfeld, 1966) has been shown to generate lordotic curvature in the quadruped lumbar spine. In some cases the manifestation of “lumbar lordosis” appeared related to changes in the anterior and posterior heights of the intervertebral discs (rather than changes in bone shape per se; Yamada et al., 1960; Nakatsukasa et al., 1995) that often resulted in intervertebral disc herniation (e.g., Sato, 1959). However, other workers have detected “lumbar lordosis” directly from changes in the wedging of the bony lumbar vertebral bodies as determined from comparisons of anterior and posterior vertebral body heights in quadrupedal animals trained to walk bipedally (e.g., macaques, Preuschoft et al., 1988). Additional researchers have noted that increasing concavity in the anterior face of the lumbar vertebral bodies (Sato, 1959; Cassidy et al., 1988), intensification of cervical lordosis and thoracic kyphosis (Yamada et al., 1960; Riesenfeld, 1966), and an increase in articular surface areas of the lumbar vertebrae (dorsal-ventral diameter) (Nakatsukasa et al., 1995) and auricular surface of the sacrum (Riesenfeld, 1966; Nakatsukasa et al., 1995), may also accompany the acquisition of a lordotic lumbar spine in bipedally trained animals. Moss (1961) extended research on the spine to the head, and observed that the crania of bipedal rodents exhibited greater rotation of the splanchnocranium relative to the neurocranium during growth (i.e., it becomes increasingly “unflexed”) beyond what is typical during neonatal rat growth (Moss, 1958).
Such studies demonstrate that experimental animal models have the potential to validate links between skeletal form and locomotion [function] by way of mechanically imposing bipedal loads on a quadrupedal body plan. Further, they implicate the potential role of developmental plasticity (modifiability of an organism during development in response to different environmental conditions) in the evolution of modern human traits associated with bipedalism (Shefelbine et al., 2002; West-Eberhard, 2003; Mozek et al., 2011). However, while it is clear that bipedal behaviors impact axial skeletal morphology in some ways that are consistent with the evolutionary appearances of these adaptations to bipedalism observed in hominins, relatively less is known about the specific loading conditions that might underpin these adaptations. In many previous studies altered animals (e.g., with forelimbs amputated) moved freely around their enclosures, were housed in restricted environments (e.g., narrow cylinders necessitating bipedalism) (Riesenfeld, 1966), or wore garments (e.g., plaster trousers) that necessitated upright stance and/or locomotion (Nathan et al., 1964). As a result, animals engaged in uncontrolled upright (and semi-upright, see below) behaviors (e.g., leaning, standing, walking) for unspecified and uncontrolled amounts of time. Further, it has been demonstrated that some methods (e.g., forelimb amputation) intended to induce bipedal behaviors do not actually produce trunk postures in bipedal animals that differ from that of their quadrupedal counterparts, nor do altered rats take on bipedal postures more frequently than control rats in their cage environment (Moravec and Cleall, 1987; Bailey et al., 2001). Experimental approaches that explicitly control for behavioral duration and the amount of hind limb mechanical loading offer an opportunity to link presumed morphological adaptations, like vertebral body wedging for mechanical loading offer an opportunity to link presumed morphological adaptations, like vertebral body wedging, to loading regimes more refined than bipedalism versus quadrupedalism, thereby offering valuable information that can elucidate the process of “bipedalization.” For example, some authors have suggested that bipedalism originated as a postural adaptation for feeding from tall bushes or small trees (Hunt, 1994, 1996). Work aimed at differentiating the skeleton’s response to a variety of bipedal loading behaviors (e.g., stance vs. walking) may improve our ability to make inferences about the evolutionary origins of the form of locomotion that later became characteristically obligate in Homo by helping us generate predictions for how bone should respond to specific mechanical loading environments.

The overarching goal of the present study was to shed light on the evolutionary acquisition of axial skeletal features in hominins by expanding on previous work aimed at determining how skeletal morphology responds to bipedal loading behaviors in an experimental animal model. To do so, we integrated three approaches that depart from the approaches of most prior studies. First, given that previous work on the axial skeleton has focused primarily on the vertebral column (but see Moss, 1958, 1961), we extended analyses to the cranial base by incorporating measures of foramen magnum position (herein, FMP) and orientation (herein, FMO). Second, we quantified features using metrics established in the paleoanthropological literature (see also Preuschoft et al., 1988; Nakatsukasa et al., 1995) to build on most earlier work that did not report statistics and/or relied on visual descriptions of morphological differences, and to direct focus to aspects of axial skeletal morphology meaningful to discussions of human evolution. Third, we evaluated axial skeletal morphological changes associated with a variety of loading regimes (“fully loaded bipedal walking,” “partially loaded bipedal walking,” “standing bipedally,” “quadrupedal walking,” and “no exercise control”; see below for category definitions) that tests the role of both amount and direction of loading (using controlled conditions and behavioral durations) to expand analyses beyond a quadrupedal versus bipedal dichotomy.

Based on previous work, we tested the hypothesis that axial skeletal morphology will differ between all bipedal loading behavior groups, and the “quadrupedal walking” and “no exercise control groups.” We also tested the null hypothesis that axial skeletal morphology would be similar among all bipedal loading behavior groups. Below, we provide background and specific predictions for how we expect differences in mechanical loading to translate to changes in six axial skeletal features, including (1) FMP, (2) FMO, (3) lumbar vertebral body dorsal wedging, cranial surface area of the (4) penultimate lumbar and (5) first sacral vertebral bodies, and (6) mediolateral sacral breadth.

MATERIALS AND METHODS

Sample

The rodent sample (female Sprague-Dawley rats; Rattus norvegicus, Muridae; Harlan Laboratories, Inc., Indianapolis, IN) used in this study were housed at the University of Arizona Animal Care Facility in a temperature and humidity controlled environment under a 12 hour day/night light cycle with ad libitum access to food and water. Animals were euthanized by CO₂ overdose at the end of the experiment. All methods and protocols were approved by the University of Arizona IACUC (10-164).

Experimental Procedure

Methods for this experiment are described in detail in Foster (2014, In press). Briefly, rats were acquired at three weeks of age and allowed a 1-week acclimation period. At four weeks of age, rats were trained to bipedally walk or stand over a period of 12 weeks using a harness system mounted to a treadmill (Fig. 1). Rats were randomly assigned to one of five experimental groups (n = 14/group) that differed in the amount and directionality of induced loading (a bipedal or quadrupedal gait): (1) “fully loaded bipedal walking” (targeted to ~90% body weight; change in the direction and amount of mechanical load); (2) “partially loaded bipedal walking” (targeted to ~45% body weight, which is the average amount supported by rats hind limbs when quadrupedal (Giszter et al., 2008); i.e., change in the direction of load); (3) “standing bipedally” (targeted to ~90% body weight; i.e., change in the direction and amount of load, only postural support); (4) “quadrupedal walking” (unaltered walking; no postural support); and (5) “no exercise control” (rats remain in cages). Rats were exposed to their assigned behaviors over a 12-week experimental period, exercising five days a week in 60 minute bouts. In the first week of exercise, rats underwent behavior training using increasing time intervals each day until they could comfortably engage in the assigned behavior for the full
60 minute duration. Rats in the exercise groups walked at a speed determined to be a visually comfortable pace (that is, a speed that was fast enough to maintain focus on the activity but did not elicit signs of distress) for the bipedal walking groups (~0.13 m/sec).

Bipedal rats received postural support from a bar that ran horizontally across the trackway (to hold on to with their forelimbs for stability) and a vertical force on the torso. The vertical force on the torso derives from a wire attached to a rat jacket (SAI Infusion Technologies, Lake Villa, IL) that is connected to a hanging scale above each rat and permits monitoring of the load during the experiment. Each scale is attached to a runner above each of the four lanes (to accommodate fore-aft movement when on the treadmill) and is connected to a data logger, recording at 2 Hz, to monitor and record hind limb loading during each exercise bout. The amount of hind limb loading was adjusted by altering the height of the scale and subsequently the amount of upward force on the torso. Use of the horizontal bar for postural stability resulted in some variation in the weight supported by the hind limbs over each gait cycle and throughout the 60 minute exercise period. Any weight taken off the hind limbs and onto the forelimbs by pushing up or hanging off the cross bar resulted in a manual adjustment in the height of the scale, and as a result, an alteration of the amount of upward force on the torso. Adjustments were not the primary means of ensuring proper loading during exercise, as most corrections were accomplished by behavioral encouragements to induce rats to take on the desired posture (i.e., touching the rat with a gloved hand). Any weight not recorded by the scale was assumed to be on the treadmill belt. Scales were calibrated daily by placing four calibration weights of known mass on each scale to measure the voltage captured by the data logger. A least-squares linear calibration curve was used to fit the data and produce a formula to calculate the amount of load measured by each hanging scale for each rat, over each exercise period, for the 12-week experiment. Rats in the "quadrupedal walking" group were not subject to any upward force on the torso, and were exercised using a normal gait in the harness for the same period as other experimental groups. Body mass for all rats was measured on each experimental day for all groups but the "no exercise control" group, which was measured every three weeks.

Micro-Computed Tomography (μCT) Scanning

To track changes in axial skeletal morphology, in vivo μCT scans were taken before the start of the experiment (Week 0) and at the end of the 12-week experimental period (Week 12) using a small animal scanner (Inveon, Siemens Medical Solutions, Malvern, PA). Rats were anesthetized using isoflurane (3% induction, 1.5% maintenance using 1.5 L of O2 per min). Images were acquired at 80 kV and 500 μA with 440 exposures at 475 msec per projection, using a binning factor of 4, and the total rotation of the gantry was 220°. Low magnification was used in an attempt to accommodate whole body scans (but see below) at a pixel size of 105 μm. μCT scans were reconstructed using COBRA software (Exxim, Pleasanton, CA) and slice images were standardized using Hounsfield units (Schneider et al., 1996). Prior to each scanning period, a distilled water phantom was scanned using the protocol described above and reconstructed using Inveon Research Workplace (Siemens Medical Solutions, Malvern, PA). After reconstruction, a measurement of the voxel (a 3D pixel) intensity was taken every 20 slices to determine average voxel intensity for water, which was applied to a scale factor of (1,000/(mean voxel value)) and used to reconstruct each scan.

Because the original project for which scan data were collected focused on postcranial morphology (in particular the hind limbs) (Foster, 2014; Foster, In press), the rostralmost part of the cranium was omitted from the majority of scans in the original data set. Since the cranial base is an anatomical region of interest in the present study, and measures required for calculating metrics of FMP and FMO include landmarks located on the splanchnocranium (e.g., inferiormost point on the zygomatic arch for the FMO reference plane), crania were isolated from adult cadaveric rodents (frozen at the end of the experimental period) and individually scanned. Scans were obtained only for the “fully loaded bipedal walking” and the “quadrupedal walking” individuals as there were additional costs associated with obtaining these scans specifically for this study.
Metrics and Predictions

Foramen magnum position (FMP) and orientation (FMO). Metrics have already been devised for quantifying the position and inclination of the foramen magnum (as basion: the anteriormost border of the foramen magnum in the midsagittal plane) in morphologically and taxonomically diverse mammalian samples that include representatives of Muridae (the family to which the rodent species *R. norvegicus* used in this experiment belongs) (Russo and Kirk, 2013, 2017; Ruth et al., 2016) (Fig. 2). These and other studies (e.g., Kimbel et al., 2004; Wolpoff et al., 2006) have also specifically established the utility of 2D photographs for collecting such data. Therefore, we followed the literature in collecting the measurements required to calculate FMP and FMO from 2D image captures of 3D crania oriented in *norma basilaris* (for FMP) and *norma lateralis* (for FMO) in AMIRA (FEI Visualization Sciences Group, Hillsboro, OR).

To obtain 2D image captures of 3D crania in *norma basilaris*, a reference plane that roughly approximated the Frankfurt Horizontal was delineated. Because *R. norvegicus* exhibits a myomorphous zygomaseteric condition in which the orbital and temporal fossae are broadly confluent, we followed Russo and Kirk (2013) in adjusting our horizontal reference plane definition as the inferiormost point on the external auditory meatus and the inferiormost point on the superior surface of the zygomatic arch. In *norma basilaris*, this rodent reference plane resembles that reference plane used for primates in the anthropological literature by yielding an approximately orthogonal view of the basicranium and palate (Russo and Kirk, 2013, 2017). To obtain 2D image captures of 3D crania in *norma lateralis*, we placed 3D landmarks on osteometric points positioned along the cranial midline (prosthion-basion-opisthion) to generate a plane that approximated the midsagittal plane. The cranium was then rotated such that this plane was positioned orthogonal to the viewer (i.e., in *norma lateralis*). A basion-opisthion chord was drawn to approximate the orientation of the opening of the foramen magnum. This final view captured both our horizontal reference plane and the basion-opisthion chord. A scale bar was also introduced to the image.

2D image captures from AMIRA were then imported into NIH ImageJ software (http://imagej.nih.gov/ij/) to take linear and angular measurements for quantifying FMP and FMO. For each image of crania in *norma basilaris*, measurement units were calibrated using the scale bar, images were rotated until the prosthion-opisthocranion chord was vertical, and a horizontal “basion line” was drawn through basion perpendicular to the prosthion-opisthocranion chord (following Russo and Kirk, 2013,

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**Fig. 2.** Panels A and B depict the measurements taken on each rodent cranium in (A) *norma basilaris* and (B) *norma lateralis*. In (A) Measurement 1: cranial length, as the distance between prosthion and posterior cranial vault; Measurement 2: cranial width, as the distance between right and left zygion; Measurements 3 and 4: basion line to the posterior margins of the distalmost molars; Measurements 5 and 6: basion line to the anterior margins of the temporal fossae; Measurement 7: basion line to the posterior margin of the hard palate in the mid-sagittal plane (shifted slightly left in figure so Measurement 8 is visible); Measurement 8: basion line to the sphenocapsoidal synchondrosis. In (B) FMO is foramen magnum orientation measured as the angle between a horizontal reference plane and a chord connecting basion and opisthion. Panel C depicts the 3D landmarks obtained for the penultimate lumbar vertebra (PUL) and the sacrum in ventral (left) and dorsal (right) views. In (C) Landmark 1: ventrocranialmost edge of the PUL vertebral body; Landmark 2: dorsocranialmost edge of the PUL vertebral body; Landmark 3: right lateroalmost edge of the PUL vertebral body; Landmark 4: left lateroalmost edge of the PUL vertebral body; Landmark 5: ventrocaudalmost edge of the PUL vertebral body; Landmark 6: dorsocaudalmost edge of the PUL vertebral body; Landmark 7: ventrocranialmost edge of the first sacral (S1) vertebral body; Landmark 8: dorsocranialmost edge of the S1 vertebral body; Landmark 9: right lateroalmost edge of the S1 vertebral body; Landmark 10: left lateroalmost edge of the S1 vertebral body; Landmark 11: lateroalmost visible (in 3D view) edge of the left sacral ala; Landmark 12: lateralmost visible (in 3D view) edge of the right sacral ala. Landmarks 11 and 12 are slightly obscured by the iliac blades in the 2D image above. See also Methods for description of measurements and definitions.
Eight linear measurements were taken on each image of crania in *norma basilaris* (Fig. 2A). Maximum cranial length was measured as the distance from prosthion to posterior cranial vault (Measurement 1, Fig. 2A). Bzygomatic width was measured as the distance between right and left zygion (Measurement 2, Fig. 2A). Basion position (basion line in Fig. 2A) was measured relative to multiple osteological landmarks, including the posterior most margins of the distal molars (Measurements 3 and 4, Fig. 2A), the anterior most margins of the temporal fossae (Measurements 5 and 6, Fig. 2A), the posterior margin of the hard palate at midline (Measurement 7, Fig. 2A), and the sphenoorbital synchondrosis (Measurement 8, Fig. 2A). An average value was obtained for bilateral measurements (Measurements 3 and 4, Measurements 5 and 6) for use in subsequent analyses. For each image of crania in *norma lateralis*, FMO was measured as the angle between the horizontal reference plane described above and the basion-opisthion chord (Fig. 2B) (Russo and Kirk, 2017).

**Lumbar and sacral vertebrae.** Twelve 3D landmarks were placed on the penultimate lumbar and first sacral vertebrae for each rodent individual in AMIRA (Fig. 2C). We chose to examine the penultimate vertebrae within the lumbar spine because modern humans typically exhibit dorsal wedging of the penultimate and last lumbar vertebrae (although females may exhibit an additional dorsally wedged antepenultimate lumbar vertebra; Whitcome et al., 2007). While vertebral centroid size gradually increases moving inferiorly in the vertebral column in modern humans (and potentially other mammals as well; Cartmill and Brown, 2014), centroid size actually decreases between the penultimate and ultimate lumbar vertebra (Davis, 1961; Shapiro, 1993a). Landmark coordinates were exported and linear inter-landmark distances were obtained in PAST (PAleontological STatistics; Hammer et al., 2001) and used to calculate our variables of interest for the penultimate lumbar vertebra and the sacrum.

**Lumbar lordosis as indicated by vertebral body wedging.** Lumbar lordosis is expressed as the angle between the cranial endplate of the fifth pre-sacral vertebra and the cranial endplate of the first sacral vertebra (Cobb, 1948; Been et al., 2012), and can be estimated from measures of “wedging” that compare ventral and dorsal craniocaudal lengths of individual vertebral bodies (Robinson, 1972; Digiovanni et al., 1989; Latimer and Ward, 1993; Shapiro, 1993b; Sanders, 1998; Whitcome et al., 2007). Specifically, dorsally wedged lumbar vertebrae exhibit shorter dorsal relative to ventral craniocaudal body lengths, and so when seriated, a stack of wedged vertebrae produces lordotic curvature because the summed dorsal lengths are less than the summed ventral lengths. Here, lumbar vertebral body wedging was calculated from linear measurements derived between 3D landmarks as: wedging angle = $2 \arctan(|a - b|/2c)$, where $a$ is the craniocaudal height of the vertebral body at its dorsal midline (distance between landmarks 2 and 6 in Fig. 2C), $b$ is the craniocaudal height of the vertebral body at its ventral midline (distance between landmarks 1 and 5 in Fig. 2C), and $c$ is the dorsoventral diameter of the vertebral body cranial articular surface (distance between landmarks 1 and 2 in Fig. 2C) (Digiovanni et al., 1989; Whitcome et al., 2007).

**Articular surface areas of the penultimate lumbar and first sacral vertebrae.** The efficacy of weight transmission through the anterior elements (i.e., bodies) of the vertebral column can be assessed by quantification of body articular surface areas (Davis, 1961; Pal and Routal, 1986, 1987; Latimer and Ward, 1993; Shapiro, 1993a). Large vertebral articular surface dimensions serve to reduce axial compressive forces thereby facilitating the transmission of upper body weight to the lower limbs during bipedal stance and locomotion (Schultz, 1953; Jungers, 1991; Kapandji, 2008). Articular surface areas for the cranial surfaces of the penultimate lumbar and first sacral vertebrae were calculated using the formula for the area of an ellipse (Shapiro, 1993a; Whitcome et al., 2007; Russo and Shapiro, 2013): vertebral body cranial surface area = $\pi \times c^2 \times d/2$, where $c$ is the dorsoventral diameter of the vertebral body cranial articular surface (distance between landmarks 1 and 2 for the lumbar vertebra, and distance between landmarks 7 and 8 for the first sacral vertebra; Fig. 2C) and $d$ is the mediolateral diameter of the vertebral body cranial articular surface (distance between landmarks 3 and 4 for the lumbar vertebra, and distance between landmarks 9 and 10 for the sacrum; Fig. 2C).

**Sacral alar mediolateral breadth.** The mediolaterally wide sacra of modern humans increases the coronal distance between the sacroiliac joints (SIJ) and vertically aligns them with the hip joints (parasagittally), thereby resisting the tendency for the pelvic bones to rotate around the SIJ and reducing stresses on the pubic symphysis (Leutenegger, 1977). Along with expanded retroauricular iliac tuberosities and vertical shortening of the ilia, a wide and horizontally oriented (i.e., positioned more perpendicular to the lumbar spinal column) sacrum also provides increased dorsal surface area and leverage for attachment of the erector spinae musculature that serves to maintain upright trunk posture (Gregory, 1928; Aiello and Dean, 2002). Moreover, a wide sacrum accommodates ventromedial rotation of the iliac blades into the sagittal plane without impinging on the pelvic viscera (Lovejoy, 1988). Sacral alar mediolateral breadth was measured as the distance between the visible lateralmost aspects of the left and right sacral alae (distance between landmarks 11 and 12; Fig. 2C) following the literature (Sanders, 1998; Russo and Shapiro, 2013).

**Predictions.**

1. If an anterior position of the foramen magnum reflects the perpendicular balancing of the head atop a vertical neck in bipedal stance, then the “fully loaded bipedal walking” group should exhibit more anteriorly positioned foramina magna than the “quadrupedal walking” loading behavior group.

2. If the anteroinferior inclination of the foramen magnum reflects the perpendicular orientation of the head atop a vertical neck in bipedal stance, then the “fully loaded bipedal walking” group should exhibit more anteroinferiorly oriented foramina magna than the “quadrupedal walking” group.
3. We predict that all bipedal groups ("fully loaded bipedal walking," "partially loaded bipedal walking," and "standing bipedally") will exhibit dorsally wedged penultimate lumbar vertebrae, which would reflect bony shape changes associated with the adoption of a lordotic lumbar spine. Because the spines of the "quadrupedal walking" and "no exercise control" rodents should not be lordotic, these loading behavior groups should lack body wedging or exhibit ventral body wedging (the latter would reflect the acquisition of a kyphotic lumbar spine).

4. We predict that all bipedal groups ("fully loaded bipedal walking," "partially loaded bipedal walking," and "standing bipedally") will exhibit penultimate lumbar and first sacral vertebral bodies with greater articular surface area than the "quadrupedal walking" or the "no exercise control" groups.

5. We predict that the bipedal groups ("fully loaded bipedal walking," "partially loaded bipedal walking," and "standing bipedally") will exhibit mediolaterally broader sacra than the "quadrupedal walking" or "no exercise control" groups.

Statistical Analysis

All postcranial measures used to compare experimental groups in this study were calculated as a percent difference between measurements taken at the first scan prior to the initiation of the experiment and the final scan [i.e., (Week 12–Week 0)/Week 12] with the exception of the cranial metrics (see below). Larger percent values represent greater changes in axial skeletal morphological dimensions relative to the initial condition, whereas smaller percent values represent less change in axial skeletal morphological dimensions relative to the initial condition. These analyses were performed for lumbar body vertebral wedging, articular surface areas of the penultimate lumbar and the first sacral vertebral bodies, and sacral alar mediolateral breadth. We performed an additional analysis for evaluating lumbar lordosis as indicated by vertebral body wedging because angular measurements should be dimensionless within the sample and, therefore, do not require size adjustments. As such, we were able to directly compare wedging angle values for rodents at the end of the 12-week experiment across loading behavior groups. Negative wedging angles indicate lordosis, whereas positive wedging angles indicate kyphosis.

As mentioned, scans for rodent crania could not be obtained for specimens prior to the end of the 12-week experiment. Because of this limitation, comparisons of metrics for FMP and FMO were limited to adults from only two loading behavior groups ("fully loaded bipedal walking" and "quadrupedal walking"). Because we did not have additional scans for calculating percent changes, we size-adjusted distances from the basion line to the distalmost molar, posterior hard palate, anterior temporal fossa, and sphenoccipital synchondrosis using a measure of "cranial size," calculated as the geometric mean of cranial length and width (Measurements 1 and 2 in Fig. 2A) to make comparisons among adults of different body sizes (Russo and Kirk, 2013, 2017). Used in previous studies to size-adjust measures of FMP in nonprimate samples (Russo and Kirk, 2013, 2017), these measurements of cranial length and width from photographs reliably approximate measurements taken directly on specimens using calipers. To size-adjust each measure, we divided the linear distance between basion and the anterior landmark by cranial size to create a ratio. Henceforth, these ratios are referred to as "molar ratio" (distance from posterior molar to basion line/cranial size), "temporal fossa ratio" (distance from anterior temporal fossa to basion line/cranial size), " palate ratio" (distance from posterior hard palate to basion line/cranial size), and "basioccipital ratio" (distance from sphenoccipital synchondrosis to basion line/cranial size). Lower ratio values indicate relatively anteriorly positioned foramen magna and shortened basioccipital segments, whereas higher ratio values indicate relatively posteriorly positioned foramina magna and more elongate basioccipital segments. FMO values were not size-adjusted because angular measurements should be dimensionless within the sample. Lower FMO angular values indicate a more anteroinferiorly inclined foramen magnum, and higher FMO angular values indicate a more posteriorly inclined foramen magnum.

Univariate analyses were performed using SPSS v.24 (IBM). Because analyses of FMP and FMO were limited to the "fully loaded bipedal walking" and the "quadrupedal walking" groups, we examined mean differences between these groups using an independent samples t-test. For all other variables, we compared group (>2 groups) means for each variable by differences by conducting a one-way analysis of variance (ANOVA) followed by Fisher’s LSD pairwise comparisons, with significant differences recognized if P < 0.05. All pairwise comparisons were one-tailed because we had directional predictions of how bipedal rats should differ from quadrupedal rats for each variable.

RESULTS

Mechanical Loading

The amount of hind limb loading, calculated as the mean percentage of body weight experienced by the hind limbs, differed for each rat over each 60 minute bout. The "fully loaded bipedal walking" group experienced 90.2% (±7.2%) of body mass, the "partially loaded bipedal walking" group experienced 54.5% (±8.9%) of body mass, and the "standing bipedally" group experienced 78.5% (±8.2%) of body mass. The "standing bipedally" group experienced hind limb loading that was lower than expected compared to the other bipedal groups because these rats required more monitoring and behavioral encouragement to ensure they maintained standing bipedal posture for the entire 60 minute duration as they did not have the behavioral stimulus from a constantly moving treadmill belt. As described in the methods, placing a gloved hand in the trackway typically resulted in rats standing upright and taking on the desired posture. It is worth noting that the loading percentages for the "fully loaded bipedal walking" and "partially loaded bipedal walking" groups were contained within one standard deviation from the targeted mean (see Foster, In press).

Foramen magnum position (FMP) and orientation (FMO)

Figure 3 shows mean (±95% confidence intervals [CI]) molar, palate, temporal fossa, and basioccipital ratios quantifying FMP for the rats at the end of the 12-week
experiment. For all four ratios, the “fully loaded bipedal walking” (FB) and “quadrupedal walking” (Q) loading behavior groups, (A) based on the distalmost molars (molar ratio), (B) based on the posterior hard palate (palate ratio), (C) based on the anterior temporal fossae (temporal fossa ratio), and (D) based to the sphenoccipital synchondrosis (basioccipital ratio). See text and Figure 2A for more detail concerning measurements used to calculate ratios. Values shown represent means (denoted by position of group abbreviation) and 95% confidence intervals. Arrows to the right of each panel indicate the directionality of our one-tailed test prediction: ratio values should be lower in the “FB” group than the “Q” group. Low values indicate that the foramen magnum is relatively anteriorly positioned, and high values indicate that the foramen magnum is relatively posteriorly positioned.

Lumbar lordosis as Indicated by Vertebral Body Wedging

The percent change (prior to exercise and at the end of Week 12) in the wedging angle of the penultimate lumbar vertebra differed significantly among loading behavior groups (F-statistic: 15.528, df = 4, P < 0.001; Fig. 5 and Table 1). The percent change for the “fully loaded bipedal walking” group was significantly greater than all other rodent groups (P < 0.001; Table 1). The percent change was significantly greater in the “standing bipedally” group compared to the “quadrupedal walking” (P < 0.001) and the “no exercise control” (P < 0.001) groups (Table 1), though in the direction opposite to that of the “fully loaded bipedal walking” group (Fig. 5).

At the end of the 12-week experiment, the wedging angle of the penultimate lumbar vertebra differed significantly among loading behavior groups (F-statistic: 25.908, df = 4, P < 0.001; Fig. 5 and Table 2). The “fully loaded bipedal walking” and
FMO values should be lower in the relatively posterior FMO. See text and Figure 2B for measurement details. Values indicate a relatively anteroinferior FMO, and high values indicate a relatively posterior FMO. Arrow to right indicates the directionality of our one-tailed test prediction: the bipedal groups should have the lowest values. For the right panel, negative wedging angle values indicate that vertebrae are ventrally wedged (kyphotic), and high values indicate relatively greater percent change, in PUL vertebral wedging angle. Right panel shows a comparison of mean vertebral wedging angle values among rodents at the end of the 12-week experiment; arrow to right indicates the directionality of our one-tailed post hoc test prediction: the bipedal groups should have the lowest values. For the right panel, negative wedging angle values indicate that vertebrae are dorsally wedged (lordotic) and positive wedging angle values indicate that vertebrae are ventrally wedged (kyphotic). Values shown represent means (denoted by position of group abbreviation) and 95% confidence intervals. See text and Figure 2C for measurement details.

**Articular Surface Areas of the Penultimate Lumbar and the First Sacral Vertebrae**

The percent change in the cranial articular surface area of the penultimate lumbar (P-statistic: 6.817, df = 4, P < 0.001; Fig. 6 and Table 1) and first sacral (P-statistic: 8.828, df = 4, P < 0.001; Fig. 7 and Table 1) vertebrae differed significantly among loading behavior groups. The percent change in the cranial articular surface area of the penultimate lumbar vertebra was significantly lower in the “quadrupedal walking” group than all other groups (P < 0.05; Fig. 6 and Table 1). The percent change in the cranial articular surface area of the penultimate lumbar vertebra was statistically similar among the “partially loaded bipedal walking,” “standing bipedally,” and “fully loaded bipedal walking” groups (P > 0.05; Fig. 6 and Table 1). The percent change in the cranial articular surface area of the penultimate lumbar vertebra for the “no exercise control” group was significantly higher than all other groups (P < 0.05) with the exception of the “standing bipedally” group (P = 0.108; Fig. 6 and Table 1).

The percent change in the cranial articular surface area of the first sacral vertebra for the “quadrupedal walking” group was significantly lower than all other loading behavior groups (P < 0.05; Fig. 7 and Table 1), and the percent change for the “no exercise control” group was significantly higher than all other groups (P < 0.05; Fig. 7 and Table 1). The percent change in the cranial articular surface area of the first sacral vertebra was statistically similar among the “partially loaded bipedal walking,” “standing bipedally,” and “fully loaded bipedal walking” groups (P > 0.05; Fig. 7 and Table 1).

**Sacral Alar Mediolateral Breadth**

The percent change in values for the mediolateral breadth of the sacral alae differed significantly among
loading behavior groups (F-statistic: 2.997, df = 4, P < 0.001; Fig. 8 and Table 1). The percent change in sacral alar mediolateral breadth for the “quadrupedal walking” group was significantly lower than all other groups (P < 0.05; Fig. 8 and Table 1). Values for percent change in sacral alar mediolateral breadth were statistically similar (P > 0.05) among all other groups (Fig. 8 and Table 1).

**DISCUSSION**

Consistent with our first hypothesis, our findings for the rodent model evaluated here validate observations by previous workers (e.g., Goff and Landmesser, 1957; Nathan et al., 1964; Nakatsukasa et al., 1995) that quadrupedal animals acquire bipedal traits in the axial skeleton when trained to walk or stand on two legs. Our specific results demonstrate that rodents trained to engage in bipedal behaviors generally have more anteriorly positioned foramina magna, more dorsally wedged penultimate lumbar vertebrae (“fully loaded bipedal walking” group), penultimate lumbar and first sacral vertebrae having greater cranial articular surface areas, and sacra exhibiting greater mediolateral widths, when compared to quadrupedal rodents (and often “no exercise control” rodents). Refuting our second (null) hypothesis, we observed that the expression of some bipedal features of the axial skeleton differed among bipedal loading behaviors groups. Specifically, only the “fully loaded bipedal walking” exhibited dorsally wedged penultimate lumbar vertebrae among the bipedal loading behavior groups. Below, we discuss our findings for each of the variables examined in this study in the context of our three study approaches and their implications for the paleoanthropological literature.

Previous workers that have investigated changes in the axial skeleton of quadrupeds in response to bipedal loading behaviors have focused primarily on the vertebral

**TABLE 1.** Results for ANOVA and post hoc pairwise comparisons of percent change for postcranial variables among loading behavior groups. Bold typeface indicates significant (p < 0.05) result.

| Percent change in wedging angle of the penultimate lumbar vertebra | Partially loaded bipedal walking | Standing bipedally | Quadrupedal walking | No exercise control |
|---|---|---|---|---|
| F-statistic = 15.528 | Fully loaded bipedal walking | <0.001 | <0.001 | <0.001 | <0.001 |
| DF = 4 | Partially loaded bipedal walking | — | 0.299 | 0.055 | 0.051 |
| P-value < 0.001 | Standing bipedally | — | — | 0.017 | 0.017 |
| | Quadrupedal walking | — | — | — | 0.464 |

| Percent change in cranial articular surface area of the penultimate lumbar vertebra | Partially loaded bipedal walking | Standing bipedally | Quadrupedal walking | No exercise control |
|---|---|---|---|---|
| F-statistic = 6.817 | Fully loaded bipedal walking | 0.0063 | 0.023 | 0.004 |
| DF = 4 | Partially loaded bipedal walking | — | 0.040 | 0.002 |
| P-value < 0.001 | Standing bipedally | — | — | 0.108 |
| | Quadrupedal walking | — | — | <0.001 |

| Percent change in cranial articular surface area of the first sacral vertebra | Partially loaded bipedal walking | Standing bipedally | Quadrupedal walking | No exercise control |
|---|---|---|---|---|
| F-statistic = 8.828 | Fully loaded bipedal walking | 0.216 | 0.027 | <0.001 |
| DF = 4 | Partially loaded bipedal walking | — | 0.108 | 0.007 |
| P-value < 0.001 | Standing bipedally | — | <0.001 | 0.010 |
| | Quadrupedal walking | — | — | <0.001 |

| Percent change in mediolateral breadth of the sacrum | Partially loaded bipedal walking | Standing bipedally | Quadrupedal walking | No exercise control |
|---|---|---|---|---|
| F-statistic = 2.997 | Fully loaded bipedal walking | 0.495 | 0.016 | 0.104 |
| DF = 4 | Partially loaded bipedal walking | — | 0.439 | 0.026 |
| P-value < 0.001 | Standing bipedally | — | — | 0.017 |
| | Quadrupedal walking | — | — | <0.001 |

**TABLE 2.** Results for ANOVA and post hoc pairwise comparisons of penultimate lumbar vertebral wedging angle among loading behavior groups at the end of the 12-week experiment. Bold typeface indicates significant (p < 0.05) result.

| Partially loaded bipedal walking | Standing bipedally | Quadrupedal walking | No exercise control |
|---|---|---|---|
| F-statistic = 25.908 | Fully loaded bipedal walking | <0.001 | <0.001 | <0.001 |
| DF = 4 | Partially loaded bipedal walking | — | 0.361 | 0.027 | 0.016 |
| P-value < 0.001 | Standing bipedally | — | — | 0.011 | 0.006 |
| | Quadrupedal walking | — | — | — | 0.397 |
As such, one aim of our study was to extend analyses to the cranial base by incorporating measures of foramen magnum position (FMP) and orientation (FMO). In doing so, we followed Russo and Kirk (2013, 2017) who established methods for quantifying FMP as the position of basion relative to four osteological landmarks, including the distalmost molar, the posteriormost aspect of the bony palate at midline, the anteriormost aspect of the temporal fossa, and the sphenoccipital synchondrosis. These landmarks can be reliably identified on a morphologically and taxonomically diverse comparative mammalian sample, including murid rodents, the family to which the experimental animal used in this study belong. Our results demonstrated that for all four measures of relative basion position (molar ratio, palate ratio, temporal fossa ratio, and basioccipital ratio), “fully loaded bipedal walking” rodents had significantly lower indices, indicating more anteriorly positioned foramina magna and shorter basioccipital regions, than “quadrupedal walking” rodents (Fig. 5). This observation is consistent with the findings of prior comparative studies demonstrating that extant bipedal primates (i.e., Homo), bipedal marsupials (e.g., Macropus), and bipedal members of at least three rodent clades (Dipodidae, Heteromyidae, Anomaluridae) exhibit more forwardly shifted foramina magna than their quadrupedal relatives, indicating that an anteriorly positioned foramen magnum evolved in concert with bipedal locomotion at least five times within Mammalia (Russo and Kirk, 2013, 2017). That these evolutionary patterns are also reflected in an experimental animal model has significance for the application and interpretation of the FMP metrics used here in the paleoanthropological literature (see also Russo and Kirk, 2013, 2017).

Many researchers accept that the relatively anterior position of the foramen magnum in humans (compared to other extant apes) is likely an adaptation for maintaining balance of the head atop a vertical neck during bipedalism and/or the assumption of orthograde trunk postures (Topinard, 1890; Dart, 1925; White et al., 1994; Kimbel and Rak, 2010; Russo and Kirk, 2013, 2017; Kimbel et al., 2014). However, that the position as well as orientation of the foramen magnum may be linked to other structural and/or functional systems in the body (e.g., brain expansion and/or reorganization, facial size) has contributed to ongoing debate concerning the functional anatomy of this region (Biegert, 1957, 1963; Kimbel and Rak, 2010; Russo and Kirk, 2013, 2017; Ruth et al., 2016; Villamil, 2017). For example, Ruth et al. (2016) recently rejected the use of Russo and Kirk’s (2013) metrics for assessing FMP, asserting these metrics instead captured changes in other regions of the skull across mammals (e.g., brain size in strepsirrhine primates, masticatory apparatus size in marsupials). They further contended that both measures of FMP and FMO could be captured by a single metric (“foramen magnum angle”) that they devised (Ruth et al., 2016). Russo and Kirk (2017) responded to these critiques by Ruth et al. (2016) using an expanded data set, and demonstrated that in fact brain size (i.e., encephalization quotient) had no discernible effect on either FMP or FMO, and that the relationship between FMP and FMO was weak or nonexistent within the mammalian clades they (and Ruth et al., 2016) examined (rodents, marsupials, primates).

It was not the goal of this study to further directly test alternative hypotheses for observed changes in FMP and FMO, however, our findings from evaluation of these metrics in our experimental rodent model offer an additional perspective on the topic. Here, we demonstrate that FMP differs in the ways predicted among members of a single
rodent species grouped by loading regime using Russo and Kirk’s (2013, 2017) metrics based on reference landmarks representative of both the basicranium (e.g., sphenoccipital synchondrosis) and the sphenobasal region. Specifically, although the rodents employed in this study (Sprague-Dawley rats) are outbred (as opposed to using genetically similar rats from an inbred line, in which a functional signal could respond differently), they offer the opportunity to evaluate variation in the norm of reaction—the range of variation in the phenotype outcome that is the product of genes and the environment. With the exception of assigned loading behavior regimes, all other environmental variables (e.g., access to food and water, cage environment and number of cage mates, frequency of cage changes, etc.) were kept constant in this experiment, though activities in the cage were not monitored or quantified. Although not directly measured in this study, resultant adult morphotypes across loading behavior groups are not expected to differ in other ways (e.g., face size) that would potentially confound our measurement or interpretations of FMP (the one other morphological variable we did collect data for—body size—does not statistically differ among adult rodents; see Supporting Information). Moreover, that the metrics of Russo and Kirk (2013, 2017) capture changes in FMP in the specific ways we predicted, lends additional support to their utility for evaluating FMP across comparative mammalian samples1. In our view, our results for comparisons of cranial base measures in a rodent model species with individuals parceled into different mechanical loading regimes provides compelling evidence that differences in postural and locomotor behaviors are functionally linked with FMP.

In contrast to FMP, there were no significant differences between the “fully loaded bipedal walking” and “quadrupedal walking” groups in our measure of FMO. Thus, our results do not support a direct functional link between the anteroposterior inclination of the foramen magnum and bipedal locomotion. Put another way, in the absence of variation in other cranial features (e.g., brain size, face size) that could potentially interact with the basicranium to influence FMO, locomotion alone does not appear to drive morphological distinctiveness in the orientation of the foramen magnum in our rodent species parceled into different loading behavior groups. This observation is generally consistent with studies of FMO across mammals, including nonhuman primates, marsupials, and rodents (when taking other cranial structures into account) that vary in locomotor mode (Ruth et al., 2016). The lack of congruency between results for FMP and those for FMO in our experimental rodent sample is consistent with a recent comparative mammalian study that demonstrates a weak to nonexistent relationship between the two variables (Russo and Kirk, 2017). This potential functional (and evolutionary) disconnect between the position and orientation of the foramen magnum has been discussed at length elsewhere (Kimbel and Rak, 2010; Russo and Kirk, 2017).

It is worth noting that limitations in our data set precluded the ability for us to evaluate changes in FMP among the other loading behavior regimes (“partially loaded bipedal walking,” “standing bipedally,” and “no exercise control”). Nonetheless, based on our results from the other regions of the axial skeleton (e.g., sacral mediolateral breadth) in which all bipedal loading regime groups are distinguished from the “quadrupedal walking” group, we hypothesize that these cranial data available for the “standing bipedally” and “partially loaded bipedal walking” groups, these groups would likely also exhibit more forwardly shifted foramina magna compared to the “quadrupedal walking” rodents. If that were the case, such a finding would be consistent with other results of Russo and Kirk (2013) who demonstrated that orthograde trunk postures (and not necessarily bipedalism) can potentially select for a more anteriorly positioned foramen magnum in strepsirrhine primates (e.g., Propithecus) (see also Kimbel and Rak, 2010 for discussion).

We additionally sought to quantify features using metrics established in the paleoanthropological literature (see also Preuschoft et al., 1988; Nakatsukasa et al., 1995; and discussion above) to simultaneously build on most earlier work that did not report statistics and/or relied on visual descriptions of differences, and to direct focus to aspects of axial skeletal morphology meaningful to discussions of human evolution. Moreover, we extended analyses beyond a quadrupedal versus bipedal dichotomy by evaluating axial skeletal morphological changes associated with a variety of loading regimes that test the role of both amount and direction of loading using a controlled experimental approach. Our findings corroborate previous observations that bipedal walking in quadrupedal mammals, such as monkeys (baboons and macaques) (e.g., Nathan et al., 1964; Hayama, 1986; Preuschoft et al.,

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1 It is additionally worth noting that Neaux et al. (2017) recently corroborated the use of Russo and Kirk’s (2013, 2017) metrics for distinguishing between bipeds and non-bipeds in the hominin fossil record.
1988; Hayama et al., 1992), goats (Slijper, 1942a, 1942b, 1946), and rodents (rats and mice) (e.g., Riesenfeld, 1966), may generate lordotic lumbar curvature. In this study, the “fully loaded bipedal walking” group exhibited the largest (~300%) change in the vertebral wedging angle over the 12-week experiment, generating significantly more dorsally wedged penultimate lumbar vertebrae than any other loading behavior group (Fig. 5; Tables 1 and 2). While our metric for quantifying vertebral body wedging (wedging angle; see calculation above) differed from Preschoff et al. (1988) (wedging ratio: ventral/dorsal length), their results from macaques trained to walk bipedally similarly revealed changes in the wedging of the bony lumbar vertebral bodies. Noticeably, the “quadrupedal walking” and “no exercise control” groups exhibited greater percent changes than the “partially loaded bipedal walking” and “standing bipedally” groups. However, their morphological transformation occurred in the direction opposite to that of the “fully loaded bipedal walking” group. That is, their developmental change was associated with the acquisition of more ventrally wedged or kyphotic lumbar vertebral bodies.

Some previous workers determined that the manifestation of “lumbar lordosis” in their experimental animal models appeared related to changes in the anterior and posterior heights of the intervertebral discs (IVDs) rather than changes in bone shape per se (e.g., Yamada et al., 1960; Nakatsukasa et al., 1995). For example, some rodents trained to walk bipedally exhibit increases in IVD protrusions (particularly in the dorsal direction), including frequent breakages of the annulus fibrosus and concomitant prolapse of the nucleus pulposus (Sato, 1959; Yamada et al., 1960); though, notably, these IVD deformations were sometimes observed on both the ventral and dorsal body facies suggesting that these observations might reflect a general response to increased weight bearing in the lumbar region (Sato, 1959). In modern humans, IVDs may contribute up to 35% of total lumbar columnar length over which lordosis can occur (Aiello and Dean, 1990). Even so, IVDs contribute considerably less (17%) to modern human lumbar lordosis than wedging of the intervertebral bodies (83%; Been et al., 2010). And, in comparisons of IVD “wedging” between modern humans and quadrupedal macaques, IVD wedging was comparable while vertebral body wedging significantly differed (Been et al., 2010). Thus, while we cannot rule out that IVD “wedging” may have also contributed to the lordosis exhibited by the rodents examined here, it is likely that any contribution would be minimal or that IVD deformations might reflect a more general response to increased weight-bearing than specifically lordosing the lumbar spine. Moreover, we selected our metrics based on those used to examine the hominian fossil record with intact seriated vertebral columns preserving soft tissue remains. Notwithstanding, our results demonstrate that the bony vertebral bodies themselves (either in addition to, or independent of, the intervertebral discs) can respond to different loading behaviors in an experimental animal model as demonstrated here.

Dorsally wedged lumbar vertebrae are indicative of a lordotic lumbar spine, which facilitates bipedal walking by positioning the torso over the base of support to maintain balance in the sagittal plane (Been and Kalichman, 2014). Following Digiovanni’s (1989) methods for measuring vertebral body wedging, Whitcome et al. (2007) suggested the presence of and sexual dimorphism in lumbar lordosis in Australopithecus africanus, one of the few extinct hominins preserving series of adult lumbar vertebral specimens complete enough for this analysis (e.g., Sts 14 and Stw 431). Likewise, Latimer and Ward (1993) suggested a hominin-like pattern of thoracic kyphosis and lumbar lordosis through a comparison of measures of vertebral wedging (using the same metric) along the remarkably complete vertebral column of the later (~1.45 Ma) subadult Homo ergaster skeleton (KNM-WT 15000). Our results from the application of the same method show that bipedal loading behaviors can generate dorsally wedged lumbar vertebrae in an experimental rodent model. Interestingly, neither the “partially loaded bipedal walking” nor the “standing bipedally” group acquired dorsally wedged vertebrae. That is, only the “fully loaded bipedal walking” group exhibited negative values for wedging angle of the penultimate lumbar vertebra, indicative of vertebral body dorsal wedging (lordosis), while all other groups exhibited positive values for wedging angle of the penultimate lumbar vertebra, indicative of vertebral body ventral wedging (kyphosis). These observations may indicate that upright bipedal posture (i.e., “standing bipedally”), or even walking with a partially loaded bipedal spine (i.e., “partially loaded bipedal walking”) does not necessitate the acquisition of a lordotic lower spine. Put another way, our results suggest that dorsal wedging only occurs as a result of bipedal walking with a change in the load direction and a change in the amount of mechanical load, and does not occur with only a change in the direction of load or from sustained bipedal postures. More specifically, the absence of dorsal wedging in the “partially loaded bipedal walking” group might be attributed to the fact that despite the change in loading direction, the amount of loading this group was subjected to (average of 54.5% body weight; Foster, In press) was targeted to reflect the average amount supported by rat hind limbs in quadrupedal walking (~45%; Giszter et al., 2008). That “fully loaded bipedal walking,” (hind limbs supported, on average, approximately 90.2% of body weight), might be required to generate dorsally wedged lumbar vertebrae is also consistent with observations for other mammals that use sustained bipedal postures (e.g., while feeding), but do not engage in sustained bipedal walking. Cartmill and Brown (2017) assessed if bipedal standing, but not bipedal locomotion, influenced lumbar vertebral body wedging in gerenunks (Litocranius walleri), which are bovids known to use bipedal standing postures while foraging on low branches. Their results demonstrated that measures of lumbar vertebral body wedging did not differ between gerenunks and their non-bipedal relatives. Taken together, these lines of evidence appear to implicate nearly full hind limb loading during bipedal walking, and not bipedal standing or assisted (i.e., shared forelimb and hind limb loading) bipedal walking, as one pathway toward generating dorsally wedged lumbar vertebrae. Even so, that an incipient lordotic spine appears early in fetal development (Reichmann and Lewin, 1971), and that the lumbo-sacral angle is delayed or minimal in formation in humans who begin to walk relatively late or never walk due to a pathologic condition (Abitol, 1987a) but is nonetheless present, suggests a significant genetic contribution to lumbar lordosis in modern humans that warrants further investigation (Dryden et al., 2008). Our study demonstrates that experimental mechanical loading on the quadrupedal axial
skeleton has the potential to reveal how specific behaviors and adaptations are linked, and could potentially implicate an initial role of plasticity in the earliest bipeds that was later taken over through genetic accommodation (West-Eberhard, 2003, 2005).

Our results further show that all bipedal rodent groups exhibit greater percent changes in the cranial articular surface areas of the penultimate lumbar and the first sacral vertebrae (Figs. 6 and 7), as well as in the mediolateral breadth of the sacral alae (Fig. 8), than the “quadrupedal walking” group (Table 1). Noticeably, the overall percent changes are considerably less in the “fully loaded bipedal walking” group for these three variables (~30%–40%; Figs. 6–8) than was observed for the penultimate lumbar vertebral wedging (~300%; Fig. 5). In contrast to dorsal wedging, which only appeared to characterize the “fully loaded bipedal walking” group, a relatively greater percent change in cranial articular surface area of the penultimate and first sacral vertebrae and in the mediolateral breadth of the sacral alae was observed for all bipedal loading behavior groups compared to the “quadrupedal walking” loading behavior group. In other words, in our sample, these traits are consistent with a signal of adaptation to bipedal posture generally, and not specifically bipedal walking. When dorsoventral and mediolateral joint surface linear dimensions are examined independently (and not in conjunction as a measure of area), it is revealed that areal changes are driven by enlargement in both the mediolateral and the dorsoventral diameters (Supporting Information: Figs. S1–S4). This additional observation somewhat contrasts that of Nakatsukasa et al. (1995) who observed that differences in centrum dimensions between macaques trained to walk bipedally and control macaques were driven primarily by changes in the dorsoventral direction although, percent changes in our values were slightly greater for the dorsoventral diameter (Supporting Information Figs. S1 and S3) than the mediolateral diameter (Supporting Information Figs. S2 and S4).

Functionally, larger vertebral body articular surface areas serve to dissipate forces over a greater area, thereby reducing peak axial compressive forces throughout the vertebral column (Schultz, 1953; Jurung, 1991; Kapandji, 2008); and, a wider sacrum aligns the sacroiliac joints with the hip joints and helps to increase dorsal surface area for attachment of the erector spinae muscles that serves to maintain upright trunk posture during bipedal stance and locomotion (Gregory, 1928; Aiello and Dean, 2002). Indeed, our results suggest that a variety of bipedal loading behaviors, including “standing bipedally,” “partially loaded bipedal walking,” and “fully loaded bipedal walking,” may generate changes in both vertebral articular surface areas and sacral mediolateral breadth. Even so, it is worth mentioning that the measure of sacral breadth often employed in the paleoanthropological literature (e.g., Robinson, 1972; Johanson et al., 1982; see also citations within Russo and Shapiro, 2013) and, thus, examined here, inherently includes measurement of both the alae and the first sacral vertebral cranial articular surface mediolateral diameter, the latter of which is also used to calculate cranial articular surface area. It is therefore possible that changes in sacral mediolateral breadth as examined here are driven by changes in the mediolateral dimensions of the cranial articular surface of the first sacral vertebral body. When cranial articular surface mediolateral breadth of the first sacral vertebra is removed (i.e., subtracted from) the measurement of sacral breadth (i.e., the distance between the left and right lateral most aspects of the sacral alae), all bipedal loading behavior groups exhibit greater percent change values in sacral breadth than the “quadrupedal walking” group (Supporting Information Fig. S5), however, these differences are no longer statistically significant. Thus, the differences we observed for the original measure of sacral breadth among loading behavior groups appear to reflect changes in both mediolateral width of the sacral alae, in the strict sense, as well as the mediolateral diameter of the cranial articular surface of the first sacral vertebra.

Extinct hominins, including A. afarensis (e.g., A.L. 288-1) and A. africanus (e.g., Sts 14), appear to exhibit relatively small nonhuman ape-like intervertebral body joint surfaces (but large prezygapophysyal articular facets) compared to modern humans (Sanders, 1998; Russo and Shapiro, 2013), but preserved Australopithecus sacra are consistently mediolaterally wider than those of nonhuman hominoids (Abitolb, 1987b; Russo and Shapiro, 2013 and citations therein). That a mediolateral wide sacrum is characteristic of all known hominins suggests that this feature is functionally significant for both Australopithecus-like and modern human-like bipedalism (Russo and Shapiro, 2013). This inference is seemingly corroborated by the results of our experimental study that show all bipedal loading behaviors induced here appear to produce significant changes in sacral alar mediolateral breadth in comparison to the “quadrupedal walking” behavior in rodents. Contrarily, we did not observe any differences (with the exception of the pairwise comparison between the “standing bipedally” and “partially loaded bipedal walking” group for the penultimate lumbar vertebra; Table 1) among bipedal loading behaviors for penultimate lumbar and first sacral vertebral articular surface areas differences that could shed light on the relatively smaller joint surfaces observed for Australopithecus (and by extension presumably other early hominins) in comparison to modern humans. One ostensible explanation might be differences in the “baseline” positional behaviors between rats and, in our opinion, the expected hominid ancestral condition (i.e., pronograde-adapted vs. orthograde-adapted torso morphology). We also did not examine prezygapophysyal or sacroiliac joint size in this study to assess how bipedal loading behaviors might translate to changes in relative joint sizes (i.e., comparisons of intervertebral body vs. zygapophysyal joint surfaces; but see Riesenfeld, 1966; Nakatsukasa et al., 1995 for sacral auricular surfaces). However, an apparent discrepancy in relative joint size patterns between Australopithecus and modern humans has been previously noted (e.g., Sanders, 1998) and suggested to reflect different patterns of weight transmission throughout the vertebral column, and potentially distinct modes of bipedality (e.g., kinematic variability, such as a bent-hip, bent-knee gait) among bipedal hominins (Sanders, 1998; Russo and Shapiro, 2013), as has been discussed elsewhere in relation to other regions of the postcranial skeleton (e.g., Haile-Selassie et al., 2012). Future work using experimental animal models might focus on patterns of variation in articular surface dimensions within a skeletal region and/or assess kinematic and kinetic changes to better evaluate these hypotheses.

Finally, the “no exercise control” group delivered some confounding results when compared to the bipedal and
quadrupedal groups that are worthy of discussion. For instance, while for some analyses the “no exercise control” group was not statistically different from the “quadrupedal walking” group (e.g., the percent change in the wedging angle of the penultimate lumbar vertebra; Fig. 5 and Table 1), there were also results for which the “no exercise control” group values were consistent with any one or more of the bipedal groups, and were significantly different from the “quadrupedal walking” group (e.g., the percent change in the articular surface area of the first sacral vertebra; Fig. 7 and Table 1). It is generally unclear why results for the “no exercise control” group are not most similar to the “quadrupedal walking” group, particularly as previous work on the role of activity in generating bony adaptive change demonstrates that limb joint articular surface areas do not differ significantly between exercised and noneexercised animals (Lieberman et al., 2001).

One explanation for the “no exercise control” results might be that they represent a phenotype that is less canonicalized by behaviors outside the cage environment and by exercise. Indeed, when looking at the 95% confidence interval (CI) of variables measured by percent change, the no exercise control group almost always has a greater range of variation than any other group, with the only exception being the percent change in wedging angle of the penultimate lumbar vertebra (Fig. 5). These highly variable phenotypes within one behavior group were less influenced by activity level and specific loading behaviors than were experienced by the other groups during the 12-week experiment. As an additional explanation, previous studies of bipedally trained rodents have noted that control rodents experience greater body size growth than experimental rodents that are either altered (i.e., forelimbs amputated) and/or subject to training conditions (e.g., bipedal walking) (Cassidy et al., 1988), perhaps due to a reduced amount of stress in the former that can otherwise suppress growth. In this way, the greater percent changes we observed for the “no exercise control” rodents could reflect overall changes in body size. Body masses for the “no exercise control” group were low relative to the other groups at the beginning of the experiment, but all loading behavior groups became more similar by weeks 6 and 9 (Supporting Information Fig. S6). At the end of the experiment, the “fully loaded bipedal walking” group had the highest body masses among the experimental groups. However, a repeated-measures linear mixed-effect model found no significant differences between the experimental groups in body mass (P = 0.202; Supporting Information Fig. S6). One final possibility is that, specifically where values from the control group were similar to bipedal groups or when their variation overlapped the range of all other groups, results may reflect different rates of behaviors each rat engaged in while in the home cage environment. In particular, because the “no exercise control” group remained in their cages, this allowed for an additional 5 hours per week of unmonitored cage activity, totaling to 60 hours over the course of the experiment. In other words, the bipedal and quadrupedal experimental groups engaged in 60 hours of a narrow set of loading behaviors, while the “no exercise control” group had 60 hours to engage in a variety of behaviors. Future experiments should attempt to account for behavioral differences via automated phenotyping methods that quantify cage movement using RFID (e.g., Howerton et al., 2012; König et al., 2015; Bains et al., 2016, 2017; Noorshams et al., 2017). Nevertheless, while the “no exercise control” serves as an important and informative control group for bounding our interpretations, the most apt comparisons are seen between the bipedal and quadrupedal groups, which exhibit morphologies that are consistent with previous work and provide new insights into how behaviors are linked with traits consistent with adaptation to bipedalism in the axial skeleton.

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