



HAL
open science

Ontogeny and variability of trabecular bone in the chimpanzee humerus, femur and tibia

Zewdi J Tsegai, Matthew M Skinner, Dieter H Pahr, Tracy L Kivell,
Jean-Jacques Hublin

► **To cite this version:**

Zewdi J Tsegai, Matthew M Skinner, Dieter H Pahr, Tracy L Kivell, Jean-Jacques Hublin. Ontogeny and variability of trabecular bone in the chimpanzee humerus, femur and tibia. *American Journal of Physical Anthropology*, 2018, 167, pp.713 - 736. 10.1002/ajpa.23696 . hal-04045400

HAL Id: hal-04045400

<https://hal.science/hal-04045400>



Submitted on 24 Mar 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

RESEARCH ARTICLE

Ontogeny and variability of trabecular bone in the chimpanzee humerus, femur and tibia

Zewdi J. Tsegai¹  | Matthew M. Skinner^{2,1} | Dieter H. Pahr³ | Jean-Jacques Hublin¹ | Tracy L. Kivell^{2,1} 

¹Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany

²Skeletal Biology Research Center, School of Anthropology and Conservation, University of Kent, Canterbury, United Kingdom

³Institute for Lightweight Design and Structural Biomechanics, Vienna University of Technology, Wien, Austria

Correspondence

Zewdi J. Tsegai, Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, D-04103 Leipzig, Germany.
Email: zewdi_tsegai@eva.mpg.de

Funding information

European Research Council Starting Grant, Grant/Award Number: 336301; Max Planck Society

Abstract

Objectives: Trabecular bone structure is known to be influenced by joint loading during life. However, many additional variables have the potential to contribute to trabecular bone structure of an adult individual, including age, sex, body size, genetics, and overall activity level. There is little research into intraspecific variability in trabecular bone and ontogeny of trabecular bone structure, especially in nonhuman primates.

Materials and methods: This study investigates trabecular structure in adult and immature chimpanzees from a single population using high-resolution microcomputed tomographic scans of the proximal humerus, proximal femur, and distal tibia. Trabecular bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular spacing (Tb.Sp), and degree of anisotropy (DA) were quantified in specific regions of adult and immature chimpanzees, and color maps were generated to visualize the distribution of BV/TV throughout the joint in the metaphysis of immature specimens.

Results: The results demonstrate that variability in adult trabecular structure cannot be explained by sex or body size. During ontogeny, there is a general increase in trabecular BV/TV and Tb.Th with age, and ratios of trabecular parameters between the fore- and hindlimb may be consistent with locomotor transitions during ontogeny.

Discussion: Variation in trabecular morphology among adult individuals is not related to sex or body size, and the factors contributing to intraspecific variability, such as overall activity levels and genetic differences, require further investigation. Trabecular ontogeny in chimpanzees differs from humans in some respects, most notably the absence of a high BV/TV at birth.

KEYWORDS

cancellous bone, development, knuckle-walking, locomotion, *Pan troglodytes*

1 | INTRODUCTION

Reconstructing locomotor behavior in extinct species requires identification of plastic features that reflect actual behavior during life (Ward, 2002). The internal structure of bone is able to remodel in response to loading, a process known as bone functional adaptation (Cowin, 2001; Kivell, 2016; Ruff, Holt, & Trinkaus, 2006), and thus features of both cortical and trabecular bone may hold a functional signal that could be used to interpret behavior of extinct taxa. Experimental studies have demonstrated the ability of trabecular bone to remodel in response to changes in joint loading (Barak, Lieberman, & Hublin, 2011; Biewener, Fazzalari, Konieczynski, & Baudinette, 1996; Pontzer et al., 2006), and

several studies have identified behavioral correlates in the trabecular structure of extant (Griffin et al., 2010; MacLatchy & Müller, 2002; Maga, Kappelman, Ryan, & Ketcham, 2006; Ryan & Ketcham, 2002b; Tsegai et al., 2013) and fossil (Barak, Lieberman, Raichlen, et al., 2013b; Ryan et al., 2018; Ryan & Ketcham, 2002a; Skinner et al., 2015; Su & Carlson, 2017; Su, Wallace, & Nakatsukasa, 2013; Zeininger, Patel, Zipfel, & Carlson, 2016) primate taxa. However, we currently lack an understanding of how nonbiomechanical factors may influence trabecular structure, particularly within nonhuman primates. Here, we help to fill this gap through an analysis of how trabecular bone structure changes throughout ontogeny within the chimpanzee hind- and forelimb to provide insight into the underlying patterning of

trabecular bone, and how the structure may change in response to locomotor changes throughout development.

Cortical bone morphology of the adult humerus and femur reflects variation in human activity levels (Macintosh, Pinhasi, & Stock, 2014, 2017; Shaw & Stock, 2013; Stock & Macintosh, 2016), as well as locomotor differences in extant apes, enabling the reconstruction of limb loading in fossil taxa (Ruff, 2002, 2008; Ruff, Burgess, Ketcham, & Kappelman, 2016). Humans, gorillas and chimpanzees undergo changes in locomotion during ontogeny (Doran, 1992, 1997; Sarrinhaus, MacLatchy, & Mitani, 2014; Sutherland, 1997; Sutherland, Olshen, Cooper, & Woo, 1980), which are reflected in limb bone cross-sectional geometry at various life stages (Cowgill, Warrener, Pontzer, & Ocobock, 2010; Gosman, Hubbell, Shaw, & Ryan, 2013; Ruff, 2003a; Ruff, Burgess, Bromage, Mudakikwa, & McFarlin, 2013; Sarrinhaus, MacLatchy, & Mitani, 2016). In humans, analysis of long bone cross-sectional geometry, from neonates to adulthood, reveals ontogenetic changes in relative femoral to humeral strength (i.e., polar section modulus), and femoral and tibial diaphyseal strength and shape (i.e., subperiosteal area, and the ratio of the maximum and minimum second moments of inertia, respectively), which reflect the acquisition of bipedal locomotion (Gosman et al., 2013; Ruff, 2003a). The increased stability that is acquired as bipedal locomotion becomes more refined is also reflected in changes in femoral cross-sectional shape (Cowgill et al., 2010). In chimpanzees, there is an increase in strength of the femur compared to the humerus during ontogeny, with a significant change between infant and juvenile periods (Sarrinhaus et al., 2016). This transition coincides with a reduction in forelimb loading and an increase in hindlimb loading, as the hindlimb becomes more dominant in locomotion (Doran, 1992, 1997; Sarrinhaus et al., 2014; 2016). Similarly, young mountain gorillas, which engage in more arboreal and suspensory behavior than adults (Doran, 1997), have forelimb to hindlimb strength ratios similar to more arboreal adult Western lowland gorillas, but have relatively stronger forelimbs than hindlimbs when compared to their less arboreal adult counterparts (Ruff et al., 2013). The ratio of forelimb to hindlimb strength changes to the adult condition at around 2 years of age, which corresponds to the reduction of arboreal locomotion, and adoption of a more terrestrial, adult-like locomotor repertoire (Doran, 1997; Ruff et al., 2013). Evidence from cortical bone diaphyseal morphology demonstrates that both adult and nonadult cortical structure reflects locomotor behavior at different life stages.

In contrast to cortical bone, comparisons of trabecular architecture between the adult humeral and femoral head do not clearly distinguish bipedal humans from other primates (Shaw & Ryan, 2012; Tsegai, Skinner, Pahr, Hublin, & Kivell, 2018). In general, across primate taxa, regardless of their locomotor behavior, the femoral head has a more robust trabecular structure (i.e., higher bone volume/total volume, or BV/TV) than the humeral head (Chirchir et al., 2015; Ryan & Walker, 2010; Shaw & Ryan, 2012; Tsegai et al., 2018). However, trabecular bone does show signals of locomotor changes during ontogeny. In humans, changes in trabecular structure in the lower limb occur around the time of acquisition of bipedal locomotion; the human proximal femur (Ryan & Krovitz, 2006) and proximal tibia (Gosman & Ketcham, 2009) have a shared pattern of trabecular ontogeny that is characterized by an initially high volume of

numerous, thin trabeculae, with both BV/TV and uniformity of orientation (i.e., degree of anisotropy, or DA) reducing until 1–2 years of age. At this time, perhaps driven by commencement of bipedal locomotion, there is an increase in BV/TV and DA, reaching adult levels at around 4–7 years. The human calcaneus shares this pattern of high BV/TV, followed by a rapid reduction after birth, subsequently increasing around 3 years of age (Saers, 2017). The DA follows the inverse pattern, unlike the femur and tibia, there is an increase in DA from birth until 3 years of age, followed by a reduction until DA stabilizes at around 5 years of age (Saers, 2017). Furthermore, throughout human ontogeny, there is a reduction in the number of trabeculae and an increase in trabecular thickness (Tb.Th) in the lower limb and vertebrae, reaching adult-like values during adolescence (proximal tibia: Gosman & Ketcham, 2009; lumbar vertebra: Kneissel et al., 1997; proximal femur: Ryan & Krovitz, 2006; calcaneus: Saers, 2017).

Although these ontogenetic changes in trabeculae fit well with the onset and development of human bipedal locomotion, both the proximal humerus and proximal femur show a similar pattern of initial trabecular reduction, followed by an increase in BV/TV around 1–2 years of age, indicating the potential for genetic, rather than mechanical, influence on the timing of trabecular structural change (Ryan, Raichlen, & Gosman, 2017). In other words, these commonalities between the upper limb and lower limb bones may suggest, a “general model for trabecular development” (Ryan et al., 2017, p. 294), which may also be shared with the vertebral column (Acquaah, Robson Brown, Ahmed, Jeffery, & Abel, 2015). During this period, bone is adapting from the initial grid-like structure laid down during endochondral ossification (Carter & Beaupré, 2000; Martin, Burr, Sharkey, & Fyhrie, 2015; Scheuer & Black, 2000), and undergoing changes in both physical properties and bone mineral content (Mølgaard, Thomsen, & Michaelsen, 1998; Nafei, Danielsen, Linde, & Hvid, 2000a), while adjusting to a rapidly increasing body mass (Tanck, Homminga, van Lenthe, & Huiskes, 2001). In the ilium, characteristic adult-like trabecular organization is observed in neonates, further indicating the potential roles of genetics, or other factors such as joint morphology, in determining trabecular structure (Abel & Macho, 2011; Cunningham & Black, 2009a, 2009b). Other nonprimate, domesticated mammalian taxa also demonstrate an increase in BV/TV and DA during ontogeny (sheep tibia: Nafei, Kabel, Odgaard, Linde, & Hvid, 2000b; pig vertebra and tibia: Tanck et al., 2001; dog ulna: Wolschrijn & Weijis, 2004).

Nonetheless, in humans divergence of trabecular bone characteristics of the humerus and femur, with a higher rate of increase in BV/TV, Tb.Th, and Young's Modulus in the femur, begins at around 1 year of age, indicating that although the general pattern of ontogeny is similar between these elements, differences in the developmental trajectory between elements may reflect biomechanical changes in limb function (Ryan et al., 2017). At later stages of ontogeny, the refinement of bipedal locomotion, which involves increased stability and reduced variability (Cowgill et al., 2010; Raichlen et al., 2015), is indeed reflected in increased homogeneity of trabecular organization across the distal tibia (Raichlen et al., 2015). There is perhaps evidence of changes in trabecular morphology of the human foot related to the

acquisition of the characteristic human heel-strike and toe-off stance phases (Saers, 2017; Zeininger, 2013).

Among nonhuman primates, ontogenetic changes in trabecular structure have, to our knowledge, only been studied in the African ape foot (Zeininger, 2013). Similar to humans, African apes show an increase in BV/TV and Tb.Th between infant and adult age groups across taxa (Zeininger, 2013). However, whether the timing of trabecular structural changes in African apes is similar to that of humans is unknown. With very little comparative context, it remains unclear to what extent ontogenetic changes in human trabecular architecture are driven by biomechanical changes associated with the onset of bipedal locomotion, or by predetermined genetic transitions in bone microarchitecture.

Bone is a complex structure, with adult form being determined by a combination of factors that can change throughout life, including body mass, sex, age, hormonal, and genetic differences. For example, nonbehavioral factors, such as genetic and hormonal differences, can influence trabecular structure and/or the responsiveness of trabecular bone to mechanical load between species or populations, and even between anatomical sites (Lovejoy, McCollum, Reno, & Rosenman, 2003; Wallace, Judex, & Demes, 2015a; Wallace, Kwaczala, Judex, Demes, & Carlson, 2013; Wallace et al., 2015b; Wallace, Tommasini, Judex, Garland, & Demes, 2012). Our understanding of these factors in nonhuman primates, and their contribution to bone structure at different life stages, is limited. For example, chimpanzees and bonobos have been shown to vary in the ontogenetic timing of changes in thyroid hormone, regulating among other things somatic growth and metabolism (Behringer, Deschner, Murtagh, Stevens, & Hohmann, 2014b). Sex differences have the potential to affect bone structure at all life stages. During growth, the body mass of male and female chimpanzees diverges at around 7 years of age, with males and females showing different growth rates and ages of growth cessation (Leigh & Shea, 1995, 1996), potentially contributing to sex differences in adult bone morphology. There are locomotor differences between male and female chimpanzees from the Taï National Park (Doran, 1993), with females engaging in a greater proportion of arboreal quadrupedalism and, to a lesser extent, suspension than males, who use more quadrupedal climbing and bipedal locomotion compared to females. The percentage of locomotor time spent on different substrates, comprising the ground and five types of arboreal substrate, are similar between sexes (Doran, 1993).

In this study, we use a sample of chimpanzee (*Pan troglodytes verus*) specimens from a single population—the Taï National Forest, Cote D'Ivoire—to investigate trabecular ontogeny and within population variability in the proximal humerus, proximal femur and distal tibia. We hypothesize that:

1. Across taxa Tb.Th, trabecular separation (Tb.Sp) and BV/TV scale positively with size (Doube, Klosowski, Wiktorowicz-Conroy, Hutchinson, & Shefelbine, 2011; Fajardo et al., 2013; Ryan & Shaw, 2013), trabecular number (Tb.N) scales negatively with size (Fajardo et al., 2013; Ryan & Shaw, 2013), and there is a weak relationship between the relative uniformity of trabecular orientation (i.e., DA) and body size (Doube et al., 2011). Among humans, it has been found that Tb.Th, BV/TV, and bone mineral density of

the calcaneus are correlated with body size (Best, Holt, Troy, & Hamill, 2017), however, this is only the case in individuals who do not engage in regular running. Here, we test whether there is a relationship between body size and trabecular variables in adult chimpanzees within a single subspecies of chimpanzee, with a limited range of size variability.

2. Trabecular bone will change during ontogeny in a manner similar to that found in humans, following the model for trabecular bone development outlined by Ryan et al. (2017) and identified in other studies (Gosman & Ketcham, 2009; Ryan & Krovitz, 2006; Saers, 2017; Zeininger, 2013). Trabecular BV/TV and DA will be initially high followed by a reduction and then subsequent increase. We also predict Tb.Th will increase and Tb.N will decrease, from birth to adulthood.
3. Locomotor ontogeny in chimpanzees is characterized by a reduction in arboreal locomotion and an increase in the frequency of quadrupedal knuckle-walking with increasing age (Doran, 1992, 1997; Sarringhaus et al., 2014). It is difficult to directly characterize loading experienced by the skeleton in chimpanzees, as their complex locomotor repertoire includes a diverse range of positional and locomotor behaviors (Hunt, 1991), and there are both practical and ethical limitations to research on chimpanzee locomotion. To simplify interpretation of skeletal signals of locomotion, a dichotomy of reduced forelimb and increased hindlimb loading during ontogeny has been used; however, this may not fully capture changing loads on the skeleton. This concept is generally based on reduced torso-orthograde suspension and increased frequencies of knuckle-walking, which incurs higher vertical reaction forces on the hindlimb than the forelimb (Demes et al., 1994). Together this implies a reduction in forelimb loading and an increase in hindlimb loading. However, infants and juveniles use some hindlimb loaded modes more frequently than adults, specifically, they have higher frequencies of bipedalism, leaping and vertical climbing (for limb loading during vertical climbing: Hanna, Granatosky, Rana, & Schmitt, 2017). Despite this complexity of locomotor changes, there is morphological evidence for increased loading of the hindlimb relative to the forelimb across ontogenetic stages, as the femur increases in torsional and bending strength (i.e., polar second moment of area and polar section modulus) compared to the humerus during ontogeny (Sarringhaus et al., 2016). As such, we predict that, as with diaphyseal cross sectional properties, trabecular bone will reflect increased hindlimb (femoral head and distal tibia) loading compared to the forelimb (proximal humerus), with this change occurring after five years of age (Doran, 1992, 1997; Sarringhaus et al., 2014; Sarringhaus et al., 2016).
4. During ontogeny, the distribution of trabecular BV/TV within each metaphysis will be initially homogeneous, with increasing heterogeneity over time (Ryan & Krovitz, 2006). Previous studies have not assessed the stage in humans at which heterogeneity stabilizes, due to absence of adult or adolescent specimens in the study samples. Therefore, we make no prediction as to which age adult-like distributions of trabecular BV/TV will be reached.

2 | MATERIALS AND METHODS

2.1 | Study sample

The study sample was taken from a single population of *Pan troglodytes verus*, wild collected in the Taï National Park, Republic of Côte d'Ivoire. This is a long-term study site for which age, sex, behavioral data, and cause of death is available for some individuals (Boesch & Boesch-Achermann, 2000). Trabecular bone was analyzed in the proximal humerus, proximal femur, and distal tibia of 21 adult and 21 immature individuals (Table 1), although all elements were not present for every individual in the sample. The proximal humerus, proximal femur, and distal tibia were selected based on preservation and availability, with femora and tibiae taken from the same side where possible.

2.2 | Age categories

The locomotor repertoire of chimpanzees comprises a range of positional and locomotor modes, and changes in frequencies of these behaviors occur at different life stages (Doran, 1992, 1997; Sarrinhaus et al., 2014, 2016). As such, the study sample was divided into five age categories based on locomotor transitions reported by Sarrinhaus et al. (2014, 2016) and, for this particular population/subspecies, by Doran (1992, 1997). Age of each individual was based on the collection records, as the identification, sex, and date of birth for most individuals was known (see Supporting Information).

Age categories were defined as follows. Baby: from birth to 5 months, the period prior to onset of independent locomotion where chimpanzees are carried by the mother, although locomotor play occurs after 3 months (Doran, 1992). Infant: from 5 months until 4 years, after the commencement of locomotion but before complete independence from the mother. During this stage torso-orthograde suspension is the most frequent locomotor mode (42.8%: Sarrinhaus et al., 2014), and individuals continue to engage in higher frequencies of arboreal locomotion than adults (Doran, 1992; Sarrinhaus et al., 2014). Juvenile: 5–9 years, after independence from the mother but before the adolescent period; quadrupedal walking is the most frequent locomotor mode for this age category (42.3%: Sarrinhaus et al., 2014), however, a higher frequency of suspensory behavior is used during arboreal locomotion than in adults (Doran, 1992). Adolescent: from 10 years until epiphyseal fusion, locomotor modes are approaching adult-like frequencies of each locomotor mode. Adult: defined by complete epiphyseal fusion. The oldest individual included in the sample of known age was 38 years old. Among the individuals

of unknown age, only one male individual had pronounced tooth wear, however, the trabecular structure of this individual showed no evidence of age-related reduction in BV/TV, compared to other males in the sample. For four immature individuals in the study sample, age was estimated based on dental eruption data (Smith & Boesch, 2011; Smith, Crummett, & Brandt, 1994; see Supporting Information). One individual was considered juvenile (M2 erupted) and three were considered to be infants (two individuals with incomplete deciduous dentition and one individual with M1 erupted). One individual (MPI 15003) was very small (see Supporting Information for femoral length), and could potentially be younger than 5 months, however, to be conservative, this individual was included in the infant category.

2.3 | Scan acquisition

Microcomputed tomographic scans (micro-CT) were collected using three scanners (BIR ACTIS 225/300, diondo d3, and SkyScan 1173) housed at the Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology (Leipzig, Germany). Specimens were scanned at an isotropic voxel size of 13.7–42.8 μm . All scans were reconstructed as 16-bit TIFF image stacks. Specimens were downsampled in Avizo 6.3 (FEI Visualization Sciences Group) to reduce file size for subsequent processing stages. The downsampled resolutions, along with relative resolution, are shown in Table 1 for each age category. Adequate resolution for analysis of trabecular structure was retained, as demonstrated by the range of relative resolutions, which indicates the number of pixels representing trabeculae with mean thickness (Kivell, Skinner, Lazenby, & Hublin, 2011; Sode, Burghardt, Nissenson, & Majumdar, 2008).

2.4 | Segmentation

All specimens were reoriented into standardized positions in Avizo 6.3, and segmented using the Ray Casting Algorithm (Scherf & Tilgner, 2009). Scans were subsequently segmented into three regions (cortex, trabeculae, and internal region of the bone) using an in-house script for medtool 4.0 (www.dr-pahr.at), following Gross et al. (2014). The cortex was removed to create the inner mask, where the internal region of the bone and the trabecular bone had different grey values (Figure 1a).

2.5 | 3D morphometric mapping

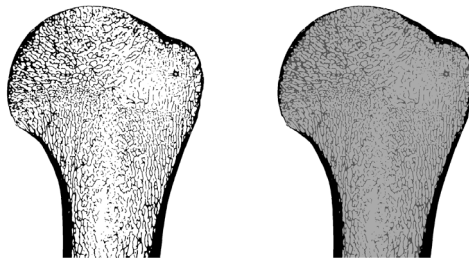
Morphometric maps of BV/TV were generated for immature individuals following Gross et al. (2014) using medtool v4.0 (www.dr-pahr.at)

TABLE 1 Study sample

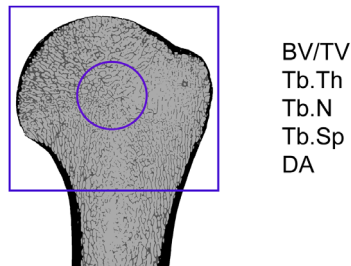
Age group	Age	No. individuals (M/F/U)	No. per element			Resolution	Relative resolution*
			Humerus	Femur	Tibia		
Adult	Fused epiphyses	8/12/1	19	21	20	0.035–0.045	4.25–8.10
Adolescent	10+ yr	2/2/0	4	4	3	0.025–0.035	5.03–11.85
Juvenile	5–10 yr	6/2/1	9	7	6	0.015–0.030	4.41–12.54
Infant	5 mo–4 yr	2/1/3	6	6	5	0.014–0.020	7.99–13.07
Baby	0–5 mo	1/0/1	2	2	2	0.014–0.015	6.48–9.82

*Relative resolution is calculated as mean trabecular thickness divided by the resolution. This demonstrates how many pixels represent trabeculae of mean thickness.

(a) Segmentation of trabecular and cortical bone



(b) Quantification of trabecular variables in ROI in adult or VOI in immature individuals



(c) Morphometric mapping

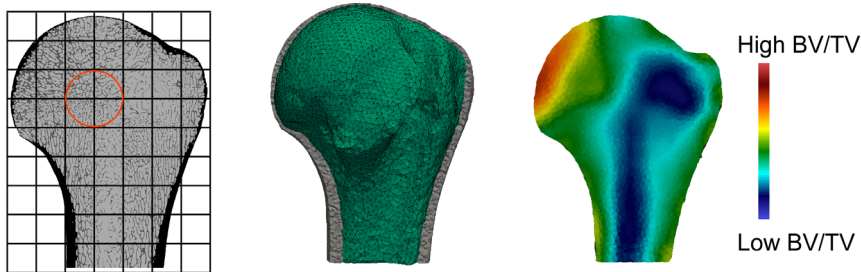


FIGURE 1 Processing stages for analysis of trabecular bone structure, shown here in the proximal humerus. (a) Mid-plane images of the segmented bone (left) and the masked image (right), where trabecular bone, cortical bone, and internal region of the bone are assigned different grey values. (b) Trabecular variables are quantified in a large ROI for adult individuals and a small spherical VOI for immature individuals. (c) Trabecular bone is quantified using a spherical VOI at each node of a three-dimensional grid (left), using a mesh of the internal region of the bone (middle), the values are interpolated to produce a three-dimensional morphometric map of BV/TV (right)

as shown in Figure 1c. A three-dimensional (3D) tetrahedral mesh was created of the internal region of the bone using CGAL (<http://www.cgall.org>). For adolescent individuals a mesh size of 1 mm was used, and a mesh size of 0.5 mm was used for younger individuals. A 2.5 mm background grid was applied in three dimensions to the inner mask, and BV/TV was quantified at each node of the background grid using a 5 mm sampling sphere. The values from each sampling sphere were interpolated and applied to elements of the 3D tetrahedral mesh in order to generate morphometric maps of BV/TV. For some specimens in the immature sample (four tibia, four femora, and six humeri) BV/TV maps were not generated, either due to the presence of matrix or, due to high porosity, the cortex could not be segmented (see Supporting Information).

2.6 | Definition of regions and volumes of interest

Definition of biomechanically-homologous subregions in trabecular analyses in adult specimens is often complex (Kivell et al., 2011;

Lazenby, Skinner, Kivell, & Hublin, 2011b; Maga et al., 2006), and this complexity only increases when investigating immature specimens. Therefore, different methods of defining subregions were applied here for adult and immature specimens. To investigate variability in adult chimpanzees, trabecular bone was quantified in large regions of interest (ROIs; following Tsegai et al., 2018), which were defined according to the following protocol (Figures 1b and 2). The proximal humerus was defined from the point at which curvature of the shaft expands towards the humeral head (Figure 2a). The femoral head was defined by the most inferior and lateral points of the femoral head (Figure 2b). The ROI in the distal tibia was defined by increasing curvature of the shaft in medial and anterior views (Figure 2c).

In immature individuals, it is difficult to define homologous regions, especially in very young individuals, due to continued longitudinal growth and changing morphology over time (Ryan & Krovitz, 2006). As such, definition of a large ROI, similar to that used for adult individuals, across an ontogenetic series is challenging. Instead, volumes of interest (VOIs) were extracted from the inner mask using

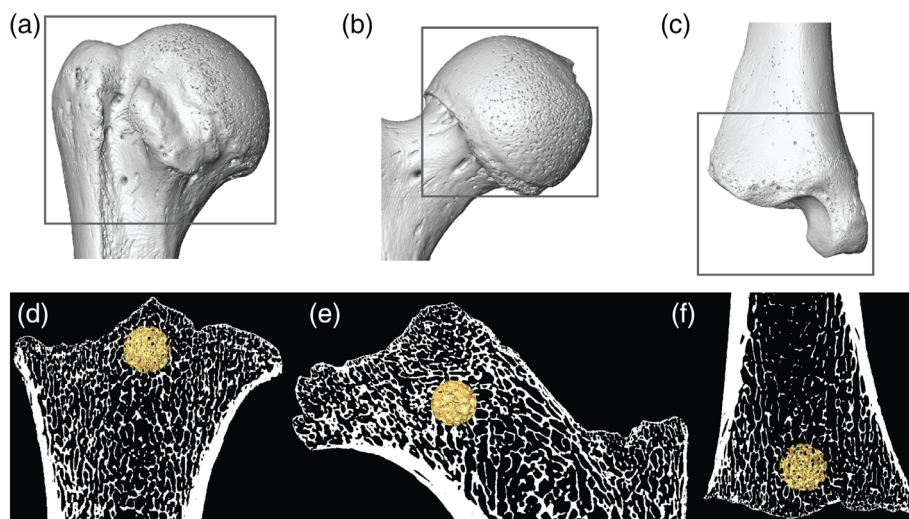


FIGURE 2 Definition of cubic ROIs in (a) the proximal humerus, (b) the proximal femur, and (c) the distal tibia of adult chimpanzees. Definition of 5 mm spherical VOIs in (d) the proximal humerus, (e) the proximal femur, and (f) the distal tibia of immature chimpanzees

medtool v4.0, and trabecular bone parameters were quantified in these subregions (Figure 2d–f). A spherical VOI, with a 5 mm diameter, was placed 5 mm deep to the epiphyseal surface in each individual, centered in the mediolateral and anteroposterior planes of the proximal humerus and distal tibia following approximately the ROI size and placement protocols of Ryan and Krovitc (2006) and Ryan et al. (2017). In the proximal femur, centering the VOI in the mediolateral and anteroposterior planes, resulted in VOIs located in clearly different locations between different age categories. Thus, in the youngest individuals ($N = 3$) VOIs were placed centered in the mediolateral and anteroposterior plane, whereas in older individuals the VOI was centered in the anteroposterior plane, the mediolateral location was determined by the most proximal extent of the metaphysis and the proximodistal location by the most medial extent of the metaphysis. This resulted in similar VOI placement between individuals. Specimens with partially fused epiphyses were included in the study, if the epiphyseal line remained clearly visible.

An advantage of this methodology is that it enables comparison of the results of the present study to previous studies of trabecular ontogeny in humans. There are potential advantages to using scaled VOIs due to the problem of oversampling (Lazenby et al., 2011b), and scaled VOIs have been applied to investigate trabecular ontogeny of the proximal and distal tibia (Gosman & Ketcham, 2009; Raichlen et al., 2015). However, for many volume based variables, including most variables quantified in this study, VOI size has less of an impact than location (Kivell et al., 2011; Lazenby et al., 2011b).

2.7 | Quantification of regional trabecular variables

Trabecular bone architectural variables were quantified in each defined ROI or VOI (Figures 1b and 2). These were quantified from the inner mask using a script available in medtool v4.0. Bone volume fraction (BV/TV) was calculated as the total number of bone voxels divided by the total number of voxels. Trabecular thickness (Tb.Th) and trabecular spacing (Tb.Sp) were calculated following the sphere fitting method of Hildebrand and Rüegsegger (1997), and trabecular

number (Tb.N) was calculated as $Tb.N = 1/(Tb.Th + Tb.Sp)$. The mean intercept length method was used to quantify the second order fabric tensor and the degree of anisotropy (DA) was calculated as $DA = 1 - [\text{smallest eigenvalue}/\text{largest eigenvalue}]$, with a DA of 0 reflecting complete isotropy and a DA of 1 representing “complete” anisotropy.

2.8 | Statistical analysis

Nonparametric tests were used for the statistical analysis, as the Shapiro–Wilk test for normal distribution found that trabecular variables were not normally distributed in the adult and immature samples. A p value of <0.05 was considered significant. All statistical tests were conducted using R v3.3 (R Core Team, 2016) and ggplot2 (Wickham, 2009) and cowplot (Wilke, 2017) were used for plot generation.

2.8.1 | Adult analysis

In the adult sample, Mann–Whitney U tests were applied to test for significant differences between sexes. As no significant differences were found between sexes in any trabecular variable, sexes were pooled for subsequent analyses. To test for significant differences in trabecular parameters between elements in the adult sample, Kruskal–Wallace tests were used, followed by post hoc Nemenyi tests for pairwise comparisons. The relationship between trabecular variables both within and between elements was tested using Spearman's rank correlation tests with a post hoc Bonferroni correction. To investigate the relationship between body size and trabecular structure among adult chimpanzees, two measures of size were used. First, the superoinferior femoral head height (FHH) was used as a proxy for body size. Although primate taxa differ slightly in the relationship between femoral head height and body mass, only one taxon is included in the study sample and there is a strong correlation between FHH and body mass in chimpanzees (Burgess, McFarlin, Mudakikwa, Cranfield, & Ruff, 2018; Jungers, 1991; Ruff, 2003b). Moreover, this measurement has been used in previous analyses of allometric relationships in trabecular bone structure (e.g., Doube et al., 2011; Ryan & Shaw, 2013). The second measure of

TABLE 2 Trabecular variables from ROIs in adult individuals

	BV/TV (%)	Tb.Th (mm)	Tb.Sp (mm)	Tb.N (mm ⁻¹)	DA
Humerus	24.82 (2.91)	0.22 (0.02)	0.69 (0.11)	1.12 (0.12)	0.16 (0.02)
Femur	37.56 (5.13)	0.28 (0.03)	0.56 (0.07)	1.20 (0.12)	0.09 (0.06)
Tibia	25.26 (3.22)	0.23 (0.02)	0.73 (0.14)	1.07 (0.15)	0.35 (0.05)
<i>p</i>	<0.01	<0.01	<0.01	0.01	<0.01

Note. Mean and standard deviation, in parentheses, for each trabecular variable in the proximal humerus, proximal femur, and distal tibia. Results of Kruskal–Wallace test for significant differences between elements, with significant differences in bold.

size was the total joint volume (TJV) included within each ROI, which was used to investigate the relationship between joint size and trabecular bone structure. TJV was found to correlate significantly with FHH in each element, using Spearman's rank correlation test (humerus: $r = 0.86$, $p < 0.01$; femur: $r = 0.75$, $p < 0.01$; tibia: $r = 0.77$, $p < 0.01$). The relationship between trabecular variables and both size proxies, FHH and TJV, were tested using Spearman's rank correlation tests with a post hoc Bonferroni correction.

2.8.2 | Ontogenetic analysis

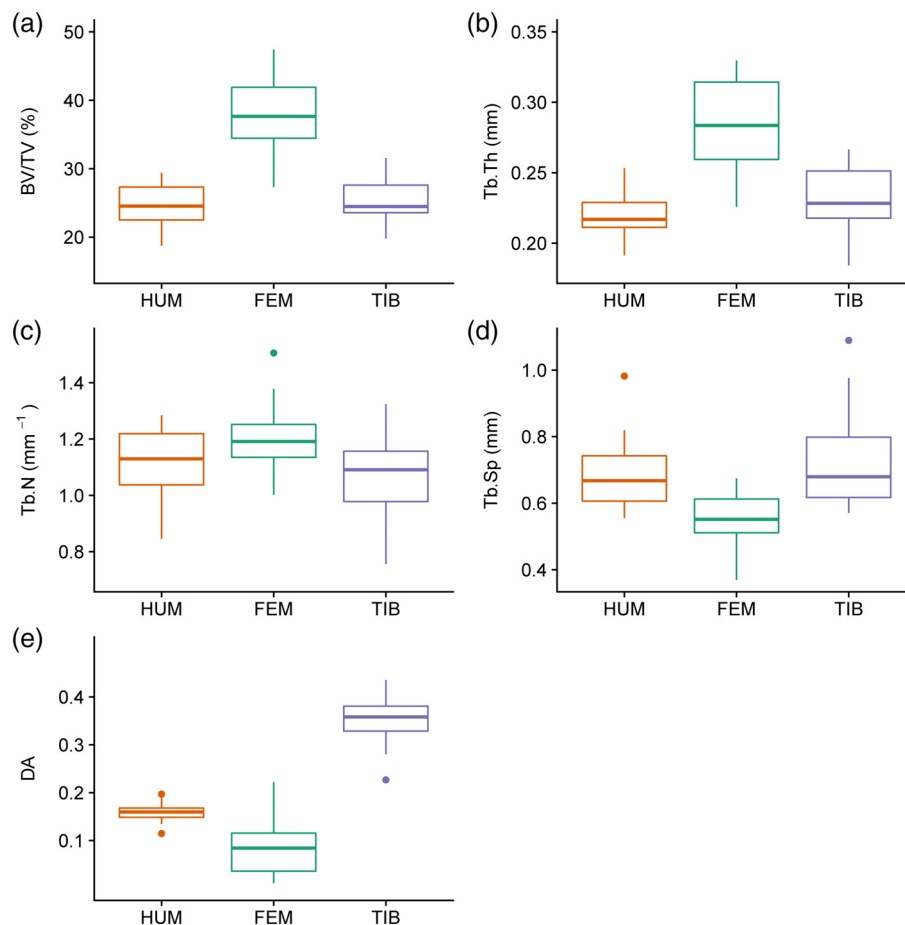
The four individuals for whom exact age was not known were included in all statistical analyses conducted by age category, but excluded from those analyses that were based on exact age. To test

for significant differences in trabecular variables between age groups, a Kruskal–Wallace test was applied, followed by post hoc pairwise Nemenyi tests. To test for changes in trabecular structure over time, OLS regressions with a post hoc Bonferroni correction of age against each trabecular variable in each element were conducted. To compare changes in trabecular structure between elements over time, the ratio of trabecular variables between elements was calculated. Kruskal–Wallace tests were applied to test for differences in these ratios between elements.

3 | RESULTS

3.1 | Adults

Mean and standard deviations of each trabecular parameter from ROIs in adult individuals are shown in Table 2, and box-and-whisker plots of trabecular variables are shown for each element in Figure 3. Mann–Whitney *U* tests find no significant differences between males and females in any trabecular parameter in the femur, humerus, and tibia, with box-and-whisker plots of trabecular variables by sex shown in Figure 4. Results of Kruskal–Wallace tests indicate significant differences between elements in all trabecular variables (Table 2) and post hoc pairwise Nemenyi tests (Table 3) reveal that this is due to differences between the proximal femur and the other two elements in

**FIGURE 3** Trabecular bone quantified from ROIs in the humerus, femur, and tibia of adult chimpanzees

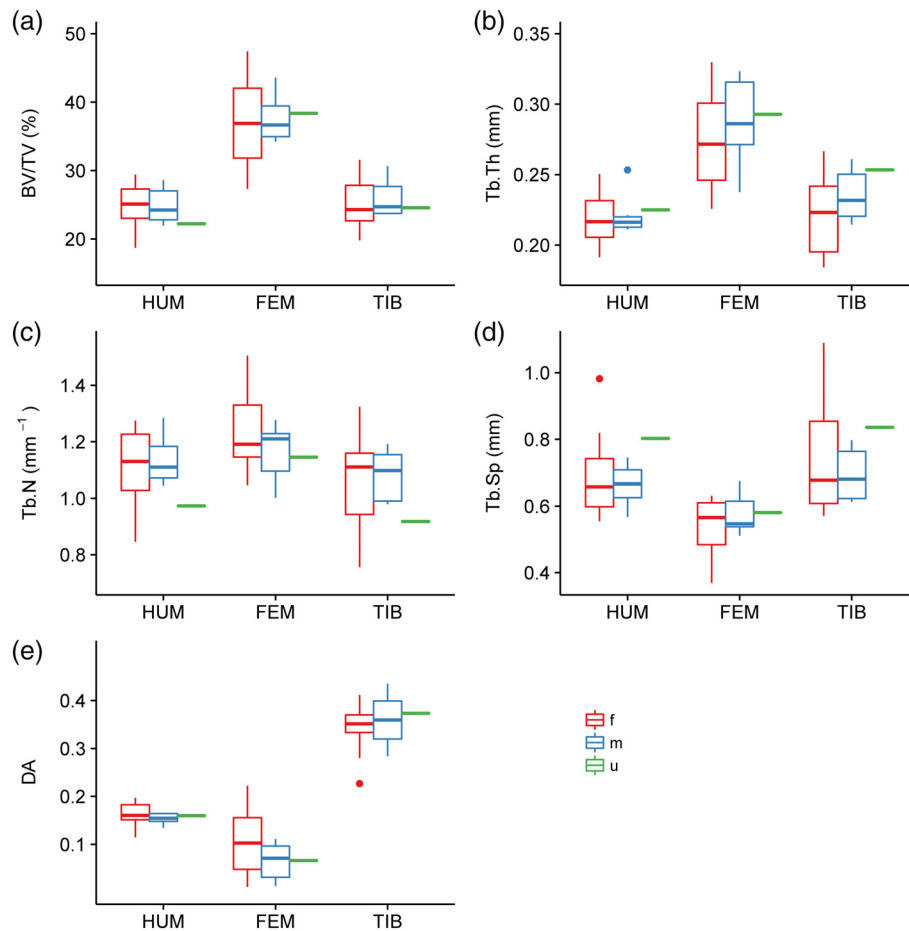


FIGURE 4 Sex specific trabecular bone structure quantified from ROIs in the humerus, femur, and tibia of adult chimpanzees. Females are shown in red, males in blue, and individuals of unknown sex in green

TABLE 3 Results of post hoc Nemenyi test to identify significant differences between elements in adult chimpanzees, with significant differences shown in bold

	BV/TV	Tb.Th	Tb.Sp	Tb.N	DA
Humerus-femur	<0.01	<0.01	<0.01	0.12	0.03
Humerus-tibia	0.94	0.45	0.75	0.61	<0.01
Femur-tibia	<0.01	<0.01	<0.01	<0.01	<0.01

the majority of trabecular variables. The only variable that differs significantly between the humerus and tibia is DA.

Results of Spearman's correlation test to identify correlations between trabecular variables in each element are shown in Table 4. There is a significant correlation between the humerus and femur in BV/TV, Tb.Th, Tb.Sp, and Tb.N, and between the humerus and tibia in Tb.N. These relationships between trabecular variables are shown in Figure 5 as the values for the femur and tibia compared to the

TABLE 4 Spearman's correlation test between elements in adult chimpanzees, with a post hoc Bonferroni correction and significant correlations in bold

	BV/TV		Tb.Th		Tb.Sp		Tb.N		DA	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Humerus-femur	0.64	0.047	0.67	0.023	0.81	<0.001	0.75	0.003	-0.43	1.000
Humerus-tibia	0.49	0.569	0.26	1.000	0.64	0.060	0.66	0.044	-0.04	1.000
Femur-tibia	0.41	1.000	0.45	0.726	0.59	0.091	0.52	0.274	0.33	1.000

humerus. Spearman's correlation test to identify the relationship between trabecular variables within each element (Table 5), shows that in both the femur and tibia, BV/TV and Tb.Th are significantly correlated, and in the femur DA is significantly correlated with both BV/TV and Tb.Th. In all elements, there is a significant correlation between Tb.Sp and Tb.N. Within the humerus there are no additional significant correlations. Spearman's correlation tests between trabecular structure and both FHH and TJV of each element find no significant relationship between either size variable and trabecular bone structure, with no R^2 value above 0.08 (Table 6). Figure 6 shows the relationship between TJV and trabecular structure.

3.2 | VOIs during ontogeny

For the immature individuals, means and standard deviations of trabecular variables measured in VOIs are shown in Table 7. Scatterplots

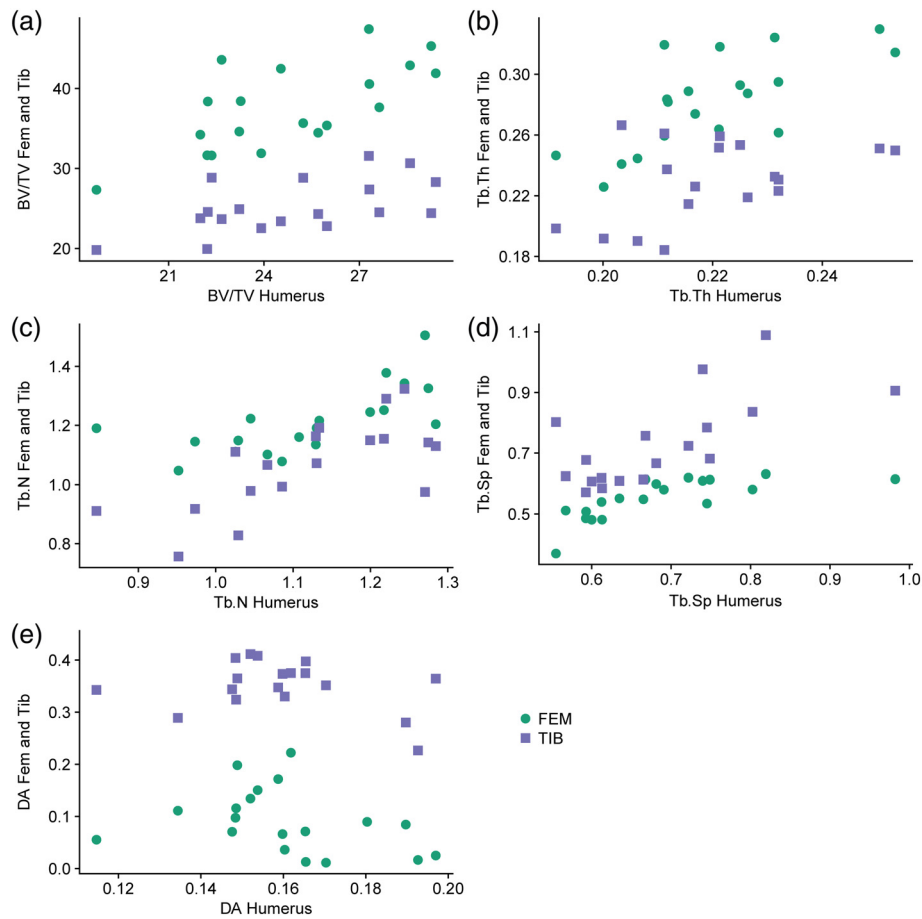


FIGURE 5 Trabecular bone structure in the humerus compared to the femur and tibia of adult chimpanzees

of trabecular variables by age are shown in Figure 7, with four individuals for whom exact age is unknown plotted in the shaded area on the right side of each plot. These plots show a general trend of increasing BV/TV and Tb.Th with age, a relatively constant Tb.N and Tb.Sp over time, and DA that is higher at a younger age in the humerus and tibia,

but in the femur remains relatively similar across age groups. There appears to be an increase in DA in all three elements in the oldest individuals. Significant differences between age categories are only present in the femur for BV/TV, Tb.Th, and Tb.N (Table 7). Post hoc Nemenyi tests (Table 8) find significant differences between the baby

TABLE 5 Spearman's correlation between trabecular variables within elements in adult chimpanzees, with a post hoc Bonferroni correction and significant correlations in bold

	BV/TV		Tb.Th		Tb.Sp		Tb.N	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Humerus								
Tb.Th	0.38	1.000						
Tb.Sp	-0.64	0.104	0.12	1.000				
Tb.N	0.54	0.547	-0.31	1.000	-0.97	<0.001		
DA	0.29	1.000	0.25	1.000	0.29	1.000	-0.29	1.000
Femur								
Tb.Th	0.75	0.002						
Tb.Sp	-0.29	1.000	0.17	1.000				
Tb.N	-0.03	1.000	-0.52	0.502	-0.91	<0.001		
DA	-0.69	0.015	-0.91	<0.001	-0.20	1.000	0.49	0.728
Tibia								
Tb.Th	0.68	0.029						
Tb.Sp	-0.52	0.598	0.02	1.000				
Tb.N	0.42	1.000	-0.18	1.000	-0.97	<0.001		
DA	0.04	1.000	-0.04	1.000	-0.31	1.000	0.34	1.000

TABLE 6 Spearman's correlation between size proxies and trabecular structure in adult chimpanzees, with a post hoc Bonferroni correction

Element	BV/TV		Tb.Th		Tb.Sp		Tb.N		DA	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Humerus										
FHH	-0.22	1.00	0.24	1.00	0.33	1.00	-0.35	1.00	-0.15	1.00
TJV	-0.30	1.00	0.24	1.00	0.35	1.00	-0.36	1.00	-0.15	1.00
Femur										
FHH	-0.20	1.00	-0.06	1.00	0.40	1.00	-0.23	1.00	0.05	1.00
TJV	-0.26	1.00	0.01	1.00	0.42	1.00	-0.27	1.00	-0.11	1.00
Tibia										
FHH	-0.41	1.00	-0.49	0.81	0.46	1.00	-0.34	1.00	-0.48	0.97
TJV	0.00	1.00	-0.12	1.00	0.21	1.00	-0.09	1.00	-0.29	1.00

Abbreviations: FHH = femoral head height; TJV = total joint volume.

and adolescent groups in both Tb.Th and Tb.N and significant differences between the infant and adolescent groups in Tb.Th. Results of OLS regressions (Table 9) show a significant relationship between age and Tb.Th and BV/TV in both the femur and tibia, but not in the humerus.

The ratio of each trabecular variable between the femur and humerus, femur and tibia, and tibia and humerus, is shown by age group in box-and-whisker plots in Figure 8. No significant differences in ratios between age groups are found in the Kruskal–Wallace tests (Table 10). However, the pattern of change over time does

demonstrate potential differences between age categories, and a different pattern between the humerus and each hind limb element.

Comparisons between age groups in the ratio of femoral to humeral trabecular variables demonstrate that both BV/TV and Tb.Th in the femur increases relative to the humerus between juvenile and adolescent age groups. Between the baby and infant age groups, there is a slight reduction in the ratio of BV/TV, but not in Tb.Th. Tb.N and Tb.Sp show an inverse pattern, having more numerous, closely spaced trabeculae in the femur than humerus in the baby group compared to older age groups. The ratio of Tb.N and Tb.Sp is highly variable among

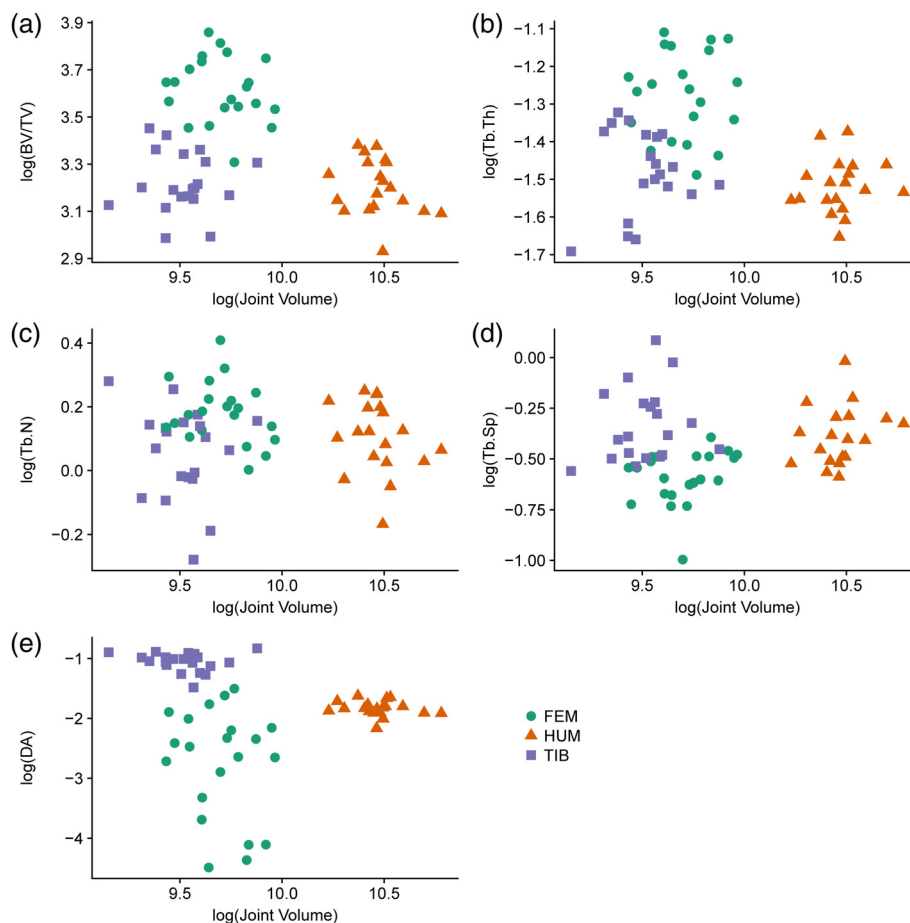
**FIGURE 6** The relationship between total joint volume and trabecular bone structure in the humerus, femur, and tibia of adult chimpanzees

TABLE 7 Trabecular variables from VOIs in immature chimpanzees

	BV/TV (%)	Tb.Th (mm)	Tb.Sp (mm)	Tb.N (mm ⁻¹)	DA
Humerus					
Baby	18.87 (3.50)	0.13 (0.02)	0.39 (0.02)	1.94 (0.15)	0.48 (0.02)
Infant	23.53 (4.52)	0.15 (0.02)	0.45 (0.08)	1.70 (0.26)	0.46 (0.06)
Juvenile	25.84 (4.62)	0.16 (0.01)	0.47 (0.07)	1.60 (0.18)	0.40 (0.06)
Adolescent	26.89 (9.74)	0.18 (0.04)	0.47 (0.08)	1.55 (0.15)	0.39 (0.05)
Significance	0.361	0.087	0.608	0.225	0.112
Femur					
Baby	20.98 (2.16)	0.14 (0.00)	0.39 (0.05)	1.89 (0.17)	0.43 (0.10)
Infant	24.73 (7.66)	0.17 (0.03)	0.53 (0.05)	1.41 (0.06)	0.35 (0.04)
Juvenile	28.22 (7.82)	0.19 (0.03)	0.53 (0.09)	1.41 (0.15)	0.33 (0.05)
Adolescent	41.43 (4.98)	0.27 (0.03)	0.51 (0.03)	1.29 (0.07)	0.37 (0.04)
Significance	0.041	0.005	0.151	0.031	0.141
Tibia					
Baby	9.33 (0.57)	0.10 (0.00)	0.53 (0.08)	1.61 (0.23)	0.55 (0.08)
Infant	12.99 (5.40)	0.14 (0.02)	0.75 (0.25)	1.18 (0.25)	0.38 (0.08)
Juvenile	15.93 (6.14)	0.14 (0.01)	0.72 (0.43)	1.30 (0.36)	0.41 (0.07)
Adolescent	20.53 (5.82)	0.16 (0.02)	0.56 (0.06)	1.38 (0.09)	0.49 (0.03)
Significance	0.177	0.070	0.227	0.109	0.078

Note. Age group mean and standard deviation, in parentheses, for each trabecular variable in the proximal humerus, proximal femur, and distal tibia, with results of Kruskal–Wallace tests for differences between age groups.

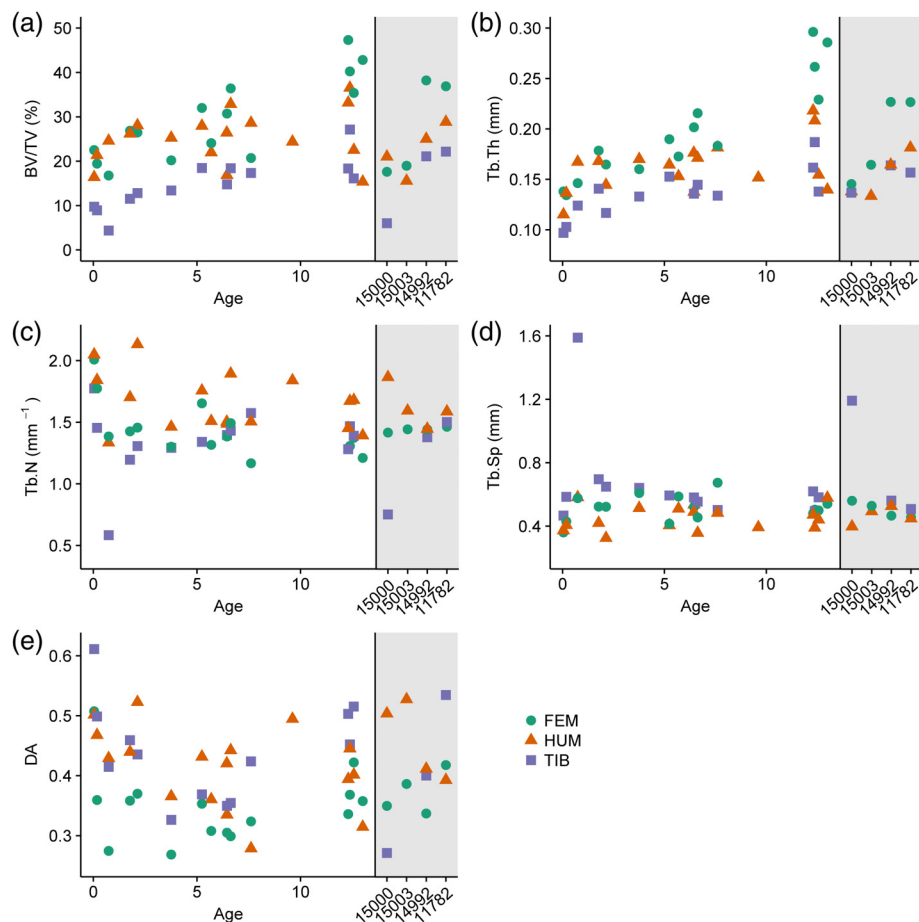
**FIGURE 7** Trabecular bone ontogeny in the proximal humerus, proximal femur, and distal tibia in immature chimpanzees. Individuals of unknown age are shown in the shaded area to the right of each plot

TABLE 8 Results of post hoc Nemenyi test between age groups in immature chimpanzees with significant differences in bold

Element	Variable	Baby	Infant	Juvenile
Femur	BV/TV			
	Infant	0.972		
	Juvenile	0.804	0.911	
	Adolescent	0.096	0.053	0.175
Tb.Th	Infant	0.559		
	Juvenile	0.208	0.810	
	Adolescent	0.006	0.030	0.171
Tb.N	Infant	0.321		
	Juvenile	0.264	0.999	
	Adolescent	0.018	0.290	0.318

the other three age groups. The ratio of femoral to humeral DA follows a similar pattern to that of BV/TV, with a decrease between baby and infant groups, and then increasing with age.

The ontogenetic pattern of tibial to humeral trabecular ratios differs from the femoral to humeral pattern. There is an increase in the BV/TV ratio between the infant and juvenile age groups, but this ratio is similar between baby and infant, and between juvenile and adolescent groups. For Tb.Th ratios, although the median shows an increase with age, there is a great deal of variability and overlap between all age groups. There is overlap between all age groups in the ratios for Tb.Sp and there is no clear pattern between age groups. The ratio of DA appears to reduce between baby and infant stages, and to subsequently increase with increasing age.

Comparisons between the two hindlimb elements, the tibia and femur, demonstrate that the ratio of BV/TV, Tb.N, Tb.Sp, and DA

TABLE 9 Results of OLS regression of age against trabecular variables with Bonferroni post hoc correction in immature chimpanzees with significant *p* values in bold

Variable	Intercept	Slope	R ²	<i>p</i>
Humerus				
BV/TV	23.24	0.31	-0.01	1.000
Tb.Th	0.15	0.00	0.17	0.884
Tb.Sp	0.43	0.00	-0.04	1.000
Tb.N	1.77	-0.02	0.06	1.000
DA	0.45	-0.01	0.11	1.000
Femur				
BV/TV	19.50	1.65	0.67	0.002
Tb.Th	0.14	0.01	0.85	<0.001
Tb.Sp	0.50	0.00	-0.05	1.000
Tb.N	1.61	-0.03	0.34	0.203
DA	0.35	0.00	-0.08	1.000
Tibia				
BV/TV	9.26	0.99	0.63	0.011
Tb.Th	0.11	0.00	0.58	0.023
Tb.Sp	0.78	-0.02	0.04	1.000
Tb.N	1.28	0.01	-0.05	1.000
DA	0.44	0.00	-0.09	1.000

remains fairly constant over time in these elements. There is a slight trend toward a lower ratio of Tb.N and a higher ratio of Tb.Sp with increasing age. However, there is a greater increase in Tb.Th in the femur compared to the tibia between the infant and juvenile, and between the juvenile and adolescent age groups.

3.3 | Morphometric maps during ontogeny

Figures 9–11 show cross-sections of segmented micro-CT data and color maps of the distribution of BV/TV in a subset of the study sample (see Supporting Information for images of the other individuals in the sample). In the proximal humerus, distal tibia, and proximal femur, the youngest individual (MPI_11787) has a relatively homogenous trabecular bone structure, with BV/TV values within a narrow range. When scaled to the range of BV/TV values in this individual, it is apparent that despite the fairly homogenous distribution, the medial region of the proximal humerus and femur, and the posterior region of the distal tibia, have the highest BV/TV. With increasing age, BV/TV increases and more regional differences become apparent, notably regions of high BV/TV on both the medial and lateral edges of the proximal humerus, femoral neck, and anterior and posterior edges of the distal tibia.

4 | DISCUSSION

Trabecular bone structure is thought to reflect behavioral differences and joint loading (Barak et al., 2011; Ehrlich & Lanyon, 2002; Lanyon, 1974; Pontzer et al., 2006), however, the precise relationship between trabecular structure and behavior in primates requires further consideration (Kivell, 2016). To accurately reconstruct behavior in fossil species, it is essential to better understand all factors that can influence trabecular structure in extant taxa. To address this, this study explores how trabecular structure changes throughout ontogeny, and intraspecific variability, within a single subspecies of chimpanzee from the Tai National Park, Republic of Côte d'Ivoire, using a multielement approach. Amongst adult chimpanzees, there is no clear link between trabecular bone structure and sex or size, emphasizing the importance of further investigation of factors that contribute to adult bone structure. These results establish that adult-like trabecular structure and distribution in chimpanzees is not present from birth but, rather, is acquired over time during ontogeny. Changes in chimpanzee trabecular structure during growth reveal both similarities and differences compared to published studies of humans.

4.1 | Variability of trabecular structure in adult chimpanzees

Numerous factors have the potential to influence intraspecific variation in trabecular structure, and consequently the degree to which trabecular architecture reflects behavioral differences. These include differences between sexes that may be due to sexual dimorphism in bone lengths (Behringer et al., 2016; Zihlman, Stahl, & Boesch, 2008) and body mass (Leigh & Shea, 1995, 1996; Smith & Jungers, 1997), or

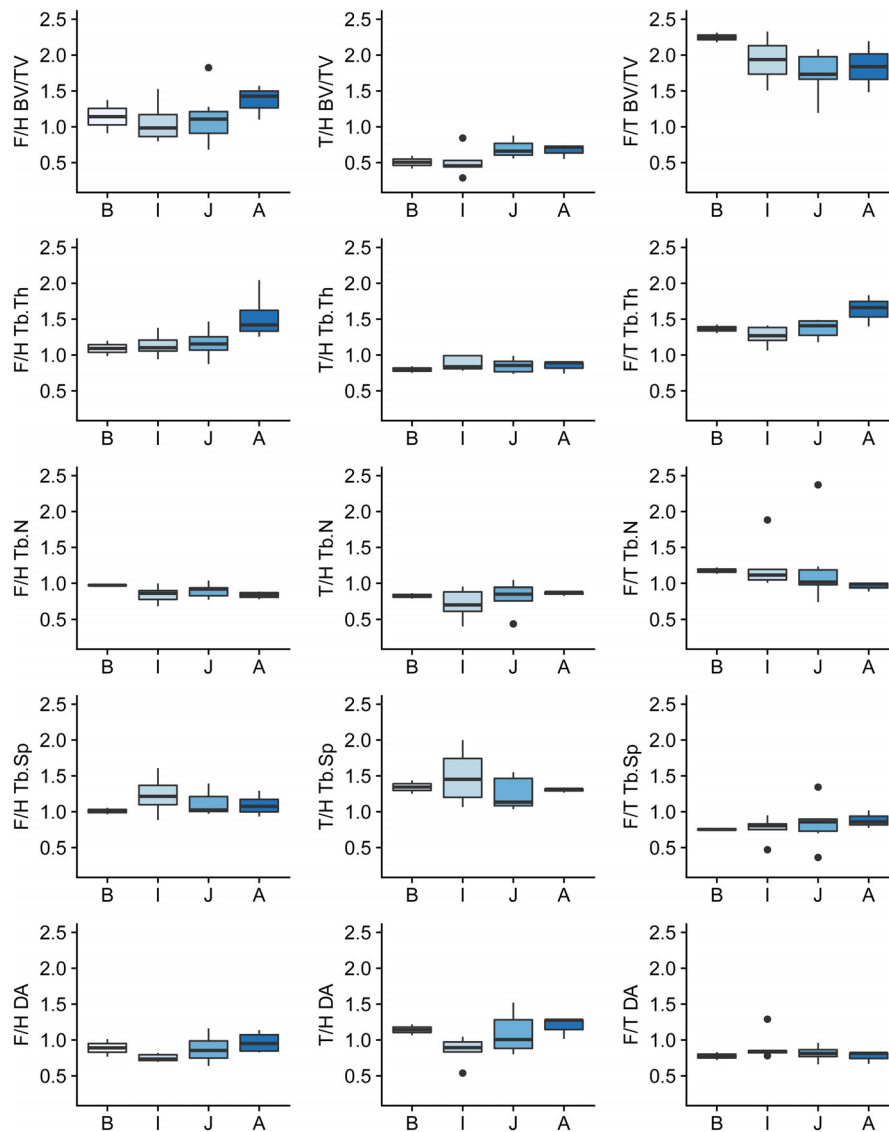


FIGURE 8 Ratios of femoral to humeral (left), tibial to humeral (center), and femoral to tibial (right) trabecular structure in each age category. B: baby; I: infant; J: juvenile; A: adolescent

TABLE 10 Results of Kruskal–Wallace tests to compare ratios of trabecular variables between age groups in immature chimpanzees

Ratio	Variable	<i>p</i>
Femur/humerus	BV/TV	0.245
	Tb.Th	0.085
	Tb.Sp	0.501
	Tb.N	0.210
	DA	0.139
Femur/tibia	BV/TV	0.745
	Tb.Th	0.156
	Tb.Sp	0.555
	Tb.N	0.183
	DA	0.459
Tibia/humerus	BV/TV	0.433
	Tb.Th	0.817
	Tb.Sp	0.865
	Tb.N	0.830
	DA	0.210

due to hormonal differences (Behringer, Deschner, Deimel, Stevens, & Hohmann, 2014a; Behringer, Deschner, Murtagh, Stevens, & Hohmann, 2014b). Trabecular structure correlates with body size both across primates (Barak, Lieberman, & Hublin, 2013a; Cotter, Simpson, Latimer, & Hernandez, 2009; Fajardo et al., 2013; Ryan & Shaw, 2013), and a broad sample of both mammalian and nonmammalian taxa (Doube et al., 2011). Trabecular structure may differ across anatomical sites for multiple reasons including, for example, as an adaptation to reduce mass distally and maximize locomotor efficiency (Chirchir, 2015; Saers, Cazorla-Bak, Shaw, Stock, & Ryan, 2016), due to differences in susceptibility to remodeling (Wallace et al., 2015b), or individual trabecular parameters varying in their contribution to Young's modulus (i.e., bone stiffness; Ulrich, van Rietbergen, Laib, & Rueggsegger, 1999). In addition, genetic differences (Patemoster et al., 2013; Wallace, Demes, & Judex, 2017), diet and the intestinal microbiome (Cao, Gregoire, & Gao, 2009; Cashman, 2007; Charles, Ermann, & Aliprantis, 2015; McCabe, Britton, & Parameswaran, 2015; Prentice, 1997; Shea et al., 2002), and activity levels (Best et al., 2017; Chirchir et al., 2015;

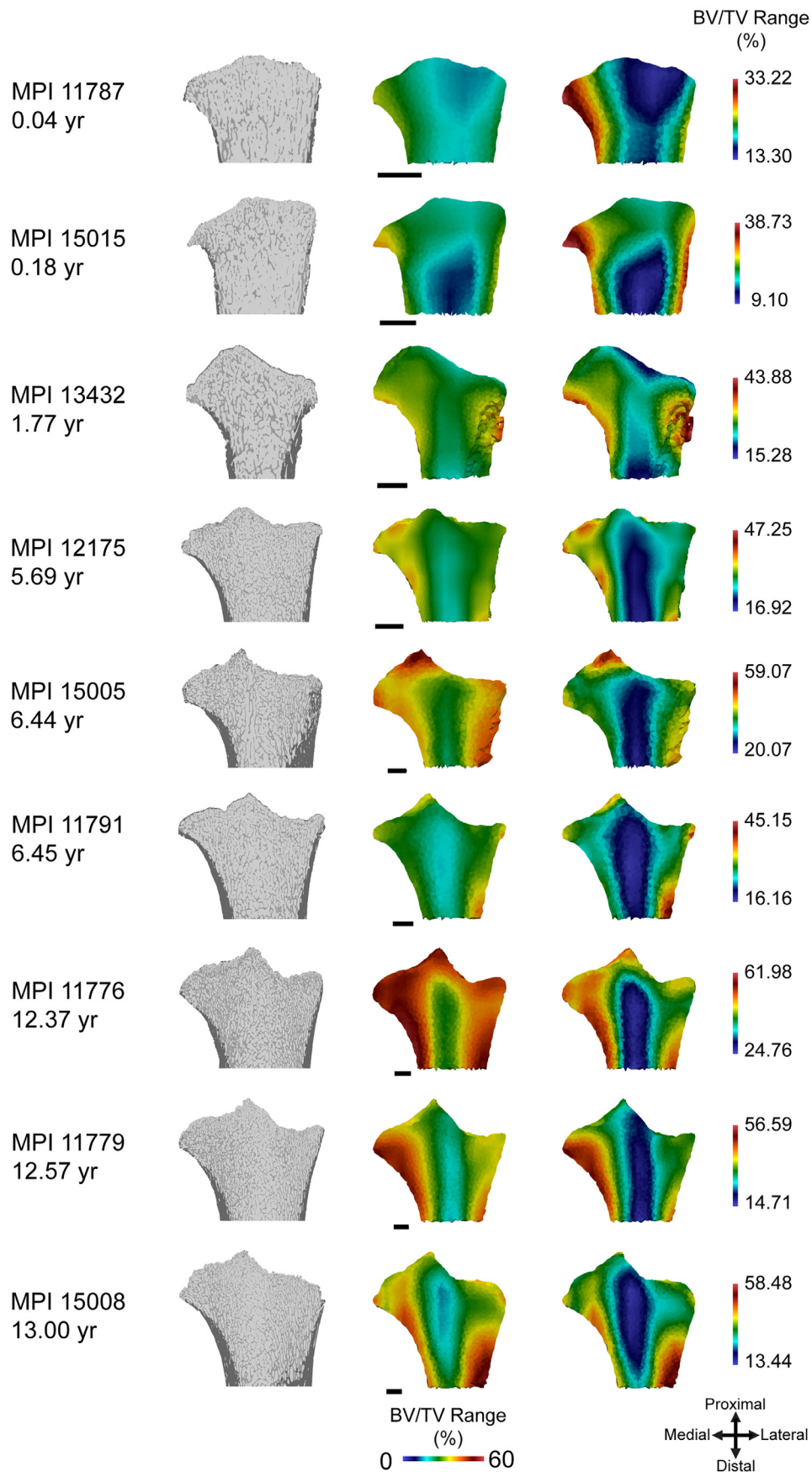


FIGURE 9 Ontogenetic changes in BV/TV of the chimpanzee proximal humerus. Shown in the midcoronal plane, segmented trabecular and cortical bone (left), trabecular structure scaled from 0% to 60% BV/TV (center) and scaled to the range of each specimen (right), with a 5 mm scale bar. The age of each individual is shown in years and specimens from the right side are flipped for comparison

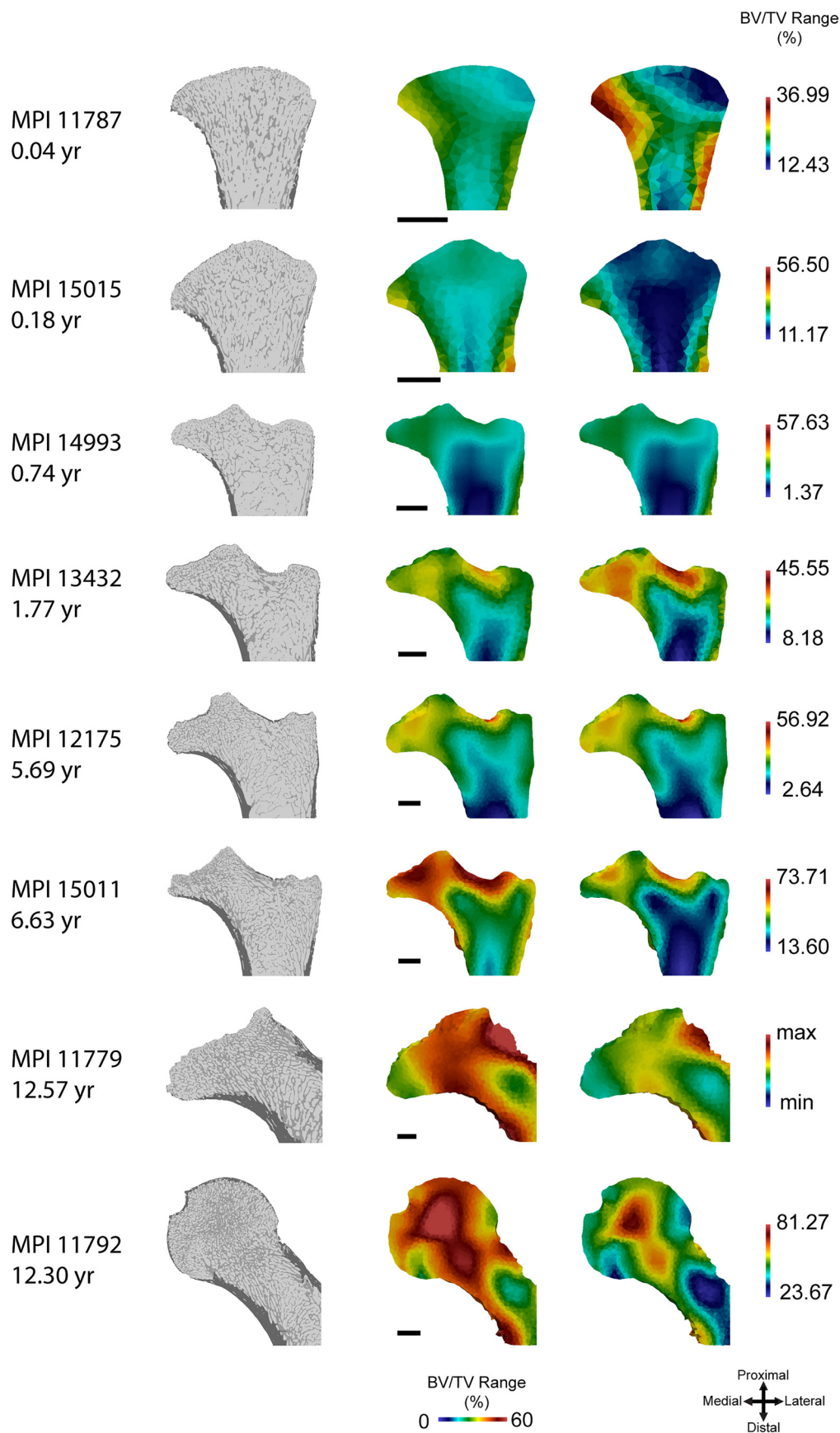


FIGURE 10 Ontogenetic changes in BV/TV of the chimpanzee proximal femur. Shown in the midcoronal plane, segmented trabecular and cortical bone (left), trabecular structure scaled from 0% to 60% BV/TV (center) and scaled to the range of each specimen (right), with a 5 mm scale bar. The age of each individual is shown in years and specimens from the right side are flipped for comparison

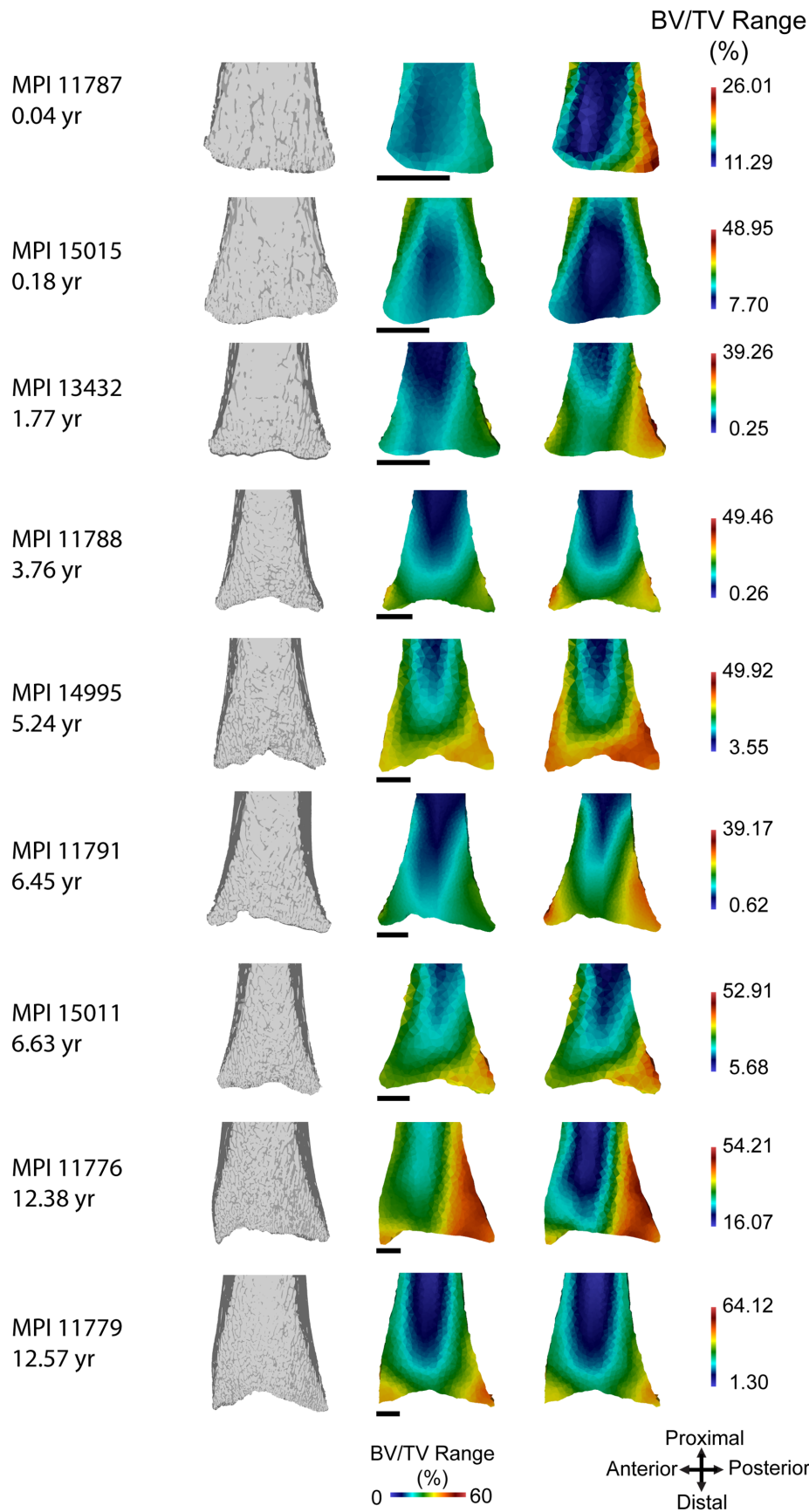


FIGURE 11 Ontogenetic changes in BV/TV of the chimpanzee distal tibia. Shown in the mid-sagittal plane, segmented trabecular and cortical bone (left), trabecular structure scaled from 0% to 60% BV/TV (center) and scaled to the range of each specimen (right), with a 5 mm scale bar. The age of each individual is shown in years and specimens from the right side are flipped for comparison

Chirchir, Ruff, Junno, & Potts, 2017; Ryan & Shaw, 2015; Saers, 2017; Saers et al., 2016) influence bone morphology.

Contrary to our prediction, we find no relationship between size proxies FHH or TJV and trabecular structure in either the proximal humerus, proximal femur, or distal tibia of chimpanzee adults. This indicates that within a species with limited size variation, body size may not be critical in determining trabecular structure. Trabecular structure does not differ significantly between sexes in the study sample in any element. Male individuals are underrepresented in the sample, with only eight males included, perhaps due to a female bias in the sex ratio of the Taï population (Boesch & Boesch-Achermann, 2000). Sex specific median values are similar with overlapping ranges of variation within all three elements and, as such, the smaller sample of male individuals is unlikely to explain the statistical results. Trabecular structure of other anatomical regions does not differ significantly between male and female adult chimpanzees in the vertebra (Cotter et al., 2009) or metacarpals (Lazenby, Skinner, Hublin, & Boesch, 2011a). Sexual dimorphism in body size is relatively low in the Taï chimpanzees, with males only having significantly longer clavicles and broader scapulae across the postcranial skeleton (Zihlman et al., 2008). Correlations between elements and trabecular variables within elements were identified, indicating the complex interrelationships between trabecular variables across the skeleton. We found DA of the proximal femur to be correlated with BV/TV, which differs from the findings of Ryan et al. (2018). This is likely due to the different methodological approach, as DA results differ between analyses which focus on the entire joint and those which quantify trabecular structure in a smaller subregion (Tsegai et al., 2018).

The absence of a relationship between trabecular structure and body size or sex in this population of adult chimpanzees suggests alternative factors, such as ontogeny, locomotor behavior, activity level and/or genetic differences, may influence adult trabecular structure. With regards to activity level, at a broad scale, across a range of mammalian taxa, longer daily travel distances are associated with higher trabecular mass (Chirchir, Ruff, Helgen, & Potts, 2016). Within living humans, comparison between runners and nonrunners reveals that only in nonrunners is body mass correlated with trabecular structure of the calcaneus, specifically trabecular density, Tb.Th and BV/TV (Best et al., 2017). In contrast, in runners, weekly running distance, number of years running and age of onset of running is correlated with Tb.Th, together explaining 68.2% of variation (Best et al., 2017). This demonstrates that behavioral differences, such as weekly running distance, have the potential to mask allometric requirements of bone structure at the intraspecific level. Across an archaeological sample, trabecular robusticity of the calcaneus, talus and first metatarsal is related to subsistence strategies based on predictions of overall activity level (Saers, 2017). The degree of sexual dimorphism in trabecular structure of the foot differs between populations, but does not clearly relate to predictions based on cross-sectional geometry of the long bones of male and female individuals, indicating a complex relationship between sex, activity level/type, and bone morphology (Saers, 2017). Across human populations, subsistence strategies requiring higher activity levels are associated with a more robust trabecular (Chirchir et al., 2015; 2017; Ryan & Shaw, 2015; Saers, 2017; Saers et al., 2016; Scherf, Wahl, Hublin, & Harvati, 2016) and cortical

(Macintosh et al., 2014, 2017; Shaw & Stock, 2013; Stock & Macintosh, 2016) structure. Although this degree of intraspecific variability is likely unique to modern humans, due to the greater degree of behavioral and technological variability (both geographical and temporal), activity levels could be an important factor determining intraspecific variability in chimpanzees.

The contribution of travel distance and locomotor repertoire to both cortical and trabecular bone structure between populations of chimpanzee is as yet uncertain. Daily travel distance varies between chimpanzee populations from 2.1 to 4.8 km at different study sites (Gruber, Zuberbühler, & Neumann, 2016), and locomotor repertoires differ between groups, in part due to variation in local ecology (Carlson et al., 2006, 2008; Hunt, 1991). Cortical bone cross-sectional geometry differs between populations and subspecies of chimpanzee, specifically at the study sites of Mahale, Gombe, Kibale, and Taï, and ecological/locomotor variables such as the degree of arboreal behavior, the amount of forest cover, elevation and incline of the terrain, as well as genetic differences, may explain bone structural differences across subspecies (Carlson et al., 2006, 2008, 2011). Comparison of trabecular structure between populations of chimpanzee has only been conducted in the first, second, and fifth metacarpals (Lazenby et al., 2011a). Taï chimpanzees have a less robust trabecular structure in the hand compared to *Pan troglodytes troglodytes* from Cameroon, which cannot be explained by either differences in body mass or daily travel distance between the subspecies. Potentially, the contribution of terrestrial knuckle-walking to the locomotor repertoire of each subspecies, or more frequent manipulation during tool use in the Taï population, contributes to this structural difference (Lazenby et al., 2011a). With regard to the Taï population, daily travel distance differs between the three study groups living in the Taï National Park, and varies seasonally within each study group (Herbinger, Boesch, & Rothe, 2001). Within two groups for whom sufficient data is available, there are no sex differences in daily travel distance (Herbinger et al., 2001), despite the distances travelled by male chimpanzees during territorial patrols (Amsler, 2010; Boesch & Boesch-Achermann, 2000; Mitani, 2009; Watts & Mitani, 2001). As the study sample was collected over a long period, a potential factor contributing to bone structure is temporal changes in home range size, which have occurred, for example due to changes in group composition, for example the number of adult males in the group, but not with fruit availability (Lehmann & Boesch, 2003). Thus, activity levels may differ across individuals in the sample.

4.2 | Ontogenetic changes in trabecular structure

Understanding how chimpanzee trabecular structure changes throughout ontogeny and how this compares with humans may provide further insight into the functional inferences that can be drawn from adult trabecular bone. We find that chimpanzee trabecular structure in the baby category is characterized by thin, numerous, closely spaced trabecular struts, with a low BV/TV compared to older age groups. In this youngest age group, trabecular structure is more uniformly oriented in the proximal humerus and distal tibia, than in the proximal femur. With increasing age, both BV/TV and Tb.Th increase in all elements, however, this is only significant for the femur and tibia.

Trabeculae become less numerous and more widely spaced with age, although this is relatively subtle, the only significant difference being higher Tb.N in the femur of the adolescent group compared to the baby group. After birth, trabeculae in the humerus and tibia become less uniformly organized, subsequently increasing in uniformity after 5 years of age. In the femur, the uniformity of orientation shows no clear pattern of change during ontogeny. These changes to trabecular structure during ontogeny may reflect locomotor transitions. Increasing BV/TV and Tb.Th of the hindlimb elements during ontogeny may reflect increased loading of these elements as the frequency of knuckle-walking increases in the locomotor repertoire. Moreover, the ratio of femoral to humeral BV/TV increases with age, suggesting increased loading of the femur in comparison with that of the humerus during ontogeny. Although comparisons of trabecular structure between elements is complex, due to differences in external joint morphology and VOI placement protocols, the higher DA of the distal tibia may reflect the more restricted movement of this joint, compared to the ball-and-socket joints included in this study (proximal humerus and femur).

In humans, there is a shared pattern of trabecular ontogeny across the proximal femur, proximal tibia and proximal humerus, with structural changes coinciding with the adoption of bipedal locomotion after 1 year of age (Gosman & Ketcham, 2009; Milovanovic et al., 2017; Ryan & Krovitz, 2006; Ryan et al., 2017). An increase in BV/TV, Tb.N, and DA occurs at around 1–2 years in the human proximal femur and proximal tibia, at the time of acquisition of bipedal locomotion (Gosman & Ketcham, 2009; Ryan & Krovitz, 2006), and BV/TV, Tb.Th and DA continue to increase into early adulthood in the proximal tibia (Gosman & Ketcham, 2009). The sample used for the proximal femur does not extend beyond 9 years (Ryan & Krovitz, 2006). The humerus follows a similar pattern of changing trabecular structure to these hindlimb elements, but differs from the femur in having a slower rate of change, reflecting increased loading of the femur with the acquisition of bipedal locomotion (Ryan et al., 2017). At a later stage of ontogeny, reduced regional variation in DA, that is, more homogeneous structure, across the distal tibia reflects increasing stability in bipedalism (Raichlen et al., 2015). These findings in humans emphasize the complex interplay between regulation of growth and biomechanical influences on the skeleton. The pattern of trabecular ontogeny identified here in chimpanzee long bones has both similarities and, contrary to our prediction, differences to the findings of previous analyses of humans. This emphasizes the potential for both different regulatory mechanisms underlying trabecular ontogeny in these taxa, and the influence of locomotion, that is, mechanical loading.

The most often discussed trabecular structural measurement is BV/TV, which explains 88% of variation in trabecular stiffness (Stauber, Rapillard, van Lenthe, Zysset, & Müller, 2006), and as such is of great biomechanical importance. Trabecular ontogeny in humans is characterized by an initially high BV/TV at birth, which drops until around 1–2 years of age, followed by an increase in BV/TV in both the proximal femur, proximal tibia, and proximal humerus. This initial reduction in BV/TV is due to a rapid reduction in Tb.N, alongside a gradually increasing Tb.Th (Gosman & Ketcham, 2009; Ryan & Krovitz, 2006; Ryan et al., 2017). This trabecular structure characteristic of

neonatal humans appears to be acquired during gestational bone development through increasing BV/TV and Tb.Th in the human vertebral column (Acquaah et al., 2015) and proximal femur (Glorieux, Salle, Travers, & Audra, 1991; Salle, Rauch, Travers, Bouvier, & Glorieux, 2002). However, Reissis and Abel (2012) found an increase in Tb.Th, but not BV/TV, during gestation in the proximal humerus and proximal femur. Unlike the pattern identified in humans, we do not find an initially high BV/TV in any of the three elements in chimpanzees at the youngest ages included in this sample, rather, BV/TV increases in all three elements during ontogeny, with a significant increase in BV/TV with age in the femur and tibia. This gradual increase in BV/TV is associated with increasing Tb.Th, also significant in the femur and tibia, similar to humans (Gosman & Ketcham, 2009; Ryan & Krovitz, 2006; Ryan et al., 2017), but with no rapid reduction in Tb.N. Tb.Sp remains relatively constant across ontogeny in all elements.

This contrast in ontogenetic changes between humans and chimpanzees is unlikely to be explained entirely by our small sample size below 1 year of age. Three individuals in the study sample are known to be younger than 1 year of age, and the suspected cause of death in these individuals is starvation (MPI_11787: 0.04 years) and respiratory disease (MPI_14993: 0.74 years; MPI_15015: 0.18 years). There are two individuals in the study sample whose age is not known, but appear to be around 1 year of age (MPI_15000 and MPI_15003), based on dental eruption and femoral length (see Supporting Information). Neither of these individuals has high BV/TV, and their values are similar to those known to be younger than 1 year of age. Younger chimpanzees in this skeletal collection have delayed dental eruption when compared to captive chimpanzees, and have a higher incidence of dental anomalies, indicating the potential influence of external factors, such as illness or nutritional stress, on their development (Smith and Boesch, 2011). As human specimens included in previous publications may also have died of disease and/or starvation, it seems unlikely, although remains a possibility, that health related factors contributed to this absence of high BV/TV in our sample prior to 1 year of age. The VOI protocol adopted in this study is similar to that of previous studies in humans (Gosman & Ketcham, 2009; Ryan & Krovitz, 2006; Ryan et al., 2017), and studies using a very different VOI protocol have found similar high BV/TV at birth in the human proximal femur (Milovanovic et al., 2017). In general, in chimpanzees there is an increase in trabecular bone robusticity (i.e., higher BV/TV and Tb.Th) with age in all three elements. This pattern is likely related to increasing body size with age, but perhaps also to increasing activity levels, such as greater locomotor independence from the mother (Doran 1992) and longer day ranges as individuals get older (Pontzer & Wrangham, 2006).

In non-primate, domesticated taxa, trabecular BV/TV follows a similar pattern to humans, being high early in ontogeny (dog ulna: Wolschrijn & Weijs, 2004), however, not all studies could determine the presence or absence of this pattern, as they did not include individuals from birth (e.g., pig mandible: Mulder, Koolstra, Weijs, & van Eijden, 2005; sheep tibia: Nafei et al., 2000b; pig vertebra and tibia: Tanck et al., 2001). In contrast, there is no postnatal reduction in BV/TV in the distal tibia and talus of Dutch warm-blooded horses, rather, BV/TV and Tb.Th increase after birth (Gorissen et al., 2018).

Acquaah et al. (2015) have suggested several potential explanations for the presence of a high BV/TV at birth in the human skeleton. First, prenatally bone structure is likely to be, primarily, under genetic regulation, whereas postnatally biomechanical loading may be more influential for bone structure. Second, development of a dense trabecular structure where bone is subsequently removed and its structure is refined, may enable greater flexibility for adaptation to mechanical load (see Tanck et al., 2001). Finally, a high BV/TV at birth may function as a calcium reserve, as human breast milk may contain lower levels of calcium than required, and this bone loss may be regulated by elevated parathyroid hormone levels at this stage of development in humans. The pattern of trabecular ontogeny in horses, which differs from that of humans and dogs, has been explained by Gorissen, Wolschrijn, van Vilsteren, van Rietbergen, and van Weeren (2016) and Gorissen, Wolschrijn, van Rietbergen, Rieppo, Saarakkala, and van Weeren (2018) as an adaptation for a precocial species whereby, in anticipation of the onset of locomotion only hours after birth, genetic factors prepare the skeleton for the mechanical loading it will experience. Potentially, all taxa could undergo a similar stage of reduction in BV/TV, but at different developmental stages, that is, at birth in humans but in utero in horses and chimpanzees. Future studies of trabecular bone structure in other mammalian taxa are required to fully understand this variability. Further, analysis of a larger sample of non-human apes at the youngest developmental stages are required to determine whether a high BV/TV and Tb.N at birth is a feature absent from their trabecular ontogeny, as the results of the present study suggest.

The uniformity of trabecular bone orientation (i.e., DA) and primary trabecular orientation is often found to reflect locomotor differences across primate taxa (e.g., Barak et al., 2013b; Barak, Sherratt, & Lieberman, 2017; Griffin et al., 2010; MacLachy & Müller, 2002; Ryan & Ketcham, 2002b; Su et al., 2013; Tsegai et al., 2017) and to reflect both experimental or natural changes in loading during ontogeny (Barak et al., 2011; Pontzer et al., 2006; Raichlen et al., 2015). DA is thought to reflect whether a joint is more stereotypically loaded in a particular direction (i.e., a high DA) or loaded from a wide range of joint positions (i.e., a low DA). In the chimpanzee humerus and tibia, DA reduces from birth until around 5 years, when it begins to increase. In contrast, DA of the femur remains relatively constant across ontogeny. The pattern in the humerus and tibia is consistent with that of the human proximal femur (Ryan & Krovitz, 2006), although the age at which DA begins to increase is earlier in humans. In humans, locomotor transitions occur at a young age, with bipedalism acquired at 1–2 years and a stable gait at 3.5–4 years (Raichlen et al., 2015; Sutherland, 1997, 1980). In contrast, in chimpanzees, locomotor changes are more gradual and occur throughout ontogeny (Doran, 1992, 1997; Sarringhaus et al., 2014, 2016), with a transition to increased terrestrial knuckle-walking at 5 years (Sarringhaus et al., 2014, 2016). Thus, in both humans and chimpanzees, changes in DA occur at a similar time to locomotor transitions, indicating that this parameter may be indicative of locomotor transitions during ontogeny. The only exception to this is the chimpanzee femur in which DA values suggest a consistency in the variability of load orientation throughout ontogeny.

Through analysis of ontogenetic changes in trabecular bone structure it is possible to characterize the process by which adult-like bone structure is obtained. A characteristic feature of the trabecular bone structure of adult primates is higher BV/TV in the femur compared to humerus (Chirchir et al., 2015; Ryan & Walker, 2010; Tsegai et al., 2018). Comparison of trabecular structure across the skeleton of chimpanzees and humans demonstrates that this similar pattern is driven by high BV/TV in the chimpanzee femur and low BV/TV in the human humerus compared to other anatomical sites (Tsegai et al., 2018). The pattern of ontogeny identified here in the chimpanzee skeleton demonstrates that adult-like trabecular ratios between the femur and humerus are not present at birth, but rather are acquired during life, with femoral and humeral BV/TV similar at birth but very different by adolescence. The femur of adult chimpanzees may have a higher BV/TV than the humerus for several reasons. Quadrupedal knuckle-walking is the largest component of the locomotor repertoire of chimpanzees (Doran, 1992, 1997; Sarringhaus et al., 2014, 2016), and although both the forelimb and hindlimb are loaded, the hindlimb experiences higher vertical ground reaction forces than the forelimb at various speeds, including both walking and galloping (Demes et al., 1994; Kimura, Okada, & Ishida, 1979). Furthermore, during vertical climbing, which has been considered to load both the forelimb and hindlimb equally (Sarringhaus et al., 2014, 2016), the hindlimb produces greater propulsive forces than the forelimb in most primates (Hanna et al., 2017; Hirasaki, Kumakura, & Matano, 1993). The relationship between vertical ground reaction forces and trabecular bone structure has not been tested. However, the domestic dog has higher vertical reaction forces on the forelimb (Kimura et al., 1979; Lee, Bertram, & Todhunter, 1999; Lee, Stakebake, Walter, & Carrier, 2004), while trabecular ash (correlated with trabecular density) (Gong, Arnold, & Cohn, 1964; Kang, An, & Friedman, 1998) and Young's modulus (Kang et al., 1998) are higher in the femoral compared to the humeral head. Thus, dogs may have a similar pattern of femoral compared to humeral trabecular structure to primates, despite the difference in vertical reaction forces. Apart from biomechanical loading of the femur, potential differences in response to loading between the forelimb and hindlimb, as demonstrated experimentally in mice (Wallace et al., 2015b), is an additional factor that could contribute to this pattern.

4.3 | Ontogenetic changes in bone distribution

Ontogenetic studies are an ideal way to identify plastic features of bony morphology to recognize phylogenetic and functional signals in the fossil record (Congdon, 2012; Kivell & Schmitt, 2009; Richmond, 1998; Tardieu, 1999; Tardieu & Trinkaus, 1994; Ward, 2002). In adult individuals, the distribution of trabecular BV/TV is consistent with habitually-loaded joint positions in the adult hand (Skinner et al., 2015; Stephens et al., 2016; Tsegai et al., 2013), and in the talus and distal tibia (Tsegai et al., 2017). During ontogeny, regional variability of several trabecular parameters increases in the human proximal femur, reflecting the development of complex regional trabecular patterns characteristic of the adult proximal femur (Ryan & Krovitz, 2006). In contrast, DA becomes increasingly homogeneous with age throughout the human distal tibia as a more stable bipedal gait is

acquired (Raichlen et al., 2015). This demonstrates that the way in which trabecular structure changes during ontogeny differs between anatomical sites, reflecting the loading environment of each joint during ontogeny. Thus, it is important to explore these changes in different skeletal sites. For example, in contrast to the limb bones discussed here, an adult-like trabecular pattern is present from birth in the human ilium (Abel & Macho, 2011; Cunningham & Black, 2009a, 2009b).

The distribution patterns of BV/TV during ontogeny in the chimpanzee proximal humerus, distal tibia, and proximal femur demonstrate that the subepiphyseal distribution of BV/TV differs at different ontogenetic stages. In all elements, the trabecular structure is initially homogenous, with increasing regional differences in BV/TV with age, reaching more adult-like distributions in juvenile or adolescent stages. The changes in BV/TV distribution that occur during ontogeny may be due to biomechanical changes caused either by locomotion and/or by the general process of growth, and the changing morphology of the external joint. During growth, trabecular bone develops beneath the epiphyseal plate via the process of endochondral ossification (Carter & Beaupré, 2000; Martin et al., 2015; Scheuer & Black, 2000), where columnar rows of chondrocytes form cartilage that is subsequently ossified to form primary trabeculae. These are then remodeled to form secondary trabeculae. This process of endochondral ossification, by which trabecular bone is formed, could explain the initially homogeneous trabecular bone distribution in all three elements (Gosman & Ketcham, 2009; Ryan & Krovitz, 2006; Ryan et al., 2017). However, BV/TV is not entirely homogeneous and all elements have regions of higher BV/TV, even before the onset of locomotion. These early appearing regions of differentiated trabecular structure could be attributed to joint morphology and nonlocomotor loading both pre- and postnatally, or to genetically predetermined patterns of deposition (Abel & Macho, 2011; Cunningham & Black, 2009a, 2009b).

During subsequent growth, trabecular bone is deposited at the metaphyseal surface, likely partly in response to the local mechanical environment. As experimental studies are often performed on immature individuals (e.g., Barak et al., 2011), it is unclear whether trabecular bone orientation is remodeled after its initial deposition, or whether adult-like morphology is entirely a result of strain during deposition (Bertram & Swartz, 1991). Although we are unable to address this question using a quantitative approach, due to the difficulty in selecting homologous regions in an ontogenetic series, qualitative comparison of both mid-slices and BV/TV maps appear to show changes in BV/TV in regions deeper to the articular surface across the ontogenetic series. As growth is not only characterized by increased bone length, via deposition at the epiphyseal plate, but also by appositional growth leading to increasing anteroposterior and mediolateral width (Carter & Beaupré, 2000; Martin et al., 2015; Scheuer & Black, 2000), it would be expected that BV/TV in regions deep to the epiphyseal plate would be characterized by reduction in BV/TV, that is, due to increases in TV with an unchanged BV. This is not the case, and BV/TV does increase deeper to the articular surface, most notably in the proximal femur, and does not appear to reduce over time with increasing size. Further research is required to compare trabecular bone distributions in ontogenetic series of both metaphyseal and epiphyseal regions in extant apes, in order to identify potential signals

of locomotor changes during ontogeny. These results suggest such analyses would be useful for understanding adult trabecular structure, and potentially the trabecular morphology of immature fossil hominoids.

ACKNOWLEDGEMENTS

This research was supported by the Max Planck Society (to Z.J.T., T.L.K., M.M.S., and J.J.H.) and European Research Council Starting Grant #336301 (to T.L.K. and M.M.S.). For access to specimens and information about individuals in the sample we thank Christophe Boesch and Roman Wittig (Max Planck Institute for Evolutionary Anthropology and Taï Chimpanzee Project). For scanning assistance, we thank David Plotzki, Patrick Schönfeld, and Heiko Temming, and for discussion we thank Nicholas Stephens. We are grateful to two anonymous reviewers whose feedback greatly improved this manuscript.

ORCID

Zewdi J. Tsegai  <https://orcid.org/0000-0001-9041-4829>

Tracy L. Kivell  <https://orcid.org/0000-0001-5087-0897>

REFERENCES

- Abel, R., & Macho, G. A. (2011). Ontogenetic changes in the internal and external morphology of the ilium in modern humans. *Journal of Anatomy*, 218(3), 324–335. <https://doi.org/10.1111/j.1469-7580.2011.01342.x>
- Acquaah, F., Robson Brown, K. A., Ahmed, F., Jeffery, N., & Abel, R. L. (2015). Early trabecular development in human vertebrae: Overproduction, constructive regression, and refinement. *Frontiers in Endocrinology*, 6, 67. <https://doi.org/10.3389/fendo.2015.00067>
- Amsler, S. J. (2010). Energetic costs of territorial boundary patrols by wild chimpanzees. *American Journal of Primatology*, 72(2), 93–103. <https://doi.org/10.1002/ajp.20757>
- Barak, M. M., Lieberman, D. E., & Hublin, J.-J. (2011). A Wolff in sheep's clothing: Trabecular bone adaptation in response to changes in joint loading orientation. *Bone*, 49(6), 1141–1151. <https://doi.org/10.1016/j.bone.2011.08.020>
- Barak, M. M., Lieberman, D. E., & Hublin, J.-J. (2013a). Of mice, rats and men: Trabecular bone architecture in mammals scales to body mass with negative allometry. *Journal of Structural Biology*, 183(2), 123–131. <https://doi.org/10.1016/j.jsb.2013.04.009>
- Barak, M. M., Lieberman, D. E., Raichlen, D., Pontzer, H., Warrener, A. G., & Hublin, J.-J. (2013b). Trabecular evidence for a human-like gait in *Australopithecus africanus*. *PLoS One*, 8(11), e77687. <https://doi.org/10.1371/journal.pone.0077687>
- Barak, M. M., Sherratt, E., & Lieberman, D. E. (2017). Using principal trabecular orientation to differentiate joint loading orientation in the 3rd metacarpal heads of humans and chimpanzees. *Journal of Human Evolution*, 113, 173–182. <https://doi.org/10.1016/j.jhevol.2017.08.018>
- Behringer, V., Deschner, T., Deimel, C., Stevens, J. M. G., & Hohmann, G. (2014a). Age-related changes in urinary testosterone levels suggest differences in puberty onset and divergent life history strategies in bonobos and chimpanzees. *Hormones and Behavior*, 66(3), 525–533. <https://doi.org/10.1016/j.yhbeh.2014.07.011>
- Behringer, V., Deschner, T., Murtagh, R., Stevens, J. M. G., & Hohmann, G. (2014b). In bonobos and chimpanzees age-related changes in urinary thyroid hormones indicate heterochrony in their development. *American Journal of Physical Anthropology*, 153, 75–76.
- Behringer, V., Stevens, J. M. G., Kivell, T. L., Neufuss, J., Boesch, C., & Hohmann, G. (2016). Within arm's reach: Measuring forearm length to assess growth patterns in captive bonobos and chimpanzees. *American*

- Journal of Physical Anthropology*, 161(1), 37–43. <https://doi.org/10.1002/ajpa.23004>
- Bertram, J. E., & Swartz, S. M. (1991). The 'law of bone transformation': A case of crying Wolff? *Biological Reviews*, 66(3), 245–273.
- Best, A., Holt, B., Troy, K., & Hamill, J. (2017). Trabecular bone in the calcaneus of runners. *PLoS One*, 12(11), e0188200. <https://doi.org/10.1371/journal.pone.0188200>
- Biewener, A. A., Fazzalari, N. L., Konieczynski, D. D., & Baudinette, R. V. (1996). Adaptive changes in trabecular architecture in relation to functional strain patterns and disuse. *Bone*, 19(1), 1–8. [https://doi.org/10.1016/8756-3282\(96\)00116-0](https://doi.org/10.1016/8756-3282(96)00116-0)
- Boesch, C., & Boesch-Achermann, H. (2000). *The chimpanzees of the Tai Forest: Behavioral ecology and evolution*. Oxford, UK: Oxford University Press.
- Burgess, M. L., McFarlin, S. C., Mudakikwa, A., Cranfield, M. R., & Ruff, C. B. (2018). Body mass estimation in hominoids: Age and locomotor effects. *Journal of Human Evolution*, 115, 36–46. <https://doi.org/10.1016/j.jhevol.2017.07.004>
- Cao, J. J., Gregoire, B. R., & Gao, H. (2009). High-fat diet decreases cancellous bone mass but has no effect on cortical bone mass in the tibia in mice. *Bone*, 44(6), 1097–1104. <https://doi.org/10.1016/j.bone.2009.02.017>
- Carlson, K. J., Doran-Sheehy, D. M., Hunt, K. D., Nishida, T., Yamanaka, A., & Boesch, C. (2006). Locomotor behavior and long bone morphology in individual free-ranging chimpanzees. *Journal of Human Evolution*, 50(4), 394–404. <https://doi.org/10.1016/j.jhevol.2005.10.004>
- Carlson, K. J., Sumner, D. R., Morbeck, M. E., Nishida, T., Yamanaka, A., & Boesch, C. (2008). Role of nonbehavioral factors in adjusting long bone diaphyseal structure in free-ranging *Pan troglodytes*. *International Journal of Primatology*, 29(6), 1401–1420. <https://doi.org/10.1007/s10764-008-9297-y>
- Carlson, K. J., Wrangham, R. W., Muller, M. N., Sumner, D. R., Morbeck, M. E., Nishida, T., ... Boesch, C. (2011). Comparisons of limb structural properties in free-ranging chimpanzees from Kibale, Gombe, Mahale, and Tai communities. In K. D'Août & E. E. Vereecke (Eds.), *Primate locomotion* (pp. 155–182). New York, NY: Springer Verlag.
- Carter, D. R., & Beaupré, G. S. (2000). *Skeletal function and form: Mechanobiology of skeletal development, aging, and regeneration*. Cambridge, UK: Cambridge University Press.
- Cashman, K. D. (2007). Diet, nutrition, and bone health. *The Journal of Nutrition*, 137(11 Suppl), 2507S–2512S.
- Charles, J. F., Ermann, J., & Aliprantis, A. O. (2015). The intestinal microbiome and skeletal fitness: Connecting bugs and bones. *Clinical Immunology*, 159(2), 163–169. <https://doi.org/10.1016/j.clim.2015.03.019>
- Chirchir, H. (2015). A comparative study of trabecular bone mass distribution in cursorial and non-cursorial limb joints. *Anatomical Record*, 298(5), 797–809. <https://doi.org/10.1002/ar.23090>
- Chirchir, H., Kivell, T. L., Ruff, C. B., Hublin, J.-J., Carlson, K. J., Zipfel, B., & Richmond, B. G. (2015). Recent origin of low trabecular bone density in modern humans. *Proceedings of the National Academy of Sciences of the United States of America*, 112(2), 366–371. <https://doi.org/10.1073/pnas.1411696112>
- Chirchir, H., Ruff, C. B., Helgen, K. M., & Potts, R. (2016). Trabecular bone mass and daily travel distance in mammals. *The FASEB Journal*, 30(1 Suppl), 779.715.
- Chirchir, H., Ruff, C. B., Junno, J.-A., & Potts, R. (2017). Low trabecular bone density in recent sedentary modern humans. *American Journal of Physical Anthropology*, 162(3), 550–560. <https://doi.org/10.1002/ajpa.23138>
- Congdon, K. A. (2012). Interspecific and ontogenetic variation in proximal pedal phalangeal curvature of great apes (*Gorilla gorilla*, *Pan troglodytes*, and *Pongo pygmaeus*). *International Journal of Primatology*, 33(2), 418–427. <https://doi.org/10.1007/s10764-012-9590-7>
- Cotter, M. M., Simpson, S. W., Latimer, B. M., & Hernandez, C. J. (2009). Trabecular microarchitecture of hominoid thoracic vertebrae. *Anatomical Record*, 292(8), 1098–1106. <https://doi.org/10.1002/ar.20932>
- Cowgill, L. W., Warrener, A., Pontzer, H., & Ocobock, C. (2010). Waddling and toddling: The biomechanical effects of an immature gait. *American Journal of Physical Anthropology*, 143(1), 52–61. <https://doi.org/10.1002/ajpa.21289>
- Cowin, S. C. (2001). The false premise in Wolff's law. In S. C. Cowin (Ed.), *Bone mechanics handbook* (2nd ed.). Boca Raton, FL: CRC Press.
- Cunningham, C. A., & Black, S. M. (2009a). Anticipating bipedalism: Trabecular organization in the newborn ilium. *Journal of Anatomy*, 214(6), 817–829. <https://doi.org/10.1111/j.1469-7580.2009.01073.x>
- Cunningham, C. A., & Black, S. M. (2009b). Development of the fetal ilium – Challenging concepts of bipedality. *Journal of Anatomy*, 214(1), 91–99. <https://doi.org/10.1111/j.1469-7580.2008.01005.x>
- Demes, B., Larson, S. G., Stern, J. T., Jungers, W. L., Biknevicius, A. R., & Schmitt, D. (1994). The kinetics of primate quadrupedalism: "hindlimb drive" reconsidered. *Journal of Human Evolution*, 26, 353–374. <https://doi.org/10.1006/jhev.1994.1023>
- Doran, D. M. (1992). The ontogeny of chimpanzee and pygmy chimpanzee locomotor behavior: A case study of paedomorphism and its behavioral correlates. *Journal of Human Evolution*, 23(2), 139–157. [https://doi.org/10.1016/0047-2484\(92\)90104-H](https://doi.org/10.1016/0047-2484(92)90104-H)
- Doran, D. M. (1993). Sex differences in adult chimpanzee positional behavior: The influence of body size on locomotion and posture. *American Journal of Physical Anthropology*, 91(1), 99–115. <https://doi.org/10.1002/ajpa.1330910107>
- Doran, D. M. (1997). Ontogeny of locomotion in mountain gorillas and chimpanzees. *Journal of Human Evolution*, 32(4), 323–344. <https://doi.org/10.1006/jhev.1996.0095>
- Doube, M., Klosowski, M. M., Wiktorowicz-Conroy, A. M., Hutchinson, J. R., & Shefelbine, S. J. (2011). Trabecular bone scales allometrically in mammals and birds. *Proceedings of the Royal Society of London B: Biological Sciences*, 278(1721), 3067–3073. <https://doi.org/10.1098/rspb.2011.0069>
- Ehrlich, P. J., & Lanyon, L. E. (2002). Mechanical strain and bone cell function: A review. *Osteoporosis International*, 13(9), 688–700. <https://doi.org/10.1007/s001980200095>
- Fajardo, R. J., Desilva, J. M., Manoharan, R. K., Schmitz, J. E., Maclatchy, L. M., & Bouxsein, M. L. (2013). Lumbar vertebral body bone microstructural scaling in small to medium-sized strepsirrhines. *Anatomical Record*, 296(2), 210–226. <https://doi.org/10.1002/ar.22632>
- Glorieux, F. H., Salle, B. L., Travers, R., & Audra, P. H. (1991). Dynamic histomorphometric evaluation of human fetal bone formation. *Bone*, 12(6), 377–381. [https://doi.org/10.1016/8756-3282\(91\)90025-E](https://doi.org/10.1016/8756-3282(91)90025-E)
- Gong, J. K., Arnold, J. S., & Cohn, S. H. (1964). Composition of trabecular and cortical bone. *The Anatomical Record*, 149(3), 325–331. <https://doi.org/10.1002/ar.1091490303>
- Gorissen, B. M. C., Wolschrijn, C. F., van Rietbergen, B., Rieppo, L., Saarakkala, S., & van Weeren, P. R. (2018). Trabecular and subchondral bone development of the talus and distal tibia from foal to adult in the warmblood horse. *Anatomia, Histologia, Embryologia*, 47(3), 206–215. <https://doi.org/10.1111/ah.12341>
- Gorissen, B. M. C., Wolschrijn, C. F., van Vilsteren, A. A. M., van Rietbergen, B., & van Weeren, P. R. (2016). Trabecular bone of precocials at birth; are they prepared to run for the wolf(f)? *Journal of Morphology*, 277(7), 948–956. <https://doi.org/10.1002/jmor.20548>
- Gosman, J. H., Hubbell, Z. R., Shaw, C. N., & Ryan, T. M. (2013). Development of cortical bone geometry in the human femoral and tibial diaphysis. *The Anatomical Record*, 296(5), 774–787. <https://doi.org/10.1002/ar.22688>
- Gosman, J. H., & Ketcham, R. A. (2009). Patterns in ontogeny of human trabecular bone from SunWatch Village in the prehistoric Ohio Valley: General features of microarchitectural change. *American Journal of Physical Anthropology*, 138(3), 318–332. <https://doi.org/10.1002/ajpa.20931>
- Griffin, N. L., D'Août, K., Ryan, T. M., Richmond, B. G., Ketcham, R. A., & Postnov, A. (2010). Comparative forefoot trabecular bone architecture in extant hominids. *Journal of Human Evolution*, 59(2), 202–213. <https://doi.org/10.1016/j.jhevol.2010.06.006>
- Gross, T., Kivell, T. L., Skinner, M. M., Nguyen, N. H., & Pahr, D. H. (2014). A CT-image-based framework for the holistic analysis of cortical and trabecular bone morphology. *Palaeontologia Electronica*, 17(3), 13.
- Gruber, T., Zuberbühler, K., & Neumann, C. (2016). Travel fosters tool use in wild chimpanzees. *eLife*, 5, e16371. <https://doi.org/10.7554/eLife.16371>

- Hanna, J. B., Granatosky, M. C., Rana, P., & Schmitt, D. (2017). The evolution of vertical climbing in primates: Evidence from reaction forces. *The Journal of Experimental Biology*, 220(17), 3039–3052. <https://doi.org/10.1242/jeb.157628>
- Herbinger, I., Boesch, C., & Rothe, H. (2001). Territory characteristics among three neighboring chimpanzee communities in the Taï National Park, Côte d'Ivoire. *International Journal of Primatology*, 22(2), 143–167. <https://doi.org/10.1023/A:1005663212997>
- Hildebrand, T., & Rügsegger, P. (1997). A new method for the model-independent assessment of thickness in three-dimensional images. *Journal of Microscopy*, 185(1), 67–75. <https://doi.org/10.1046/j.1365-2818.1997.1340694.x>
- Hirasaki, E., Kumakura, H., & Matano, S. (1993). Kinesiological characteristics of vertical climbing in *Ateles geoffroyi* and *Macaca fuscata*. *Folia Primatologica (Basel)*, 61(3), 148–156. <https://doi.org/10.1159/000156742>
- Hunt, K. D. (1991). Positional behavior in the Hominoidea. *International Journal of Primatology*, 12(2), 95–118. <https://doi.org/10.1007/Bf02547576>
- Jungers, W. L. (1991). Scaling of postcranial joint size in hominoid primates. *Human Evolution*, 6(5–6), 391–399. <https://doi.org/10.1007/BF02435532>
- Kang, Q., An, Y. H., & Friedman, R. F. (1998). Mechanical properties and bone densities of canine trabecular bone. *Journal of Materials Science: Materials in Medicine*, 9(5), 263–267. <https://doi.org/10.1023/a:1008852610820>
- Kimura, T., Okada, M., & Ishida, H. (1979). Kinesiological characteristics of primate walking: Its significance in human walking. In M. E. Morbeck, H. Preuschoft, & N. Gomberg (Eds.), *Environment, behavior and morphology: Dynamic interactions in primates* (pp. 297–311). New York, NY: G. Fischer.
- Kivell, T. L. (2016). A review of trabecular bone functional adaptation: What have we learned from trabecular analyses in extant hominoids and what can we apply to fossils? *Journal of Anatomy*, 228(4), 569–594. <https://doi.org/10.1111/joa.12446>
- Kivell, T. L., & Schmitt, D. (2009). Independent evolution of knuckle-walking in African apes shows that humans did not evolve from a knuckle-walking ancestor. *Proceedings of the National Academy of Sciences United States of America*, 106(34), 14241–14246. <https://doi.org/10.1073/pnas.0901280106>
- Kivell, T. L., Skinner, M. M., Lazenby, R., & Hublin, J.-J. (2011). Methodological considerations for analyzing trabecular architecture: An example from the primate hand. *Journal of Anatomy*, 218(2), 209–225. <https://doi.org/10.1111/j.1469-7580.2010.01314.x>
- Kneissel, M., Roschger, P., Steiner, W., Schamall, D., Kalchauer, G., Boyde, A., & Teschler-Nicola, M. (1997). Cancellous bone structure in the growing and aging lumbar spine in a historic Nubian population. *Calcified Tissue International*, 61(2), 95–100.
- Lanyon, L. E. (1974). Experimental support for the trajectorial theory of bone structure. *The Journal of Bone and Joint Surgery*, 56(1), 160–166.
- Lazenby, R. A., Skinner, M. M., Hublin, J.-J., & Boesch, C. (2011a). Metacarpal trabecular architecture variation in the chimpanzee (*Pan troglodytes*): Evidence for locomotion and tool-use? *American Journal of Physical Anthropology*, 144(2), 215–225. <https://doi.org/10.1002/ajpa.21390>
- Lazenby, R. A., Skinner, M. M., Kivell, T. L., & Hublin, J.-J. (2011b). Scaling VOI size in 3D μ CT studies of trabecular bone: A test of the over-sampling hypothesis. *American Journal of Physical Anthropology*, 144(2), 196–203. <https://doi.org/10.1002/ajpa.21385>
- Lee, D. V., Bertram, J. E., & Todhunter, R. J. (1999). Acceleration and balance in trotting dogs. *Journal of Experimental Biology*, 202(24), 3565–3573.
- Lee, D. V., Stakebake, E. F., Walter, R. M., & Carrier, D. R. (2004). Effects of mass distribution on the mechanics of level trotting in dogs. *Journal of Experimental Biology*, 207(10), 1715–1728. <https://doi.org/10.1242/jeb.00947>
- Lehmann, J., & Boesch, C. (2003). Social influences on ranging patterns among chimpanzees (*Pan troglodytes verus*) in the Taï National Park, cote d'Ivoire. *Behavioral Ecology*, 14(5), 642–649. <https://doi.org/10.1093/beheco/arg047>
- Leigh, S. R., & Shea, B. T. (1995). Ontogeny and the evolution of adult body size dimorphism in apes. *American Journal of Primatology*, 36(1), 37–60. <https://doi.org/10.1002/ajp.1350360104>
- Leigh, S. R., & Shea, B. T. (1996). Ontogeny of body size variation in African apes. *American Journal of Physical Anthropology*, 99(1), 43–65.
- Lovejoy, C. O., McCollum, M. A., Reno, P. L., & Rosenman, B. A. (2003). Developmental biology and human evolution. *Annual Review of Anthropology*, 32(1), 85–109. <https://doi.org/10.1146/annurev.anthro.32.061002.093223>
- Macintosh, A. A., Pinhasi, R., & Stock, J. T. (2014). Lower limb skeletal biomechanics track long-term decline in mobility across ~6150 years of agriculture in Central Europe. *Journal of Archaeological Science*, 52(0), 376–390. <https://doi.org/10.1016/j.jas.2014.09.001>
- Macintosh, A. A., Pinhasi, R., & Stock, J. T. (2017). Prehistoric women's manual labor exceeded that of athletes through the first 5500 years of farming in Central Europe. *Science Advances*, 3(11), eaao3893. <https://doi.org/10.1126/sciadv.aao3893>
- MacLachy, L., & Müller, R. (2002). A comparison of the femoral head and neck trabecular architecture of *Galago* and *Perodicticus* using micro-computed tomography (μ CT). *Journal of Human Evolution*, 43(1), 89–105. <https://doi.org/10.1006/jhev.2002.0559>
- Maga, M., Kappelman, J., Ryan, T. M., & Ketcham, R. A. (2006). Preliminary observations on the calcaneal trabecular microarchitecture of extant large-bodied hominoids. *American Journal of Physical Anthropology*, 129(3), 410–417. <https://doi.org/10.1002/ajpa.20276>
- Martin, R. B., Burr, D. B., Sharkey, N. A., & Fyhrie, D. P. (2015). *Skeletal tissue mechanics*. New York, NY: Springer.
- McCabe, L., Britton, R., & Parameswaran, N. (2015). Prebiotic and probiotic regulation of bone health: Role of the intestine and its microbiome. *Current Osteoporosis Reports*, 13, 1–9. <https://doi.org/10.1007/s11914-015-0292-x>
- Milovanovic, P., Djonic, D., Hahn, M., Amling, M., Busse, B., & Djuric, M. (2017). Region-dependent patterns of trabecular bone growth in the human proximal femur: A study of 3D bone microarchitecture from early postnatal to late childhood period. *American Journal of Physical Anthropology*, 164(2), 281–291. <https://doi.org/10.1002/ajpa.23268>
- Mitani, J. C. (2009). Cooperation and competition in chimpanzees: Current understanding and future challenges. *Evolutionary Anthropology*, 18(5), 215–227. <https://doi.org/10.1002/evan.20229>
- Mølgaard, C., Thomsen, B. L., & Michaelsen, K. F. (1998). Influence of weight, age and puberty on bone size and bone mineral content in healthy children and adolescents. *Acta Paediatrica*, 87(5), 494–499. <https://doi.org/10.1111/j.1651-2227.1998.tb01492.x>
- Mulder, L., Koolstra, J. H., Weijjs, W. A., & van Eijden, T. M. G. J. (2005). Architecture and mineralization of developing trabecular bone in the pig mandibular condyle. *The Anatomical Record*, 285A(1), 659–666. <https://doi.org/10.1002/ar.a.20208>
- Nafei, A., Danielsen, C. C., Linde, F., & Hvid, I. (2000a). Properties of growing trabecular ovine bone. Part I: Mechanical and physical properties. *The Journal of Bone and Joint Surgery*, 82-B(6), 910–920.
- Nafei, A., Kabel, J., Odgaard, A., Linde, F., & Hvid, I. (2000b). Properties of growing trabecular ovine bone. Part II: Architectural and mechanical properties. *The Journal of Bone and Joint Surgery*, 82(6), 921–927.
- Paternoster, L., Lorentzon, M., Lehtimäki, T., Eriksson, J., Kähönen, M., Raitakari, O., ... Ohlsson, C. (2013). Genetic determinants of trabecular and cortical volumetric bone mineral densities and bone microstructure. *PLoS Genetics*, 9(2), e1003247. <https://doi.org/10.1371/journal.pgen.1003247>
- Pontzer, H., Lieberman, D. E., Momin, E., Devlin, M. J., Polk, J. D., Hallgrímsson, B., & Cooper, D. M. (2006). Trabecular bone in the bird knee responds with high sensitivity to changes in load orientation. *Journal of Experimental Biology*, 209(Pt 1), 57–65. <https://doi.org/10.1242/jeb.01971>
- Pontzer, H., & Wrangham, R. W. (2006). Ontogeny of ranging in wild chimpanzees. *International Journal of Primatology*, 27(1), 295–309. <https://doi.org/10.1007/s10764-005-9011-2>
- Prentice, A. (1997). Is nutrition important in osteoporosis? *Proceedings of the Nutrition Society*, 56(1B), 357–367. <https://doi.org/10.1079/PNS19970038>
- Core Team, R. (2016). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Raichlen, D. A., Gordon, A. D., Foster, A. D., Webber, J. T., Sukhdeo, S. M., Scott, R. S., ... Ryan, T. M. (2015). An ontogenetic framework linking locomotion and trabecular bone architecture with applications for

- reconstructing hominin life history. *Journal of Human Evolution*, 81, 1–12. <https://doi.org/10.1016/j.jhevol.2015.01.003>
- Reissis, D., & Abel, R. L. (2012). Development of fetal trabecular micro-architecture in the humerus and femur. *Journal of Anatomy*, 220(5), 496–503. <https://doi.org/10.1111/j.1469-7580.2012.01487.x>
- Richmond, B. G. (1998). *Ontogeny and biomechanics of phalangeal form in primates* (PhD). SUNY Stony Brook, Stony Brook, NY.
- Ruff, C. (2003a). Ontogenetic adaptation to bipedalism: Age changes in femoral to humeral length and strength proportions in humans, with a comparison to baboons. *Journal of Human Evolution*, 45(4), 317–349. <https://doi.org/10.1016/j.jhevol.2003.08.006>
- Ruff, C. (2008). Femoral/humeral strength in early African *Homo erectus*. *Journal of Human Evolution*, 54(3), 383–390. <https://doi.org/10.1016/j.jhevol.2007.09.001>
- Ruff, C., Holt, B., & Trinkaus, E. (2006). Who's afraid of the big bad Wolff? "Wolff's law" and bone functional adaptation. *American Journal of Physical Anthropology*, 129(4), 484–498. <https://doi.org/10.1002/Ajpa.20371>
- Ruff, C. B. (2002). Long bone articular and diaphyseal structure in old world monkeys and apes. I: Locomotor effects. *American Journal of Physical Anthropology*, 119(4), 305–342. <https://doi.org/10.1002/Ajpa.10117>
- Ruff, C. B. (2003b). Long bone articular and diaphyseal structure in Old World monkeys and apes. II: Estimation of body mass. *American Journal of Physical Anthropology*, 120(1), 16–37. <https://doi.org/10.1002/ajpa.10118>
- Ruff, C. B., Burgess, M. L., Bromage, T. G., Mudakikwa, A., & McFarlin, S. C. (2013). Ontogenetic changes in limb bone structural proportions in mountain gorillas (*Gorilla beringei beringei*). *Journal of Human Evolution*, 65(6), 693–703. <https://doi.org/10.1016/j.jhevol.2013.06.008>
- Ruff, C. B., Burgess, M. L., Ketcham, R. A., & Kappelman, J. (2016). Limb bone structural proportions and locomotor behavior in a.L. 288-1 ("Lucy"). *PLoS ONE*, 11(11), e0166095. <https://doi.org/10.1371/journal.pone.0166095>
- Ryan, T. M., Carlson, K. J., Gordon, A. D., Jablonski, N., Shaw, C. N., & Stock, J. T. (2018). Human-like hip joint loading in *Australopithecus africanus* and *Paranthropus robustus*. *Journal of Human Evolution*, 121, 12–24. <https://doi.org/10.1016/j.jhevol.2018.03.008>
- Ryan, T. M., & Ketcham, R. A. (2002a). Femoral head trabecular bone structure in two omomyid primates. *Journal of Human Evolution*, 43(2), 241–263. <https://doi.org/10.1006/jhev.2002.0575>
- Ryan, T. M., & Ketcham, R. A. (2002b). The three-dimensional structure of trabecular bone in the femoral head of strepsirrhine primates. *Journal of Human Evolution*, 43(1), 1–26. <https://doi.org/10.1006/jhev.2002.0552>
- Ryan, T. M., & Krovitz, G. E. (2006). Trabecular bone ontogeny in the human proximal femur. *Journal of Human Evolution*, 51(6), 591–602. <https://doi.org/10.1016/j.jhevol.2006.06.004>
- Ryan, T. M., Raichlen, D. A., & Gosman, J. H. (2017). Structural and mechanical changes in trabecular bone during early development in the human femur and humerus. In C. J. Percival & J. T. Richtsmeier (Eds.), *Building bones: Bone formation and development in anthropology* (pp. 254–280). Cambridge, UK: Cambridge University Press.
- Ryan, T. M., & Shaw, C. N. (2013). Trabecular bone microstructure scales allometrically in the primate humerus and femur. *Proceedings of the Biological Sciences*, 280(1758), 20130172. <https://doi.org/10.1098/Rspb.2013.0172>
- Ryan, T. M., & Shaw, C. N. (2015). Gracility of the modern *Homo sapiens* skeleton is the result of decreased biomechanical loading. *Proceedings of the National Academy of Sciences of the United States of America*, 112(2), 372–377. <https://doi.org/10.1073/pnas.1418646112>
- Ryan, T. M., & Walker, A. (2010). Trabecular bone structure in the humeral and femoral heads of anthropoid primates. *Anatomical Record*, 293(4), 719–729. <https://doi.org/10.1002/ar.21139>
- Saers, J. P. P. (2017). *Ontogeny and functional adaptation of trabecular bone in the human foot* (doctoral thesis). University of Cambridge, UK.
- Saers, J. P. P., Cazorla-Bak, Y., Shaw, C. N., Stock, J. T., & Ryan, T. M. (2016). Trabecular bone structural variation throughout the human lower limb. *Journal of Human Evolution*, 97, 97–108. <https://doi.org/10.1016/j.jhevol.2016.05.012>
- Salle, B. L., Rauch, F., Travers, R., Bouvier, R., & Glorieux, F. H. (2002). Human fetal bone development: Histomorphometric evaluation of the proximal femoral metaphysis. *Bone*, 30(6), 823–828. [https://doi.org/10.1016/S8756-3282\(02\)00724-X](https://doi.org/10.1016/S8756-3282(02)00724-X)
- Sarringhaus, L. A., MacLatchy, L. M., & Mitani, J. C. (2014). Locomotor and postural development of wild chimpanzees. *Journal of Human Evolution*, 66, 29–38. <https://doi.org/10.1016/j.jhevol.2013.09.006>
- Sarringhaus, L. A., MacLatchy, L. M., & Mitani, J. C. (2016). Long bone cross-sectional properties reflect changes in locomotor behavior in developing chimpanzees. *American Journal of Physical Anthropology*, 160(1), 16–29. <https://doi.org/10.1002/ajpa.22930>
- Scherf, H., & Tilgner, R. (2009). A new high-resolution computed tomography (CT) segmentation method for trabecular bone architectural analysis. *American Journal of Physical Anthropology*, 140(1), 39–51. <https://doi.org/10.1002/ajpa.21033>
- Scherf, H., Wahl, J., Hublin, J.-J., & Harvati, K. (2016). Patterns of activity adaptation in humeral trabecular bone in Neolithic humans and present-day people. *American Journal of Physical Anthropology*, 159(1), 106–115. <https://doi.org/10.1002/ajpa.22835>
- Scheuer, L., & Black, S. (2000). *Developmental juvenile osteology*. London, UK: Academic Press.
- Shaw, C. N., & Ryan, T. M. (2012). Does skeletal anatomy reflect adaptation to locomotor patterns? Cortical and trabecular architecture in human and nonhuman anthropoids. *American Journal of Physical Anthropology*, 147(2), 187–200. <https://doi.org/10.1002/Ajpa.21635>
- Shaw, C. N., & Stock, J. T. (2013). Extreme mobility in the late Pleistocene? Comparing limb biomechanics among fossil homo, varsity athletes and Holocene foragers. *Journal of Human Evolution*, 64(4), 242–249. <https://doi.org/10.1016/j.jhevol.2013.01.004>
- Shea, B., Wells, G., Cranney, A., Zytaruk, N., Robinson, V., Griffith, L., ... Guyatt, G. (2002). Meta-analyses of therapies for postmenopausal osteoporosis. VII. Meta-analysis of calcium supplementation for the prevention of postmenopausal osteoporosis. *Endocrine Reviews*, 23(4), 552–559. <https://doi.org/10.1210/er.2001-7002>
- Skinner, M. M., Stephens, N. B., Tsegaï, Z. J., Foote, A. C., Nguyen, N. H., Gross, T., ... Kivell, T. L. (2015). Human-like hand use in *Australopithecus africanus*. *Science*, 347(6220), 395–399. <https://doi.org/10.1126/science.1261735>
- Smith, B. H., & Boesch, C. (2011). Mortality and the magnitude of the "wild effect" in chimpanzee tooth emergence. *Journal of Human Evolution*, 60(1), 34–46. <https://doi.org/10.1016/j.jhevol.2010.08.006>
- Smith, B. H., Crummett, T. L., & Brandt, K. L. (1994). Ages of eruption of primate teeth: A compendium for aging individuals and comparing life histories. *American Journal of Physical Anthropology*, 37(S19), 177–231. <https://doi.org/10.1002/ajpa.1330370608>
- Smith, R. J., & Jungers, W. L. (1997). Body mass in comparative primatology. *Journal of Human Evolution*, 32(6), 523–559. <https://doi.org/10.1006/jhev.1996.0122>
- Sode, M., Burghardt, A. J., Nissenon, R. A., & Majumdar, S. (2008). Resolution dependence of the non-metric trabecular structure indices. *Bone*, 42(4), 728–736. <https://doi.org/10.1016/j.bone.2007.12.004>
- Stauber, M., Rapillard, L., van Lenthe, G. H., Zysset, P., & Müller, R. (2006). Importance of individual rods and plates in the assessment of bone quality and their contribution to bone stiffness. *Journal of Bone and Mineral Research*, 21(4), 586–595. <https://doi.org/10.1359/jbmr.060102>
- Stephens, N. B., Kivell, T. L., Gross, T., Pahr, D. H., Lazenby, R. A., Hublin, J.-J., ... Skinner, M. M. (2016). Trabecular architecture in the thumb of *Pan* and *Homo*: Implications for investigating hand use, loading, and hand preference in the fossil record. *American Journal of Physical Anthropology*, 161(4), 603–619. <https://doi.org/10.1002/ajpa.23061>
- Stock, J. T., & Macintosh, A. A. (2016). Lower limb biomechanics and habitual mobility among mid-Holocene populations of the cis-Baikal. *Quaternary International*, 405, 200–209. <https://doi.org/10.1016/j.quaint.2015.04.052>
- Su, A., & Carlson, K. J. (2017). Comparative analysis of trabecular bone structure and orientation in south African hominin tali. *Journal of Human Evolution*, 106, 1–18. <https://doi.org/10.1016/j.jhevol.2016.12.006>

- Su, A., Wallace, I. J., & Nakatsukasa, M. (2013). Trabecular bone anisotropy and orientation in an early Pleistocene hominin talus from East Turkana, Kenya. *Journal of Human Evolution*, 64(6), 667–677. <https://doi.org/10.1016/j.jhevol.2013.03.003>
- Sutherland, D. (1997). The development of mature gait. *Gait & Posture*, 6(2), 163–170. [https://doi.org/10.1016/S0966-6362\(97\)00029-5](https://doi.org/10.1016/S0966-6362(97)00029-5)
- Sutherland, D. H., Olshen, R., Cooper, L., & Woo, S. L. Y. (1980). The development of mature gait. *Journal of Bone and Joint Surgery*, 62(3), 336–353.
- Tanck, E., Homminga, J., van Lenthe, G. H., & Huiskes, R. (2001). Increase in bone volume fraction precedes architectural adaptation in growing bone. *Bone*, 28(6), 650–654. [https://doi.org/10.1016/S8756-3282\(01\)00464-1](https://doi.org/10.1016/S8756-3282(01)00464-1)
- Tardieu, C. (1999). Ontogeny and phylogeny of femoro-tibial characters in humans and hominid fossils: Functional influence and genetic determinism. *American Journal of Physical Anthropology*, 110(3), 365–377.
- Tardieu, C., & Trinkaus, E. (1994). Early ontogeny of the human femoral bicondylar angle. *American Journal of Physical Anthropology*, 95(2), 183–195. <https://doi.org/10.1002/ajpa.1330950206>
- Tsegai, Z. J., Kivell, T. L., Gross, T., Nguyen, N. H., Pahr, D. H., Smaers, J. B., & Skinner, M. M. (2013). Trabecular bone structure correlates with hand posture and use in hominoids. *PLoS One*, 8(11), e78781. <https://doi.org/10.1371/journal.pone.0078781>
- Tsegai, Z. J., Skinner, M. M., Gee, A. H., Pahr, D. H., Treece, G. M., Hublin, J. J., & Kivell, T. L. (2017). Trabecular and cortical bone structure of the talus and distal tibia in *Pan* and *Homo*. *American Journal of Physical Anthropology*, 163(4), 784–805. <https://doi.org/10.1002/ajpa.23249>
- Tsegai, Z. J., Skinner, M. M., Pahr, D. H., Hublin, J.-J., & Kivell, T. L. (2018). Systemic patterns of trabecular bone across the human and chimpanzee skeleton. *Journal of Anatomy*, 232(4), 641–656. <https://doi.org/10.1111/joa.12776>
- Ulrich, D., van Rietbergen, B., Laib, A., & Ruegsegger, P. (1999). The ability of three-dimensional structural indices to reflect mechanical aspects of trabecular bone. *Bone*, 25(1), 55–60.
- Wallace, I. J., Demes, B., & Judex, S. (2017). Ontogenetic and genetic influences on bone's responsiveness to mechanical signals. In C. J. Percival & J. T. Richtsmeier (Eds.), *Building bones: Bone formation and development in anthropology* (pp. 205–232). Cambridge, UK: Cambridge University Press.
- Wallace, I. J., Judex, S., & Demes, B. (2015a). Effects of load-bearing exercise on skeletal structure and mechanics differ between outbred populations of mice. *Bone*, 72, 1–8. <https://doi.org/10.1016/j.bone.2014.11.013>
- Wallace, I. J., Kwaczala, A. T., Judex, S., Demes, B., & Carlson, K. J. (2013). Physical activity engendering loads from diverse directions augments the growing skeleton. *Journal of Musculoskeletal and Neuronal Interactions*, 13(3), 283–288.
- Wallace, I. J., Pagnotti, G. M., Rubin-Sigler, J., Naeher, M., Copes, L. E., Judex, S., ... Demes, B. (2015b). Focal enhancement of the skeleton to exercise correlates with responsivity of bone marrow mesenchymal stem cells rather than peak external forces. *Journal of Experimental Biology*, 218, 3002–3009. <https://doi.org/10.1242/jeb.118729>
- Wallace, I. J., Tommasini, S. M., Judex, S., Garland, T., Jr., & Demes, B. (2012). Genetic variations and physical activity as determinants of limb bone morphology: An experimental approach using a mouse model. *American Journal of Physical Anthropology*, 148(1), 24–35. <https://doi.org/10.1002/ajpa.22028>
- Ward, C. V. (2002). Interpreting the posture and locomotion of *Australopithecus afarensis*: Where do we stand? *Yearbook of Physical Anthropology*, 45, 185–215. <https://doi.org/10.1002/Ajpa.10185>
- Watts, D. P., & Mitani, J. C. (2001). Boundary patrols and intergroup encounters in wild chimpanzees. *Behavior*, 138, 299–327. <https://doi.org/10.1163/15685390152032488>
- Wickham, H. (2009). *ggplot2: Elegant graphics for data analysis*. New York, NY: Springer-Verlag.
- Wilke, C. O. (2017). Cowplot: Streamlined plot theme and plot: Annotations for 'ggplot2'.
- Wolschrijn, C. F., & Weijs, W. A. (2004). Development of the trabecular structure within the ulnar medial coronoid process of young dogs. *The Anatomical Record*, 278(2), 514–519. <https://doi.org/10.1002/ar.a.20039>
- Zeininger, A. (2013). *Ontogeny of bipedalism: Pedal mechanics and trabecular bone morphology*. (PhD), The University of Texas Austin, TX.
- Zeininger, A., Patel, B. A., Zipfel, B., & Carlson, K. J. (2016). Trabecular architecture in the StW 352 fossil hominin calcaneus. *Journal of Human Evolution*, 97, 145–158. <https://doi.org/10.1016/j.jhevol.2016.05.009>
- Zihlman, A. L., Stahl, D., & Boesch, C. (2008). Morphological variation in adult chimpanzees (*Pan troglodytes verus*) of the Taï National Park, Côte d'Ivoire. *American Journal of Physical Anthropology*, 135(1), 34–41. <https://doi.org/10.1002/ajpa.20702>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Tsegai ZJ, Skinner MM, Pahr DH, Hublin J-J, Kivell TL. Ontogeny and variability of trabecular bone in the chimpanzee humerus, femur and tibia. *Am J Phys Anthropol*. 2018;167:713–736. <https://doi.org/10.1002/ajpa.23696>