Citation for published version

Tsegai, Zewdi J. and Skinner, Matthew M. and Gee, Andrew H. and Pahr, Dieter H. and Treece, Graham M. and Hublin, Jean-Jacques and Kivell, Tracy L. (2017) Trabecular and cortical bone structure of the talus and distal tibia in Pan and Homo. American Journal of Physical Anthropology, 163 (4). pp. 784-805. ISSN 0002-9483.

DOI

https://doi.org/10.1002/ajpa.23249

Link to record in KAR

Document Version

Author's Accepted Manuscript

Trabecular and cortical bone structure of the talus and distal tibia in Pan and Homo

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Abbreviated title: Internal bone structure of talus and tibia

Keywords: Bone microstructure, Functional morphology, Locomotion, Bipedalism, Cancellous bone

Text pages: 36; **Figures:** 10; **Tables:** 6

Grant sponsorship: This research was supported by The Max Planck Society and the European Research Council Starting Grant #336301.

ABSTRACT

Objectives: Internal bone structure, both cortical and trabecular bone, remodels in response to loading and may provide important information regarding behaviour. The foot is well suited to analysis of internal bone structure because it experiences the initial substrate reaction forces, due to its proximity to the substrate. Moreover, as humans and apes differ in loading of the foot, this region is relevant to questions concerning arboreal locomotion and bipedality in the hominoid fossil record.

Materials and methods: We apply a whole-bone/epiphysis approach to analyse trabecular and cortical bone in the distal tibia and talus of Pan troglodytes and Homo sapiens. We quantify bone volume fraction (BV/TV), degree of anisotropy (DA), trabecular thickness (Tb.Th), bone surface to volume ratio (BS/BV), cortical thickness, and investigate the distribution of BV/TV and cortical thickness throughout the bone/epiphysis.

Results: We find that Pan has a greater BV/TV, a lower BS/BV and thicker cortices than Homo in both the talus and distal tibia. The trabecular structure of the talus is more divergent than the tibia, having thicker, less uniformly aligned trabeculae in Pan compared to Homo. Differences in dorsiflexion at the talocrural joint and in degree of mobility at the talonavicular joint are reflected in the distribution of cortical and trabecular bone.

Discussion: Overall, quantified trabecular parameters represent overall differences in bone strength between the two species, however, DA may be directly related to joint loading. Cortical and trabecular bone distributions correlate with habitual joint positions adopted by each species, and thus have potential for interpreting joint position in fossil hominoids.

1. INTRODUCTION

Aspects of the external bony morphology of the talus and distal tibia reflect kinematic differences between how terrestrial bipedal humans and arboreal, quadrupedal African apes load their foot and ankle during locomotion (e.g. Lewis, 1980a,b,c; Stern and Susman, 1983; Latimer et al., 1987; DeSilva, 2009; Barak et al., 2013b). These morphological differences can be related to fundamental differences in foot posture: the degree of dorsiflexion at the ankle, use of the foot in an inverted position, the general conformation of the leg, and the presence of medial and longitudinal arches of the foot. For example, compared with African apes, humans have been described as having a less mediolaterally expanded anterior distal articular surface of the tibia (Latimer et al., 1987; DeSilva, 2009), an angle close to 90 degrees between the long axis and distal articular surface of the tibia (Latimer et al., 1987; DeSilva, 2009), a more symmetric talar trochlea (Latimer et al., 1987; DeSilva, 2009), a relatively stiff mid-foot without a mid-tarsal break (Elftman and Manter, 1935; DeSilva, 2010), and a complex of features, including the medial longitudinal arch, metatarsophalangeal joints and various soft tissues, which contribute to the windlass mechanism (Griffin et al., 2015) that improves locomotor efficiency (Ker et al., 1987).

In part due to the mosaic nature of fossil hominin morphology, but also due to reliance on fragmentary or isolated postcranial elements, palaeoanthropologists often differ in their interpretations of the functional significance of various morphological features. It remains unclear, based on the morphology of the ankle, whether early hominins continued to engage in a significant amount of arboreal behaviour and whether hominin species used kinematically similar or distinct forms of bipedalism, perhaps unlike the modern human bipedal gait (e.g. Day and Wood, 1968; Lisowski et al., 1974; Lisowski et al., 1976; Oxnard and Lisowski, 1980; Stern and Susman, 1983; Latimer et al., 1987; Clarke and Tobias, 1995; Harcourt-Smith and Aiello, 2004; DeSilva, 2009; DeSilva and Throckmorton, 2010; Zipfel et al., 2011; Haile-Selassie et al., 2012; DeSilva et al., 2013; Harcourt-Smith et al., 2015; Prang, 2015, 2016). Functional interpretation of the external skeletal morphology of the foot is further complicated by the role of soft tissues in limiting or enabling adoption of different foot postures (Venkataraman, 2013a,b) and by the substantial individual variability in the flexibility of the modern human foot (Bates et al., 2013; DeSilva et al., 2015). As the foot comprises a complex system of bones, tendons, ligaments and muscles, there are potentially many different ways for it to adapt to different functions, other than by modification of external bone shape (Crompton, 2015). Even modern humans are able to access numerous resources efficiently from the arboreal environment (Kraft et al., 2014), without any apparent external morphological signal on the talus and distal tibia (Venkataraman et al., 2013a).

Analysis of internal bone structure, both cortical and trabecular bone, of the talocrural and talonavicular joint has potential to provide further insight into interpreting use of the foot in the past. While external articular morphology indicates the joint positions a species was able to adopt, the internal bone structure can provide information about how a joint was actually loaded (Ruff and Runestad, 1992; Kivell, 2016). This is because both trabecular and cortical bone structure can adapt to loading during an individual's lifetime (e.g. Lanyon, 1974; Robling et al., 2002; Pontzer et al., 2006; Ruff et al., 2006; Barak et al., 2011; Kivell, 2016), by remodelling in response to strain (Ehrlich and Lanyon, 2002). Structural adaptations can occur at the level of individual trabeculae (Schulte et al., 2013; Cresswell et al., 2015). As these individual trabeculae appear able to adapt to accommodate regional strains, it is likely that regional architectural parameters can provide information about how different areas of a joint are loaded. For example, trabecular and cortical bone distribution close to the articular surface, radiodensity patterns, and indicators of bone remodelling, correspond with predicted locations of peak loading associated with specific joint positions (Patel and Carlson, 2007; Polk et al., 2008, 2010; Mazurier et al., 2010; Zeininger et al., 2011; Carlson et al., 2013; Tsegai et al., 2013; Skinner et al., 2015).

Experimentally changing the loading regime of a joint or limb by, for example, changing the angle of the joint during loading or subjecting a limb to an unnatural load, leads to predictable alterations in both cortical and trabecular bone (Robling et al., 2002; Pontzer et al., 2006; Barak et al., 2011; Cresswell et al., 2015). It is often difficult to relate bone structure, especially that of trabecular bone, directly to the biomechanical environment, i.e. to connect specific architectural variables to joint function and loading regime. Factors other than behaviour have the potential to influence, or even be the main factor determining, bone form (Bertram and Swartz, 1991; Lovejoy et al., 2003; Ruff et al., 2006; Kivell, 2016). There is still much that we do not fully understand about bone functional adaptation, including the genetic and systemic factors that shape trabecular and cortical structure (Lieberman, 1996; Carlson et al., 2008; Havill et al., 2010; Wallace et al., 2010; Paternoster et al., 2013; Wallace et al., 2013; Tsegai et al., 2016a). These include the way in which bone remodels depending upon the duration, frequency, or magnitude of the external load (e.g. Frost, 1987; Rubin and Lanyon, 1985; Skerry and Lanyon, 1995), or how these factors might vary depending on species (e.g. Turner, 2001), anatomical region (e.g. Morgan and Keaveny, 2001), age (e.g. Pearson and Lieberman, 2004) or body mass (e.g. Biewener, 1990; Doube et al., 2011). Moreover, cortical and trabecular bone may respond differently to strain or even interact to compensate for each other (Carlson and Judex, 2007). It is likely that these factors vary between even closely related species/subspecies. For example, some of the genetic differences between modern humans and Neanderthals relate to bone growth (Green et al., 2010), and changes in indirect measures of hormone levels occur at different developmental stages in humans, chimpanzees and bonobos (e.g. TT3: Behringer et al., 2014a; testosterone: Behringer et al., 2014b). All of these factors can confound our functional interpretations of variation in bone structure. However, there is a wealth of comparative, computational and in vivo research that makes clear that variation in cortical and trabecular structure reflects, at least to some degree, variation in external loading (Ruff et al., 2006; Kivell, 2016).

The hominoid foot and ankle, specifically the talocrural and talonavicular joints, are well suited to analysis of internal bone structure due to differences in foot postures adopted by modern humans and extant apes, the specific structure of the joint, and the close association of the foot with the substrate. Several studies have investigated the kinematics of the foot, during both quadrupedal and bipedal locomotion, in humans and chimpanzees (e.g. Sockol et al., 2007; Pontzer et al., 2009; Pontzer et al., 2014; O’Neill et al., 2015; Holowka et al., 2017). As modern human bipeds and chimpanzee climbers/knuckle-walkers adopt divergent foot postures (DeSilva, 2009), the loading environment within the foot and at the ankle is likely to differ between these groups. In Pan troglodytes, the ankle is loaded in dorsiflexion during both vertical climbing and during quadrupedal knuckle-walking (Sockol et al., 2007; DeSilva, 2009; Pontzer et al., 2009; Barak et al., 2013b; Pontzer et al., 2014), whereas the human ankle adopts a more neutral posture during bipedalism (Barak et al., 2013b). The chimpanzee ankle is also inverted during climbing (Lewis 1980a; Latimer et al., 1987; DeSilva, 2009). Loading at the talonavicular joint is characterised by greater mobility in Pan compared to Homo, either related to dorsiflexion (i.e. the midtarsal break) or to rotation (Elftman and Manter, 1935; DeSilva, 2010; Thompson et al., 2014; but see Holowka et al., 2017). The high joint congruity between the distal tibia and the trochlea surface of the talus (Latimer et al., 1987) indicates that the bone structure is likely to be directly related to joint use, and not to other factors such as the action of muscles, as in other regions (e.g. the humeral head), where the bony articulation itself does not maintain joint integrity. In the absence of muscle/tendon attachments on the talus itself, and thus of tensile forces caused by muscle contractions, this region also offers an opportunity to analyse the effects of locomotor forces alone on trabecular bone structure (DeSilva and Devlin, 2012). Further, as the foot is in direct contact with the substrate, it directly experiences the initial forces of locomotion, unlike more proximally located joints. The same is true for the hand, where clear trabecular signals of the direction of loading are present (Tsegai et al., 2013; Skinner et al., 2015).

Previous analyses have assessed the functional significance of trabecular and cortical bone structure of the ankle in humans (talus: Takechi et al., 1982; Sinha, 1985; Pal and Routal, 1998; Ebraheim et al., 1999; Schiff et al., 2007; Athavale et al., 2008; Nowakowski et al., 2013; talus and distal tibia: Hvid et al., 1985), and several studies have adopted a comparative approach across different taxa (talus: Su, 2011; DeSilva and Devlin, 2012; Hérbert et al., 2012; Su et al., 2013; Su and Carlson, 2017; tibia: Su, 2011; Barak et al., 2013b; Carlson et al., 2016). DeSilva and Devlin (2012) found interspecific differences in regional patterning of trabecular structure across four quadrants of the talar body, but were unable to attribute these differences to locomotor mode and a biomechanical explanation remains unclear. Analysis of more localised subregions, sampling bone directly adjacent to the articular surface, has shown regional patterning of degree of anisotropy (DA), elongation and primary trabecular orientation, which is distinct in modern humans when compared with extant apes, with fossil hominins displaying some ape-like and some human-like features (Su, 2011; Su et al., 2013; Su andCarlson, 2017). At the distal tibia, the orientation of trabecular bone in humans and chimpanzees corresponds with measurements of dorsiflexion at the ankle (Barak et al., 2013b). Previous studies have assessed cortical thickness and radiodensity patterns of the articular surfaces of the primate talus and distal tibia (talus: Su, 2011; tibia: Su, 2011; Carlson et al., 2016), and behavioural correlates have been identified from bone profiles and radiodensity patterns at articular surfaces of other primate and mammalian taxa and epiphyses (Patel and Carlson, 2007; Mazurier et al., 2010; Carlson et al., 2013). However, to our knowledge no previous study has comparatively analysed cortical thickness maps in both the talus and distal tibia of humans and chimpanzees.

Previous studies quantifying trabecular bone structure and/or bone strength characteristics at the ankle relied on analyses of multiple volumes of interest (Su, 2011; DeSilva and Devlin, 2012; Su et al., 2013) or on destructive methods (Sinha, 1985; Athavale et al., 2008). Interspecific analyses are often complicated by the difficulty in identifying biologically homologous regions, and differences in VOI size and location have a substantial impact on trabecular bone analysis, especially when comparing among species that vary greatly in size and in morphologically complex bones (Maga et al., 2006; Kivell et al., 2011; Lazenby et al., 2011). Moreover, trabecular bone close to the articular surface, which can be difficult to sample using VOI-based methods that require manual discrimination between cortical and trabecular bone, is more likely to be of biomechanical relevance as it experiences the initial joint reaction forces, and bone closer to the articular surface differs from that in the center of the epiphysis (Singh, 1978). Analyses of bone strength at the articular surface have not investigated the cortical and trabecular structure independently, but have instead used methods which quantify cortical bone and some of the underlying trabeculae (Patel and Carlson, 2007; Mazurier et al., 2010). In this study, we address some of these challenges by using two methodologies that allow independent quantification of the trabecular and the cortical structure. The trabecular bone analysis applied here enables quantification of trabecular structure throughout the bone or in a pre-defined region of the epiphysis, however, statistical comparisons cannot be conducted between groups. For cortical bone, we use a method that is able to compare cortical thickness across the bone/epiphysis between groups, but does not allow quantification of trabecular structure further than around 5mm beneath the cortex. By combining these complementary methodologies, we are able to analyse patterns of both cortical and trabecular bone in the human and chimpanzee talus and distal tibia. As a result, we are able to generate a fine scale, nuanced analysis through the visualisation of regional patterning of both cortical and trabecular bone, which may provide detailed information about joint loading.

In this study, we measure trabecular and cortical bone of the talus and distal tibia in Pan troglodytes verus and Homo sapiens. We test the following predictions in how trabecular bone structure and distribution, and cortical thickness and distribution differ between Pan and Homo. First, as both the talocrural and talonavicular joint are used in a greater range of positions in Pan, and both joints are less mobile in Homo, we predict a higher DA in humans in both the talus and tibia (Barak et al., 2013b; Su, 2011; Su et al., 2013; Thompson et al., 2014; Su and Carlson, 2017; but see Holowka et al., 2017). Second, following the findings of previous trabecular studies that sedentary modern humans have a generally low BV/TV and cortical thickness (Ruff et al., 1993; Lieberman, 1996; Ruff, 2005; Chirchir et al., 2015; Ryan and Shaw, 2015; Scherf et al., 2015; Chirchir et al., 2017), we predict an overall lower BV/TV and thinner cortex in Homo. Third, we hypothesise that the regional distribution of both cortical and trabecular bone will reflect differences in habitual peak loading of the talocrural and talonavicular joints. More specifically, that at the talocrural joint Pan will show a pattern of BV/TV and cortical thickness that reflects use of the foot in dorsiflexion and inversion, and at the talonavicular joint a greater degree of mobility. In Homo, the trabecular bone distribution and cortical thickness will reflect less mobility, and a more neutral ankle position.

2. MATERIALS AND METHODS

2.1 Sample

This study analysed trabecular and cortical bone morphology of the tibia and talus of two species with divergent modes of locomotion: Pan troglodytes verus and Homo sapiens. The sample, detailed in Table 1, included fifteen wild P. t. verus individuals (tibiae: N = 10; tali: N = 13; of which N = 8 were paired) whose skeletal remains were collected from the Taï National Park, Cote d'Ivoire, and ten H. sapiens individuals (tibia: N = 8; tali: N = 9; of which N = 7 were paired) from an 18th - 19th century cemetery in Inden, Germany. Adult specimens were used, based on fusion of the epiphyses throughout the skeleton and no external signs of pathology or senescence related changes were present. The right side was chosen where both talus and tibia were available and free from damage, otherwise the left side was used.

2.2 Computed tomography

High resolution micro-computed tomography (CT) scans were collected with a BIR ACTIS 225/300 CT scanner for the tibiae and with a SkyScan1173 CT scanner for the tali, using an acceleration voltage of 130kV and 100µA and either a 0.5mm brass or 1mm aluminium filter, at the Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology (Leipzig, Germany). Isotropic acquisition voxel sizes were 25-36 microns for the tibia and talus of Homo and 19-30 microns for the tibia and talus of Pan. Each scan was reconstructed as a 2048 x 2048 16-bit TIFF image stack from 2500 projections with three-frame averaging. Following reconstruction, all specimens were reoriented into standardised positions using AVIZO 6.3® (Visualization Sciences Group, SAS) and segmented using a Ray Casting Algorithm (Scherf and Tilgner, 2009).

Prior to segmentation, all Pan specimens were resampled to 35 microns and all Homo specimens to 40 microns, due to processing constraints. The relative resolutions, a measure of how adequately the average trabecular strut is represented (i.e. mean trabecular thickness [mm] / resolution [mm]), are shown in Table 1. The average for the entire sample of 7.57 (range: 5.46 – 11.59) is consistent with previous studies of trabecular bone structure (Sode et al., 2008; Kivell et al., 2011; Tsegai et al., 2013), and is appropriate for microstructural analysis.

2.3 Analysis of trabecular bone microstructure

To quantify trabecular bone, each material in the scan (Fig. 1a), i.e. cortical bone, trabecular bone, air and the internal bone cavity, were segmented automatically using an in house script in medtool v3.9 (www.dr-pahr.at), following Gross et al. (2014). Morphological filters were used to separate these regions, and the kernel size used was adjusted for each individual according to its measured trabecular thickness, enabling an accurate, subject-specific segmentation. This resulted in three data sets that were used in subsequent processing steps: (1) the trabecular bone (Fig. 1b), (2) the inner region of the bone and, (3) the inner mask (Fig. 1c), which contains the internal region of the bone where internal bone cavity and trabecular bone are represented by different grey values and the cortex has been removed. This automated segmentation was problematic in two locations in the talus, at the inferior talar neck and at the subtalar joint surfaces, due to their complex morphology. Thus the results from these regions are treated with caution. The proximal boundary of the distal tibia was defined as the point at which curvature of the shaft begins in both medial and anterior views, which is at the proximal extent of the fibular notch, and is an equivalent location across the sample.

From the trabecular only mask (Fig. 1b), trabecular thickness (Tb.Th), bone surface area (BS), and bone volume (BV) were quantified using the BoneJ plugin (version 1.3.12; Doube et al., 2010) for ImageJ v1.46r (Schneider et al., 2012). Bone surface to volume ratio (BS/BV) was subsequently calculated.

The inner region of the bone was used to create a 3D tetrahedral mesh with a mesh size of 1mm, using CGAL 4.4 (CGAL, Computational Geometry, http://www.cgal.org). The inner mask (Fig. 1c) was used to calculate BV/TV throughout the bone to generate 3D colour maps of bone distribution, and to calculate the overall bone volume fraction (BV/TV) and degree of anisotropy (DA) using medtool v3.9. A rectangular background grid, with a grid size of 2.5mm, was applied and a spherical VOI with a diameter of 5mm was used to measure BV/TV at each node of the grid. A sphere size of 5mm is appropriate as enough trabecular struts are sampled to adequately quantify trabecular parameters (Gross et al., 2014). To create a 3D colour map of bone distribution, the BV/TV values at each node were interpolated to assign each element in the 3D mesh of the trabecular region a BV/TV value (Fig. 1d). The colour maps were visualized in Paraview v4.0.1 (Ahrens et al., 2005). The overall BV/TV value was calculated as the mean of the values for each element in the 3D mesh, and thus is the average for the whole bone/epiphysis. The mean intercept method (Whitehouse, 1974; Odgaard, 1997) was used to calculate the mean fabric tensor, the arithmetic mean of all second order fabric tensors normalised using the determinants. The extracted eigenvalues and eigenvectors were then used to calculate the DA (DA = 1 – [smallest eigenvalue/largest eigenvalue]), whereby a DA of 1 indicates complete anisotropy and a DA of 0 complete isotropy.

2.4 Analysis of cortical bone microstructure

To compare cortical thickness between Pan and Homo in the talus and distal tibia, cortical bone thickness maps were generated for each specimen (following Treece et al., 2010; Treece et al., 2012; Tsegai et al., 2016b). This was accomplished via semi-automatic segmentation of the cortical surface, from the unsegmented CT data (Fig. 1e-f) in Stradwin v5.1a (Treece, Gee, Cambridge; http://mi.eng.cam.ac.uk/~rwp/stradwin). Following definition of the surface, around 15,000 independent measurements of cortical thickness were calculated throughout the bone (Fig. 1f) and mapped onto a subject specific surface (Fig. 1g). Subsequently, each surface was registered to a canonical surface using wxRegSurf v13 (Fig. 1h). The canonical surface used was an average of the entire sample, each species was averaged separately and then the average of the two resulting surfaces was used, to prevent the difference in sample size affecting the average morphology. After registration to the canonical surface, mean thickness maps were generated for each species.

2.5 Statistical analysis

For trabecular bone analysis, all statistical tests were performed using R v3.0.3 (R Core Team, 2016) and ggplot2 was used for generating plots (Wickham, 2009). Shapiro-Wilk test for normality showed that the data were not normally distributed and thus non-parametric tests were used. Mann-Whitney U tests were used to test for statistical differences in trabecular bone parameters between Homo and Pan. A principal component analysis was conducted to determine which parameters contributed to interspecific differences in the talus and in the tibia. All variables were included in the principal component analysis: Tb.Th, BV/TV, DA, BS/BV, and cortical thickness. As there are large differences in the variances of these variables, prior to analysis the data was centered and scaled to unit variance. Principal components were subsequently derived by singular value decomposition of the resulting data matrix. Spearman's correlation test and RMA regression were used to test for correlation between trabecular parameters and cortical thickness in the talus and distal tibia. To test the relationship between size and trabecular bone parameters, OLS $\log_{10}$ regressions and Pearson's correlation tests were conducted for each trabecular parameter against the size of the epiphysis/bone for each taxon. The size of each bone was represented as the geometric mean of several measurements, both of overall bone size and of the size of the articular surfaces. For the talus, these measurements were the anteroposterior length, mediolateral width and dorsoplantar height of the talus, the anteroposterior length and mediolateral width of the talar trochlea, and the dorsoplantar height and mediolateral width of the talar head. For the tibia, a geometric mean was derived from the maximum anteroposterior length and maximum mediolateral width of the distal tibia, the anteroposterior length and mediolateral width of the distal articular surface, the anteroposterior length, mediolateral width and proximodistal height of the medial malleolus. Pearson's correlation test was used to compare trabecular parameters between paired tibia and tali in each taxon. Statistical parametric mapping was used to identify regional cortical thickness differences between the two species (Friston et al., 1995), using the SurfStat package (Worsley et al., 2009), by fitting a general linear model (GLM) to the data. This model determined whether cortical thickness differences could be explained by species (covariates of interest) or other factors (confounding covariates). As there is risk of systematic misregistration due to shape differences, non-rigid shape coefficients were included as confounds in the GLM (Gee and Treece, 2014; Gee et al., 2015). Bone size, however, was strongly correlated with species and therefore not included as a confound in the GLM. Statistical parametric maps were generated using F statistics and the corresponding p-values were corrected for multiple comparisons using random field theory to control for the chance of false positives. Relative cortical thickness was calculated for each specimen, by subtracting the individual mean value from each individual thickness measurement and dividing by the standard deviation. In this way, relative patterns of cortical thickness could be analysed, despite considerable interspecific differences in absolute cortical thickness. For all statistical tests, a p value of <0.05 was considered significant.

3 RESULTS

3.1 Trabecular and cortical architecture of the talus and tibia

Means and standard deviations of measured trabecular and cortical parameters and Mann-Whitney U test results are shown in Table 2, and extracted regions of trabecular bone, visualizing structural differences, are shown in Figure 2. Mann-Whitney U test results (Table 2) find that the trabecular structure of Pan differs from that of Homo in having a significantly greater BV/TV and lower BS/BV in both the talus and the tibia. The trabecular structure is more divergent in the talus than in the tibia: with the talus of Pan having significantly thicker, less uniformly-oriented trabeculae (i.e. lower DA). The cortex of Pan is significantly thicker in both the talus and the tibia compared to Homo.

Correlations between parameters in the talus and tibia of each taxon are reported in Table 3. Significant correlations between variables differ both between taxa and between skeletal regions. As such, all parameters were included in the analysis, although correlations between parameters may lead to overemphasis of the contribution of these variables. Table 4 shows the results of the principal component (PC) analysis, and Figure 3 shows the plot of PC1 against PC2 for both the talus and tibia. Together, PC1 and PC2 explain 92.90% and 90.85% of the variance for the talus and tibia, respectively and in both analyses, Homo and Pan are clearly separated. All four trabecular parameters and cortical thickness contribute equally to PC1 in the talus, distinguishing Pan, with greater BV/TV, Tb.Th and cortical thickness, but lower DA and BS/BV, from Homo. PC2 is driven by Tb.Th and BS/BV, but only separates out particular individuals within each taxon. In the tibia, separation along PC1 is largely determined by

BV/TV, BS/BV and cortical thickness. Along PC2, most Pan individuals are distinguished from Homo in having lower Tb.Th and higher DA.

3.2 Allometry

The results of the $\log_{10}$ OLS regressions of each parameter against the geometric mean, a proxy for bone size, are shown for Pan and Homo in Table 5 and Figures 4 and 5. There were no significant correlations between any trabecular parameter and bone size. However, the relationship between size and trabecular and cortical structure does differ between species and between the talus and tibia (Figs. 4-5).

3.3 Correlation between the talus and tibia

Paired tali and tibiae were used to compare trabecular and cortical bone parameters between the talus and tibia in seven Homo and eight Pan specimens (Table 6 and Fig. 6). Within Pan, all parameters other than DA are strongly correlated across the joint (i.e. $r > 0.70$), whereas in Homo, only Tb.Th and BS/BV are strongly and significantly correlated.

3.4 Distribution of trabecular bone in the talus and distal tibia

Figure 7 shows BV/TV colour maps for the talus of one representative individual of Homo and Pan. Images of the full sample are included in the Supporting Information.

On the dorsal surface of the talus (Fig. 7 a and f), all Pan specimens share a region of high BV/TV on the lateral edge of the trochlea. In some individuals this extends posteriorly along the edge, and in others it is more anteriorly confined. Some, but not all, specimens have an additional region of higher BV/TV on the medial trochlea, which is not consistent in its location or antero-posterior extent (see Supporting Information). In Homo, there is no consistent pattern of trabecular bone distribution on the dorsal surface of the trochlea as this region is highly variable across the sample. All individuals of both Pan and Homo have a region of high BV/TV on the dorsal surface of the talar neck, although this is much more pronounced in Pan. In a transverse plane, where the superior portion of the talus has been removed (Fig. 7 b and g), there is a region of high BV/TV at the neck in Pan, although, as mentioned above, the inferior region of the neck must be interpreted with a certain degree of caution due to problems segmenting trabeculae from cortex. In Homo, there is no localised region of high BV/TV in the neck, but instead an anteroposterior trajectory of bone running through the head and neck, which is absent in Pan. The region of high BV/TV at the articular surface of the talar head (i.e. at the talonavicular joint), is more localized in Homo than in Pan. This is clearly seen in anterior view (Fig. 7 c and h), where Homo has a point of high BV/TV located dorsally on the head, in contrast to Pan, where there is a band running mediolaterally across the head. In the coronal (Fig. 7 d and i) and sagittal (Fig. 7 e and k) planes of Homo, the centre of the talar body contains a relatively higher BV/TV than in Pan. Also, in the sagittal plane (Fig. 7 e and k) there is a distinct trajectory of high BV/TV running antero-posteriorly through the talar head of Homo that is not found in Pan. Instead, the Pan neck has a region of high BV/TV on the dorsal surface. Comparison of the individual BV/TV scales shows that Pan has a higher BV/TV than Homo in both its minimum and maximum values.

Colour maps of the BV/TV distribution in the distal tibia of Homo and Pan are shown in Figure 8 and results for the entire sample are included in the Supporting Information. On the distal articular surface of the tibia (Fig. 8a and e), some specimens of Homo have a high concentration of BV/TV confined to the medial side of the articular surface and in other individuals it is centrally located. This is in contrast to Pan, where there are consistently three regions of higher BV/TV: anterolateral, anteromedial and posterocentral. When viewed in the mid-sagittal plane of the distal tibia (Fig. 8 b and f), the anteromedial and posterior concentrations of bone are visible in Pan, in contrast to the more central and continuous area of high BV/TV in Homo. On the anterior edge of the distal tibia (Fig. 8 c and g), Pan has a high concentration of bone extending across the edge that is absent in Homo. In the mid-coronal plane (Fig. 8 d and h), Pan contains a relatively greater BV/TV in the centre of the medial malleolus, compared to Homo. Unlike the talus, the range of BV/TV is more similar between the two species (Fig 7 and Fig 8, scale bars).

3.5 Distribution of cortical bone in the talus and distal tibia

Mean relative cortical thickness maps for the talus and distal tibia of Pan and Homo, along with regions of significant differences, are shown in Figures 9 and 10. In contrast to the trabecular bone maps, these figures do not show the cortical thickness in just one individual, but rather the mean of all individuals by taxon. As Pan has a greater cortical thickness in both the talus and the distal tibia, results are presented for relative cortical thickness values, equalized by subtracting the mean value from each cortical thickness value and dividing by the standard deviation for every individual in the sample.

Visual comparison between the relative cortical thickness maps of the talus in Homo (Fig. 9a) and Pan (Fig. 9b), show that the regions of thickest cortical bone differ between the two species. On the talar head, Homo has a dorsally located region of highest relative thickness, whereas in Pan the region of high thickness runs mediolaterally along the dorsal half of the articular surface. At the trochlea, Pan has a higher cortical thickness on the lateral edge, whereas in Homo it is the centromedial region that has the highest mean thickness. Pan and Homo share thick cortical bone around the region of the talar neck, however, in Pan this extends around the entire dorsal region of the neck, whereas in Homo it is confined to the dorso-lateral side. In Homo the centre of the posterior subtalar articular surface has the thickest cortical bone, whereas in Pan the cortical bone is thickest anterolaterally on this articular surface. Differences between Pan and Homo are shown in Figure 9c, and regions where these differences reach significance are shown in Figure 9d. There are several regions with significant differences located at the articular surfaces of the talus. Pan has relatively thinner bone compared to Homo on the anterior surface of the talar head, on the anteromedial region of the talar trochlea and on the dorsal edge of the talar head, and relatively thicker bone compared to Homo in a band anterolaterally on the posterior subtalar articular surface.

Cortical thickness maps, showing relative cortical thickness are shown for Homo and Pan in Figure 10a and b, respectively. In distal view, Homo has thickest cortical bone the along the medial edge of the distal articular surface and the distal end of the medial malleolus. Both taxa share regions of thicker cortical bone on the distal end of the medial malleolus and the medial edge of the distal articular surface. This region on the medial articular surface is relatively thicker anteriorly in Pan, whereas in Homo this feature extends along the medial border of the articular surface. Pan has two additional regions of thicker cortical bone on the anterolateral and posterocentral regions of the distal articular surface. Comparisons of relative cortical thickness values between Homo and Pan are shown in Figure 10c and regions with significant differences are shown in Figure 10d. At the distal articular surfaces, Pan has significantly thicker cortex at the anteromedial corner, extending along the anteromedial edge of the medial malleolus. There is significantly thicker cortical bone on the distal surface of the medial malleolus in Pan compared to Homo.

4 DISCUSSION

We analysed the internal bone structure of the talus and distal tibia in bipedal Homo and arboreal, quadrupedal Pan. We find that trabecular and cortical bone, both the measured parameters and the regional distribution of bone, differed, often significantly, between the two taxa in ways that are potentially related to variation in joint position and load distribution during locomotion. In addition to these differences, we find further support for previously proposed systemically weaker trabecular and cortical bone in recent humans (Ruff et al., 1993; Lieberman, 1996; Ruff, 2005; Chirchir et al., 2015; Ryan and Shaw, 2015; Scherf et al., 2015; Chichir et al., 2017).

4.1 Identifying functional signals in internal bone structure

The relationship between bone form and mechanical loading is complex. It may be influenced by numerous factors that affect bone growth and structure, which are likely to differ systematically between species and, as such, bone structure should be considered within the broader context of what is already known about the bone architecture of each species. In both the talus and distal tibia of Homo, we find support for our prediction that bone is relatively weak, having a lower BV/TV, a higher BS/BV and thinner cortices, compared with the more robust Pan. BV/TV is the strongest predictor of trabecular bone stiffness, or Young’s modulus; it alone explains 87-89% of variance in stiffness (Stauber et al., 2006; Maquer et al., 2015). Cortical bone thickness is also related to bone strength, as thin cortices are associated with increased fracture risk (Augat and Schorlemmer, 2006). The difference in trabecular

BV/TV and cortical thickness between Pan and Homo is consistent with previous findings for the talus and distal tibia (talus: Su, 2011; DeSilva and Devlin, 2012; Su and Carlson, 2017; tibia: Su, 2011; Barak et al., 2013b), and with the trabecular morphology of other anatomical regions (e.g. third metacarpal: Tsegai et al., 2013; calcaneus: Maga et al., 2006; Zeininger et al., 2016; first and second metatarsal: Griffin et al., 2010; systemic: Chirchir et al., 2015). As the biomechanical environment of different joints in the human and chimpanzee are likely to vary given their divergent modes of locomotion, this consistent difference across several anatomical sites may be part of a systemic pattern (i.e. in all regions of the skeleton) and not due to specific locomotor, or other, behaviour. This gracility of the modern human skeleton may be associated with increased sedentism following the adoption of agriculture, as early hominins and recent hunter gatherers/foragers have a more robust skeleton (Ruff et al., 1993; Lieberman, 1996; Ruff, 2005; Chirchir et al., 2015; Ryan and Shaw, 2015; Scherf et al., 2015). Analysis of the relationship between these structural parameters and size are limited by small sample sizes.

There are aspects of bone structure that appear likely to reflect joint function and thus can be of use for reconstructing behaviour in the fossil record. Here, we find support for our prediction that the human talus has a significantly higher DA than in Pan. However, contrary to our predictions, we find no significant difference for the distal tibia. During human bipedalism the mid-foot forms a relatively rigid lever during push off (Morris, 1977), compared with the flexibility of the chimpanzee mid-foot (Elftman and Manter, 1935; Susman, 1983; Thompson et al., 2014; but see Holowka et al., 2017). There is also less mobility at the ankle of Homo than in Pan (Latimer et al., 1987). The less aligned trabeculae of the Pan talus are consistent with being more able to withstand forces from multiple directions associated with a wider range of joint positions, whereas the more highly aligned trabecular structure of the Homo talus appears to reflect more stereotypical loading (Su, 2011; DeSilva and Devlin, 2012; Su et al., 2013; Su and Carlson, 2017). In contrast to previous studies (Su, 2011; Barak et al., 2013b), we do not find a higher DA in the distal tibia of Homo, but rather higher (although not significantly so) mean DA in Pan. However, Su (2011) found that trabeculae in Homo were significantly more uniformly aligned in the talus compared with the tibia, suggesting that more similar DA values in the Homo and Pan distal tibia are not unexpected.

DA may hold a functional signal for different types of behaviour that engender more or less stereotypical loads at a joint. Regional differences in DA have been useful in distinguishing between primate locomotor groups, with the structure of the proximal femur being consistent with inferred differences in loading in leaping and slow climbing strepsirrhines (Ryan and Ketcham, 2002a,b; MacLatchy and Muller, 2002; Ketcham and Ryan, 2004). The trabecular structure of the human foot is generally more highly aligned than other apes (first and second metatarsal: Griffin et al., 2010; calcaneus: Maga et al., 2006; Zeininger et al., 2016; but see Kuo et al., 2013; talus: Su, 2011; Su et al., 2013; Su and Carlson, 2017). It seems unlikely that this would relate to differences in activity level between the taxa, and there are no consistent differences in DA in the proximal femur (Ryan and Shaw, 2015) or humerus (Scherf et al., 2015) between human populations with different activity levels (i.e. engaging in the same behaviours but at different frequencies). Adult trabecular structure could reflect individual or interspecific differences in loading during puberty, at a time when bone is more responsive to strain (e.g. Pettersson et al. 2010; for cortical bone see Pearson and Lieberman, 2004). However, homologous regions of trabecular bone in adolescent and adult humans have not been sampled, as many studies exploring ontogeny have investigated changes in structure between non-adult groups (Ryan and Krovitz, 2006; Ryan et al., 2007; Gosman and Ketcham, 2009; Raichlen et al., 2015). DA in the proximal tibial metaphysis and in the ilium continue to change between adolescence and adulthood (Gosman & Ketcham, 2009; Abel & Macho, 2011). Moreover, chimpanzees reach adult-like locomotor behaviour by adolescence (Doran, 1992; Sarringhaus et al., 2014), while humans reach this point during early childhood (e.g. Sutherland et al., 1980; Beck et al., 1981; Raichlen et al., 2015). Trabecular orientation in the talus also shows plasticity later in life, as degeneration of articular cartilage, i.e. changes at the joint surface that affect loading, is associated with differences in trabecular orientation in humans (Schiff et al., 2007). This indicates that DA in adult humans and chimpanzees is likely to reflect adult behaviour patterns, as loading from locomotion has remained generally consistent during much of the later growth period. Together these results suggest that the high degree of trabecular alignment throughout several elements of the human foot may be a behavioural signal related to the stereotypical loading of terrestrial bipedality. We suggest that, using our methodology, DA may provide functional information about loading in the talus, but not the tibia.

4.2 The relationship between joint position and bone distribution

We predicted that differences in the cortical and trabecular bone distribution maps would reflect variation in dorsiflexion and inversion of the talocrural joint and the degree of mobility at the talonavicular joint. The colour maps of cortical and trabecular bone support some, but not all, of these predictions. These results are based on mean cortical thickness distribution maps and significant differences, and on BV/TV distribution maps for each individual. Generation of mean morphometric maps for BV/TV was not conducted due to the complexity of registering 3D meshes while ensuring homology.

4.2.1 Dorsiflexion

Dorsiflexion at the ankle is characteristic of both climbing and knuckle-walking in chimpanzees compared to the more neutral ankle posture adopted by humans during bipedalism. We find no clear signal of dorsiflexion in trabecular and cortical bone of the talar trochlea, but are able to identify differences in internal bone structure of the distal tibia that we propose are related to degree of dorsiflexion. In chimpanzees, during knuckle-walking the angle between the long axis of the tibia and the foot is 75.2 degrees, compared with 85.6 degrees in normal human bipedalism (Barak et al., 2013b). During vertical climbing the degree of dorsiflexion is much greater, with an angle between the long axis of the tibia and the foot of 44.5 degrees (DeSilva, 2009). The external morphology of the talar trochlea and the distal articular surface of the tibia is associated with this difference in loading of the ankle (DeSilva, 2009; but see Venkataraman et al., 2013a). It might be expected that the distribution of trabecular bone and cortical bone in the talar trochlea of Pan would be more anteriorly distributed, reflecting this difference in joint angle. However, we find no clear signal across the study sample in either the trabecular or cortical bone distribution maps. This is consistent with previous studies that did not identify differences in BV/TV across quadrants of the talar body (DeSilva and Devlin, 2012), or higher BV/TV and cortical thickness in the anterior talar trochlea (Su, 2011; Su and Carlson, 2017).

In contrast to the talus, we did find that the trabecular and cortical bone structure of the distal tibia reflected the differences in joint position between Homo and Pan. Pan shows two regions of higher BV/TV and thicker cortical bone, located at the anterior portion of the distal articular surface of the tibia, one lateral and one medial. In addition, the anterior edge of the distal articular surface has a higher BV/TV, which extends up anteriorly through the epiphysis. This is in contrast to Homo, where BV/TV maps show a more central concentration of trabecular bone. In Homo, the cortex is thickest on the medial edge of the articular surface, adjacent to the medial malleolus. In several (but not all) individuals in the study sample (see Supporting Information), this medial region also has a high BV/TV. Although direct comparison between results from different subregions is complex, some of these findings are supported by the results of Su (2011). Fewer significant differences in BV/TV and cortical thickness are found across the Homo tibia compared to Pan, and Pan has generally higher BV/TV anteriorly and posteriorly. This is not the case for cortical thickness, where both Homo and Pan have thicker bone on the antero- and postero- medial regions, and in Pan, the posterocentral region of the articular surface (Su, 2011). Perhaps also relevant to the degree of flexion at the ankle, there is a region of high BV/TV and cortical thickness posterocentrally on the distal articular surface in Pan, with the region of high BV/TV extending into the bone. This could indicate increased loading during plantarflexion in Pan compared to Homo, however, this is not supported by kinematic data. Previous findings in the distal tibia of Pan also found that the posterior region has a higher BV/TV than the central region, and thicker cortical bone was found in the posterocentral region (Su, 2011; Su and Carlson, 2017).

In the absence of detailed kinematic data on joint contact areas, in particular for Pan (for humans see Wan et al., 2006; Bae et al., 2015), our understanding of the differences in the loading of the trochlea in these two species is limited. Moreover, we must make assumptions about which aspects of a species' locomotor, or other, behaviour contribute most to the remodelling of bone. Previous studies in humans have identified areas of contact and distribution of pressure on the talus using a finite element simulation of the human foot during walking (Bae et al., 2015) and on both the talar trochlear and distal articular surface of the tibia under pressure using dual orthogonal fluoroscopy (Wan et al., 2006; Caputo et al., 2009; Bischof et al., 2010). During human bipedalism, ground reaction forces (GRF) peak at two phases, first after heelstrike and before midstance, and second at toe off (Bae et al., 2015; Alexander, 2004), with contact pressure and strain increasing throughout the stride, peaking at toe off (Bae et al., 2015). After heelstrike, during the first peak in GRF, there is contact between the cartilage of the talus and tibia on the latero-central trochlea (Wan et al., 2006; Bae et al., 2015). During stride, the area of contact moves anteriorly (Wan et al., 2006; Bae et al., 2015) and the point of highest pressure moves anterocentrally until toe off, when both the contact area and point of highest pressure are located on the anterior of the trochlea, just lateral to the midline (Bae et al., 2015). At the distal tibia, contact is located antero-posteriorly at heel strike, moving anteriorly across the medio-lateral extent of the articular surface at mid-stance, and at heel strike in the anterolateral half of the distal articular surface of the tibia (Wan et al., 2006). Although some of the human sample in this study have a region of high BV/TV on the anterior talus, just lateral to the midline, near the location of highest pressure (Bae et al., 2015), this is not always the region of highest BV/TV, and does vary within the sample. There is also no direct correspondence between regions of contact and areas with thicker cortices. There are several potential explanations for why the trabecular and cortical bone structure of the talar trochlea does not, as expected, reflect differences in dorsiflexion at the ankle. Firstly, experimental measures of cartilage contact and pressure may not necessarily correspond to the regions experiencing the greatest forces during life. Secondly, modern humans differ greatly in their gait. For example, there is inter-individual variation in the presence of a mid-tarsal break, and intra-individual variation between strides (Bates et al., 2013; DeSilva et al., 2015). There is also variability in foot strike patterns, with individuals making initial contact with the fore-foot, midfoot or heel, that could also contribute to variability in loading of the trochlea (e.g. during running: Lieberman et al., 2010; Hatala et al., 2013). Thirdly, differences in the external morphology of the talus may accommodate the different distribution of forces, i.e. different shaped tali absorb loads differently, thus cortical thickness and trabecular architecture do not directly reflect differences in joint position.

Due to interest in adaptations of the human skeleton to bipedal locomotion, many biomechanical analyses of Pan have focused on bipedal walking (e.g. Susman, 1983; Thorpe et al., 2004; Wang et al., 2014; O'Neill et al., 2015), although several studies have investigated kinematics of knuckle-walking in bonobos (e.g. Vereecke et al., 2003; D'Août et al., 2004; Schoonaert et al., 2016). Although no in vivo measurements of joint movement or cartilage contact are available for Pan, there is evidence of force transmission due to contact between the anterior edge of the distal tibia and the neck of the talus. This can be observed when manipulating dry, associated tibia and tali, where in an extreme position of dorsiflexion the ankle joint retains congruity while there is contact between the talar neck and the anterior border of the tibia in African apes, but not in Homo (Latimer et al., 1987). Modern humans who regularly adopt crouched positions develop squatting faces on the talus and tibia (Boulle, 2001). The BV/TV distribution may reflect this and indicate high loads transmitted through this region. On the medial and lateral side of the talar neck and on the anteroinferior border of the tibia, Pan has regions of high BV/TV, which are absent in Homo. This may reflect habitual loading of these regions in an ankle dorsiflexed to such a degree that force transmission occurs between the antero-inferior edge of the distal tibia and the talar neck.

4.2.2 Talonavicular mobility

We find a clear signal of differences in joint mobility at the talonavicular joint in the trabecular and cortical bone structure. Two features in which human bipedalism is distinct from ape quadrupedalism are, firstly, weight transfer from the lateral to medial side of the foot during midstance; and secondly, in having a rigid mid-foot, so that the foot acts as a lever during toe off (Elftman and Manter, 1935). The medial side of the midtarsal joint (the talonavicular joint) is more mobile than the lateral side (calcaneocuboid and cuboid-MT5 joints), during stance phase the talus rotates, along with the leg and calcaneus, creating a close packed talonavicular joint (Elftman, 1960; Siegler et al., 1988; Scott and

Winter, 1991). Although investigations of mid-foot mobility in Pan have largely focused on the mid-tarsal break at the lateral side (DeSilva, 2010), there is greater movement at the talonavicular joint which, during passive dorsiflexion of the foot, is characterised by rotation in the coronal plane (Thompson et al., 2014). Furthermore, there is greater inter-individual and intra-individual variability in mobility of the human lateral midfoot than was previously assumed (Elftman and Manter, 1935; Bates et al., 2013). During bipedalism, humans have greater midfoot mobility during push off, which is characterised by plantarflexion and adduction, whereas chimpanzees have higher dorsiflexion at the midfoot (mid-tarsal break) during the single limb support period (Holowka et al., 2017). Contrary to expectations, the human midfoot was found to be overall more mobile than that of chimpanzees (Holowka et al., 2017), however, precise kinematics of the talonavicular joint remain unknown.

There are clear differences between the study taxa in the trabecular bone distribution at the talar head, where Pan has a band of high BV/TV running mediolaterally across the talar head, and in Homo there is a localised point of high BV/TV. In cortical thickness, Pan has relatively thinner cortices at the talar head, which is significantly thinner in the central region. Previous studies have measured both trabecular bone in the medial and lateral sides of the head (DeSilva and Devlin, 2012) and trabecular bone adjacent to the neck of the talus (i.e. on the anteromedial region of the talar trochlea). When comparing the medial and lateral side of the head of the talus in humans to other species, DeSilva and Devlin (2012) found no significant difference in DA, although the trabeculae were significantly thicker in the lateral head and significantly more connected in the medial head of humans compared to other species (DeSilva and Devlin, 2012). In the anteromedial trochlea, humans have a unique orientation of trabeculae compared to other great apes, in having trabeculae with a primarily anteroinferior orientation, i.e. parallel to the talar neck; a pattern shared with an early Pleistocene biped, KNM-ER 1464 (Su, 2011; Su et al., 2013; Su and Carlson, 2017). This distinct orientation of trabeculae in bipedal species noted by Su et al. (2013) may correspond to the trajectory of bone that we show here, travelling through the talar head into the trochlea.

The trabecular and cortical distribution of the talar head reveals a clear difference in bone structure, perhaps related to differences in midfoot mobility between the study species.

4.2.3 Inversion

As well as dorsiflexion, inversion of the foot is characteristic of arboreal behaviour in Pan, including vertical climbing (DeSilva, 2009). Species that engage in more arboreal locomotion have a less symmetrical trochlea surface, where the lateral trochlea ridge is higher than the medial. This asymmetry increases the difference in the radius of curvature of the medial and lateral side, thereby increasing the arcuate path of the tibia over the talus (Latimer et al., 1987), a difference that has even been identified between more arboreal western and more terrestrial eastern gorillas (Dunn et al., 2014). Of potential interest with regard to identifying signals of inversion, is the high BV/TV on the anterolateral lip of the trochlea of the talus that is consistent throughout the sample of Pan. This region also has a slightly thicker cortex in Pan than in Homo, with Pan having relatively thinner cortical bone than Homo on the anteromedial region of the trochlea. This is consistent with previous findings of high BV/TV, but not thicker cortices, on the anterolateral two thirds of the trochlea in Pan (Su, 2011; Su and Carlson, 2017). This may reflect increased shearing stresses associated with adoption of inverted foot postures, which are also mitigated by having a higher lateral ridge of the talus. More detailed understanding of the kinematics of climbing and knuckle-walking, along with modelling of the forces experienced by the talus, may improve interpretation of this signal.

5 CONCLUSION

Identifying those features of internal bone structure that are directly related to joint loading is often problematic. Here, we find that average architectural variables (BV/TV, BS/BV and cortical thickness) that relate to overall bone strength differ between Pan and Homo. These may be part of a systemic pattern unrelated to joint function, but rather due to other factors such as overall activity levels, and therefore may not be relevant for reconstructing loading of individual joints. However, the degree to which trabeculae are uniformly oriented (DA) in the talus does correspond to variation in joint loading due to different locomotor behaviours, clearly differentiating between the more stereotypical loading regime of bipedalism in Homo and the greater range of motion and joint loading typical of arboreal behaviours in Pan. In contrast to these architectural variables quantified throughout the epiphysis/bone, more precise information about locomotor behaviour can be obtained from patterns of trabecular and cortical bone distribution. The trabecular and cortical bone distribution of the distal tibia and talus reflect differences in dorsiflexion at the ankle and range of motion at the talonavicular joint in humans and chimpanzees. Thus, the distribution of both trabecular and cortical bone in the talus and distal tibia holds potential for interpreting loading regimes and reconstructing loaded joint positions in fossil specimens.

ACKNOWLEDGEMENTS

This research was supported by The Max Planck Society (ZJT, TLK, MMS and JJH) and the European Research Council Starting Grant #336301 (TLK and MMS). We thank Christophe Boesch (Max Planck Institute for Evolutionary Anthropology) and Birgit Grosskopf (University of Göttingen) for access to specimens in their care. For assistance with CT scanning we thank David Plotzki, Heiko Temming and Patrick Schönfeld. Helpful discussions with Nicholas Stephens and comments from two anonymous reviewers greatly improved this manuscript.

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Table 1. Study sample

Taxon	Body mass (kg)[3]	Locomotor behaviour	Tibia	Talus	Paired	Scan resolution (µm)	Relative resolution[4]
Homo sapiens[1]	62.1-72.1	Biped	8	9	7	40	5.72-9.06
Pan troglodytes verus[2]	41.6-46.3	Arboreal/knuckle-walker	10	13	8	35	5.46-11.59

[1] Anthropological Collection of Institute of Zoology and Anthropology, University of Göttingen

[2] Max Planck Institute for Evolutionary Anthropology

[3] Sex specific mean body mass (F-M). Body masses from Smith and Jungers (1997)

[4] Relative resolution = mean trabecular thickness (mm)/resolution(mm)

Table 2. Mean and standard deviation of trabecular and cortical parameters in the talus and distal tibia of Homo and Pan. Results of Mann-Whitney U test between taxa are shown, with significant differences in bold.

Element	Taxon	Tb.Th (mm)	BV.TV (%)	DA	BS/BV(mm^{-1})	Cortical thickness (mm)
Talus	Homo	0.26 (0.03)	24.77 (2.17)	0.14 (0.07)	0.32 (0.05)	0.45 (0.06)
	Pan	0.31 (0.04)	34.65 (2.63)	0.02 (0.02)	0.19 (0.02)	0.88 (0.19)
	Significance	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Tibia	Homo	0.25 (0.04)	19.92 (2.87)	0.29 (0.10)	0.45 (0.08)	0.63 (0.07)
	Pan	0.23 (0.02)	24.17 (3.43)	0.32 (0.06)	0.31 (0.06)	1.13 (0.19)
	Significance	0.17	**0.02**	0.51	**<0.01**	**<0.01**

Table 3. Results of Spearman's correlation test to test relationship between trabecular parameters within Homo and Pan in the talus and distal tibia.

Element	Taxon	Parameter	Tb.Th	BV/TV	BS/BV	DA
Talus	Homo	BV/TV	0.42	**-**		
		BS/BV	-0.18	**-0.92****	**-**	
		DA	**-0.82****	-0.45	0.28	**-**
		CTh	0.57	0.50	-0.30	**-0.72***
	Pan	BV/TV	**0.59***	**-**		
		BS/BV	-0.10	**-0.80****	**-**	
		DA	**-0.98****	**-0.66****	0.16	**-**
		CTh	**0.84****	**0.63***	-0.24	**-0.80****
Tibia	Homo	BV/TV	0.71	**-**		
		BS/BV	**-0.83****	**-0.90****	**-**	
		DA	**-0.74***	-0.50	0.69	**-**
		CTh	0.31	0.07	-0.07	-0.02
	Pan	BV/TV	**0.75***	**-**		
		BS/BV	**-0.67***	**-0.95****	**-**	
		DA	**-0.71***	-0.62	0.41	**-**
		CTh	**0.82****	**0.65***	**-0.66***	-0.44

P-values indicated as $p < 0.05$ * and $p < 0.01$ **

Table 4. Results of principal component analyses showing percentage variance and loading for each principal component. The analysis was conducted separately for the talus and tibia, including Tb.Th, BV/TV, DA, BS/BV, and cortical thickness.

Element	Parameter	PC1	PC2	PC3	PC4	PC5
Talus	% variance	82.64	10.27	4.35	2.43	0.31
	(cumulative)	(82.64)	(92.90)	(97.26)	(99.69)	(100.00)
	Tb.Th	**0.41**	**0.70**	0.30	**0.50**	-0.08
	BV/TV	**0.48**	-0.28	0.09	0.08	**0.82**
	DA	**-0.44**	-0.21	**0.85**	0.16	0.07
	BS/BV	**-0.43**	**0.62**	-0.05	**-0.40**	**0.51**
	Cortical thickness	**0.46**	0.05	**0.41**	**-0.75**	-0.22
Tibia	% variance	56.45	34.41	5.41	2.88	0.86
	(cumulative)	(56.45)	(90.85)	(96.26)	(99.14)	(100.00)
	Tb.Th	0.27	**-0.65**	0.03	**-0.71**	-0.06
	BV/TV	**0.57**	0.13	**0.42**	0.06	**0.69**
	DA	-0.30	**0.62**	0.29	-0.67	0.02
	BS/BV	**-0.55**	-0.22	-0.36	-0.09	**0.72**
	Cortical thickness	**0.46**	0.37	**-0.78**	-0.20	**0**.05

High loadings (i.e. greater than 0.40) are shown in bold

Table 5. The relationship between bone structure and bone size in Homo and Pan. Results of OLS regression and Pearson's correlation for each trabecular parameter and cortical thickness against the geometric mean of several measurements, used as a proxy for bone size.

Taxon	Element	Parameter	Pearson's r	Slope	Lower 95% CI	Upper 95% CI	P-value	R^2
Homo	Talus	Tb.Th	-0.40	-0.51	-1.58	0.57	0.30	0.15
		BV/TV	-0.48	-0.52	-1.37	0.34	0.20	0.23
		DA	0.10	2.01	-4.28	8.29	0.48	0.08
		BS/BV	0.33	0.59	-1.23	2.41	0.47	0.08
		CTh	0.12	0.23	-1.23	1.70	0.72	0.02
	Tibia	Tb.Th	0.27	0.53	-1.47	2.53	0.54	0.07
		BV/TV	0.55	1.11	-0.57	2.80	0.16	0.30
		DA	0.09	-0.05	-6.71	6.61	0.99	0.00
		BS/BV	-0.51	-1.35	-3.56	0.87	0.19	0.27
		CTh	0.25	0.38	-1.19	1.95	0.57	0.06
Pan	Talus	Tb.Th	0.29	0.55	-0.79	1.89	0.39	0.07
		BV/TV	-0.05	-0.06	-0.88	0.76	0.87	0.00
		DA	-0.11	-2.97	-11.57	5.62	0.46	0.05
		BS/BV	0.12	0.25	-1.15	1.65	0.70	0.01
		CTh	0.19	0.60	-1.55	2.75	0.55	0.03
	Tibia	Tb.Th	0.37	0.74	-0.64	2.11	0.25	0.16
		BV/TV	0.05	0.16	-1.70	2.03	0.84	0.01
		DA	-0.35	-1.22	-3.98	1.54	0.34	0.11
		BS/BV	-0.04	-0.04	-3.36	3.27	0.98	0.00
		CTh	0.28	0.80	-1.32	2.93	0.41	0.09

Table 6. Results of Pearson's correlation test to test relationship of each trabecular parameter and cortical thickness between the talus and distal tibia in Homo and Pan.

Taxa	Parameter	Pearson's r	P-value
Homo	Tb.Th	0.83	**0.02**
	BV/TV	0.72	0.07
	DA	0.55	0.20
	BS/BV	0.83	**0.02**
	Cortical thickness	0.43	0.33
Pan	Tb.Th	0.86	**0.01**
	BV/TV	0.80	**0.02**
	DA	0.56	0.15
	BS/BV	0.81	**0.02**
	Cortical thickness	0.92	**<0.01**

Significant correlations are shown in bold ($p < 0.05$)

Fig 1. Processing steps for trabecular and cortical bone analysis for a Pan distal tibia. a) Segmented microCT scan. b) Segmented trabecular bone. c) Inner mask, where trabecular bone and internal region of the bone are assigned different grey values, and the cortical bone has been removed. A background grid and sampling sphere are applied to calculate trabecular structure throughout the bone. d) Tetrahedral mesh with colour scalars representing trabecular bone volume fraction. e) Unsegmented voxel data. f) Process of measurement of cortical thickness. g) Cortical thickness values mapped to a subject-specific surface. h) Each subject-specific surface (green) is registered to a canonical surface (red) for interspecific comparisons.

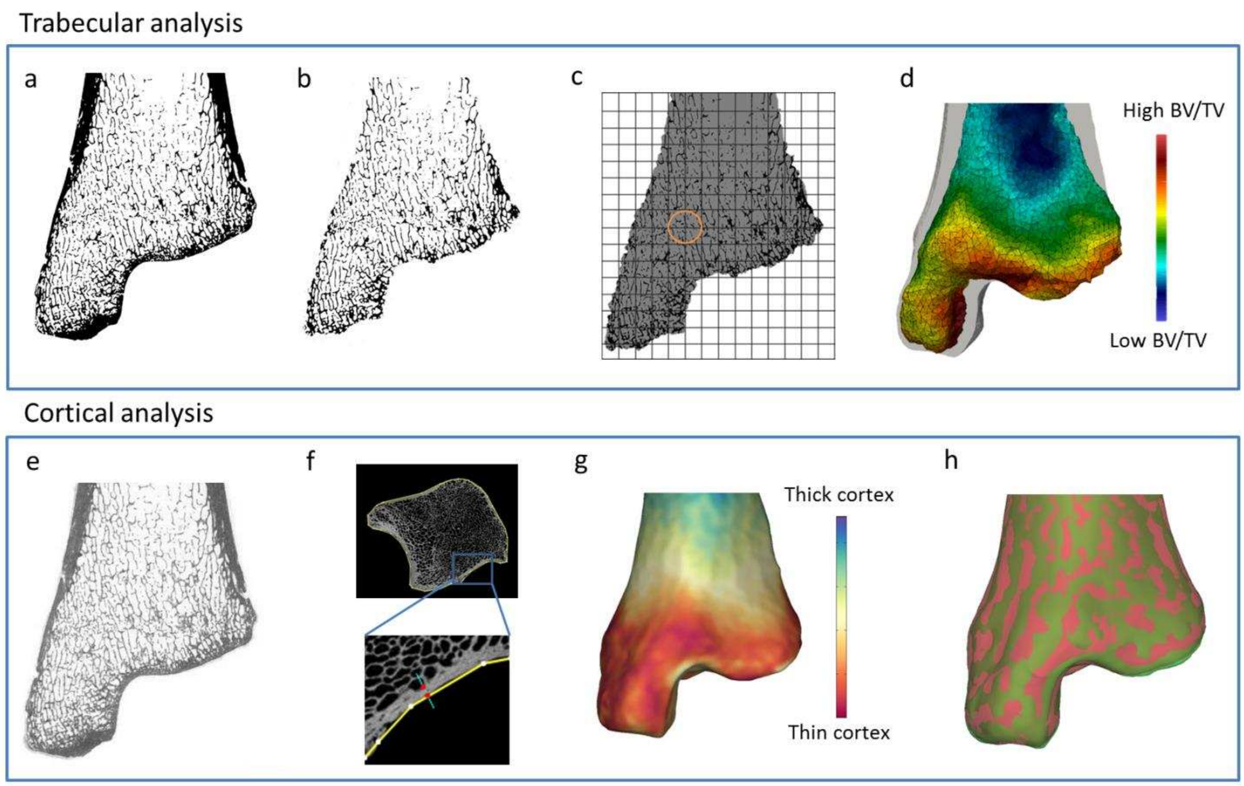

Fig 2. Extracted cubes of trabecular bone from approximately the same location in the talus and distal tibia of Homo and Pan.

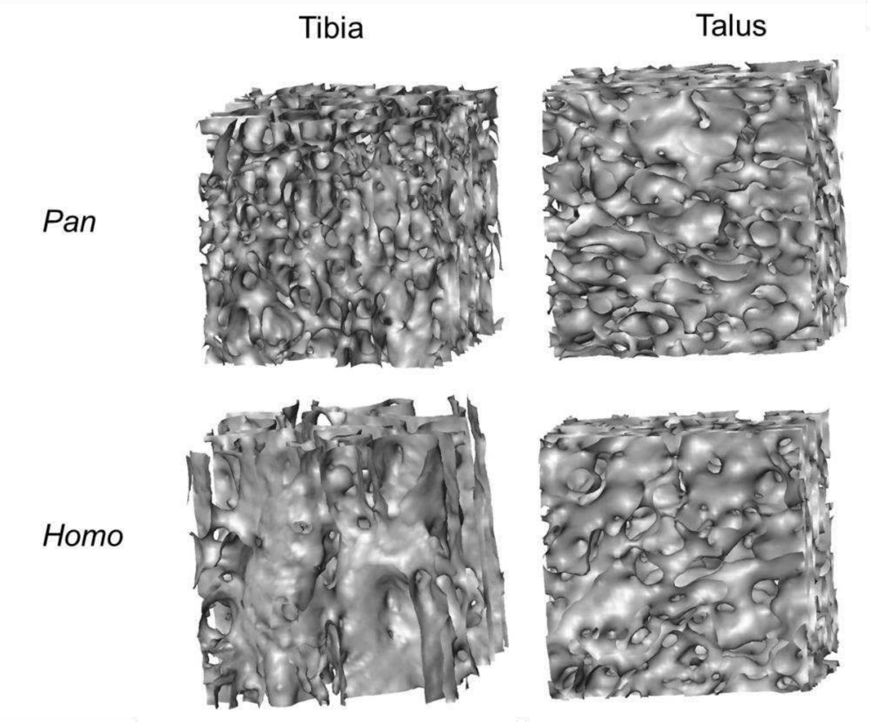

Fig 3. PC1 and PC2 for trabecular and cortical structure of the talus and distal tibia of Pan (blue) and Homo (red).

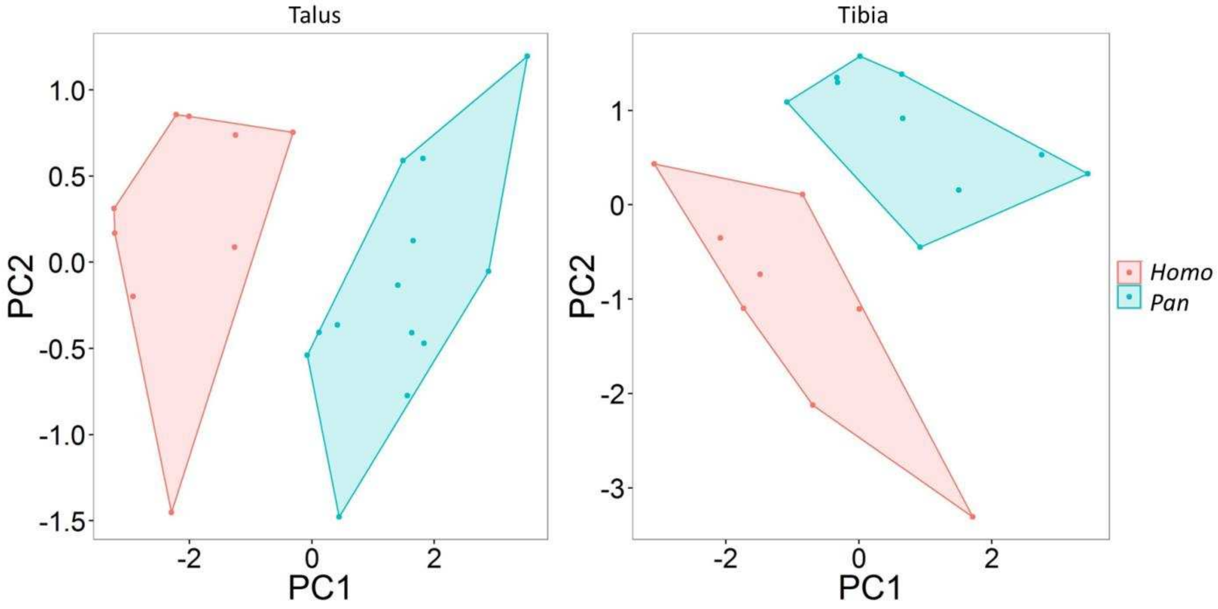

Fig 4. Relationship between talus size and trabecular and cortical parameters in Pan (blue) and Homo (red). The $\log_{10}$ OLS regression lines are shown independently for Pan (blue) and Homo (red).

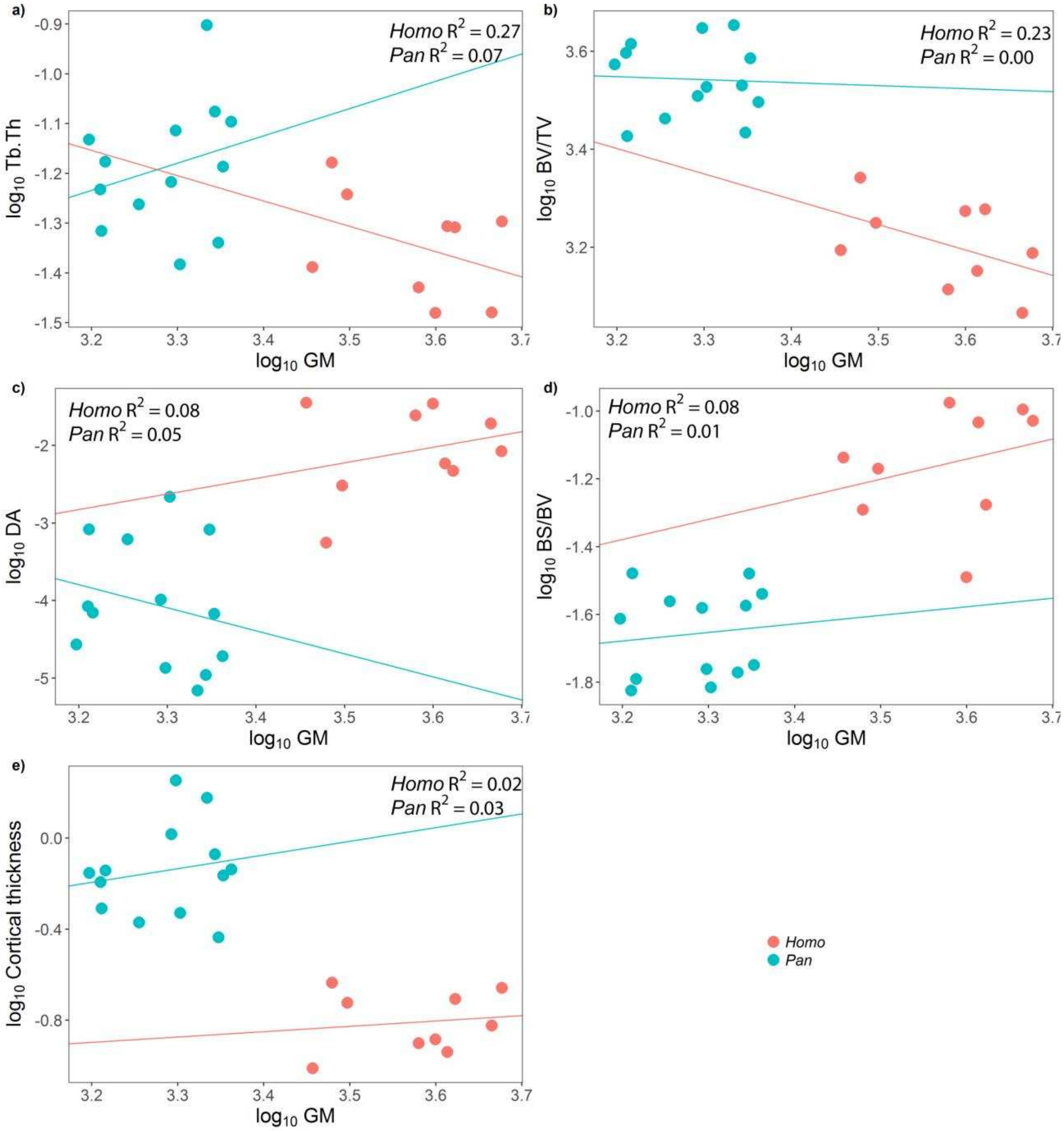

Fig 5. Relationship between tibia size and trabecular and cortical parameters in Pan (blue) and Homo (red). The $\log_{10}$ OLS regression lines are shown independently for Pan (blue) and Homo (red).

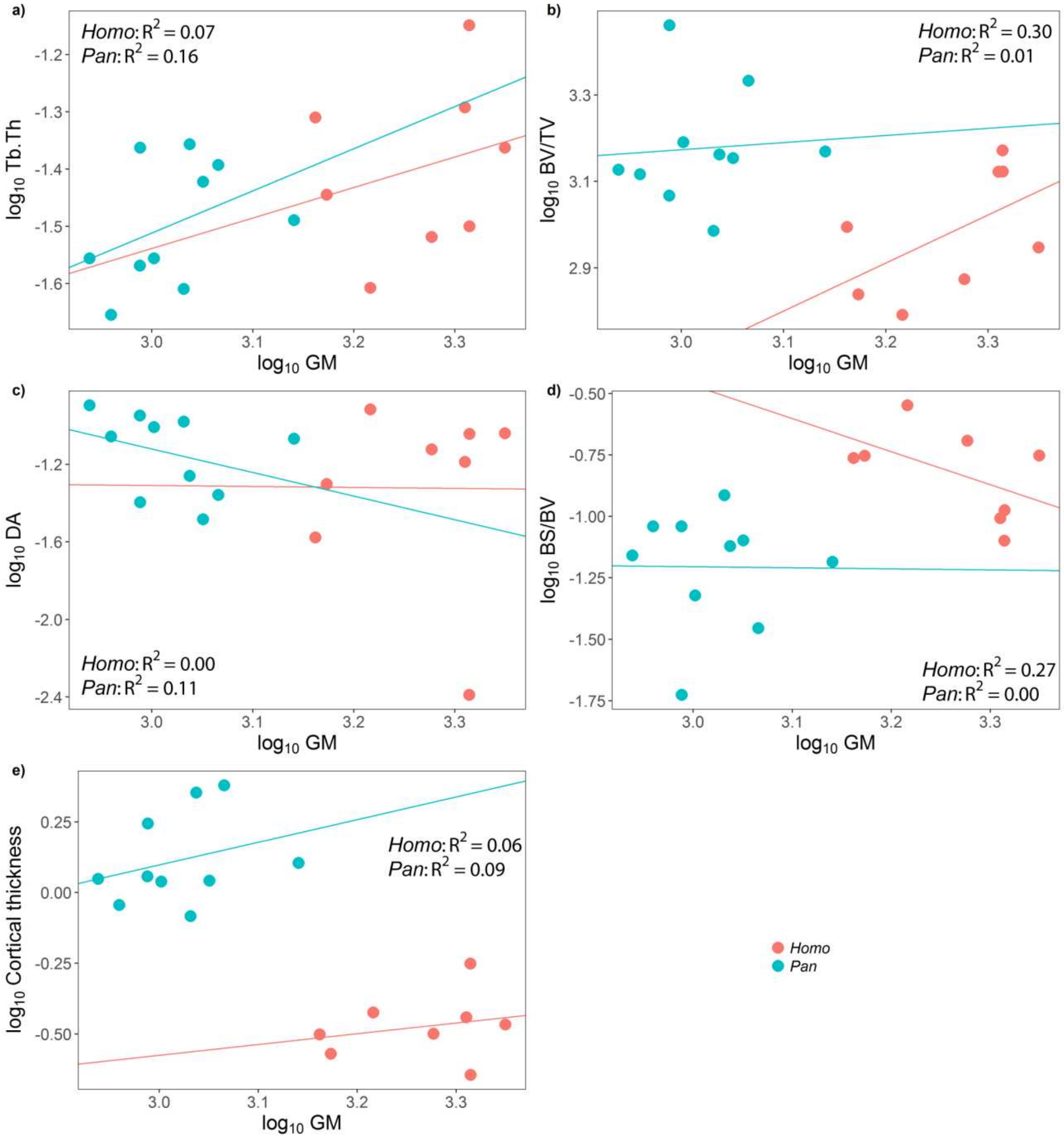

Fig 6. Comparison of trabecular and cortical structure between the talus and tibia in Pan (blue) and Homo (red). The $\log_{10}$ RMA regression lines are shown independently for Pan (blue) and Homo (red).

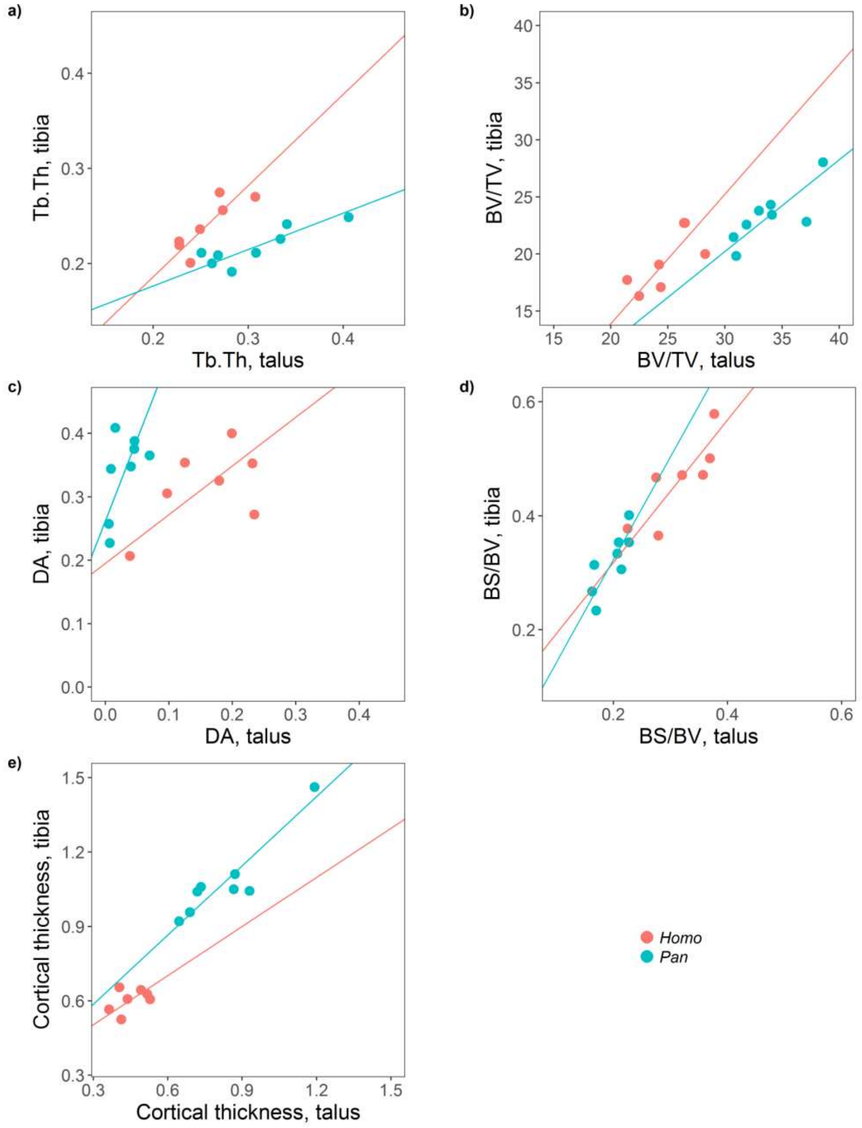

Fig 7. Morphometric maps of BV/TV in the talus in one individual of Homo (a-e) and Pan (f-j) in (from top to bottom) dorsal view, mid-transverse plane, anterior view, coronal plane (in the centre of the trochlea), and sagittal plane (in the centre of the trochlea). Each specimen is scaled to its own data range, as shown in the scale bars. Black arrows indicate regions described in the text.

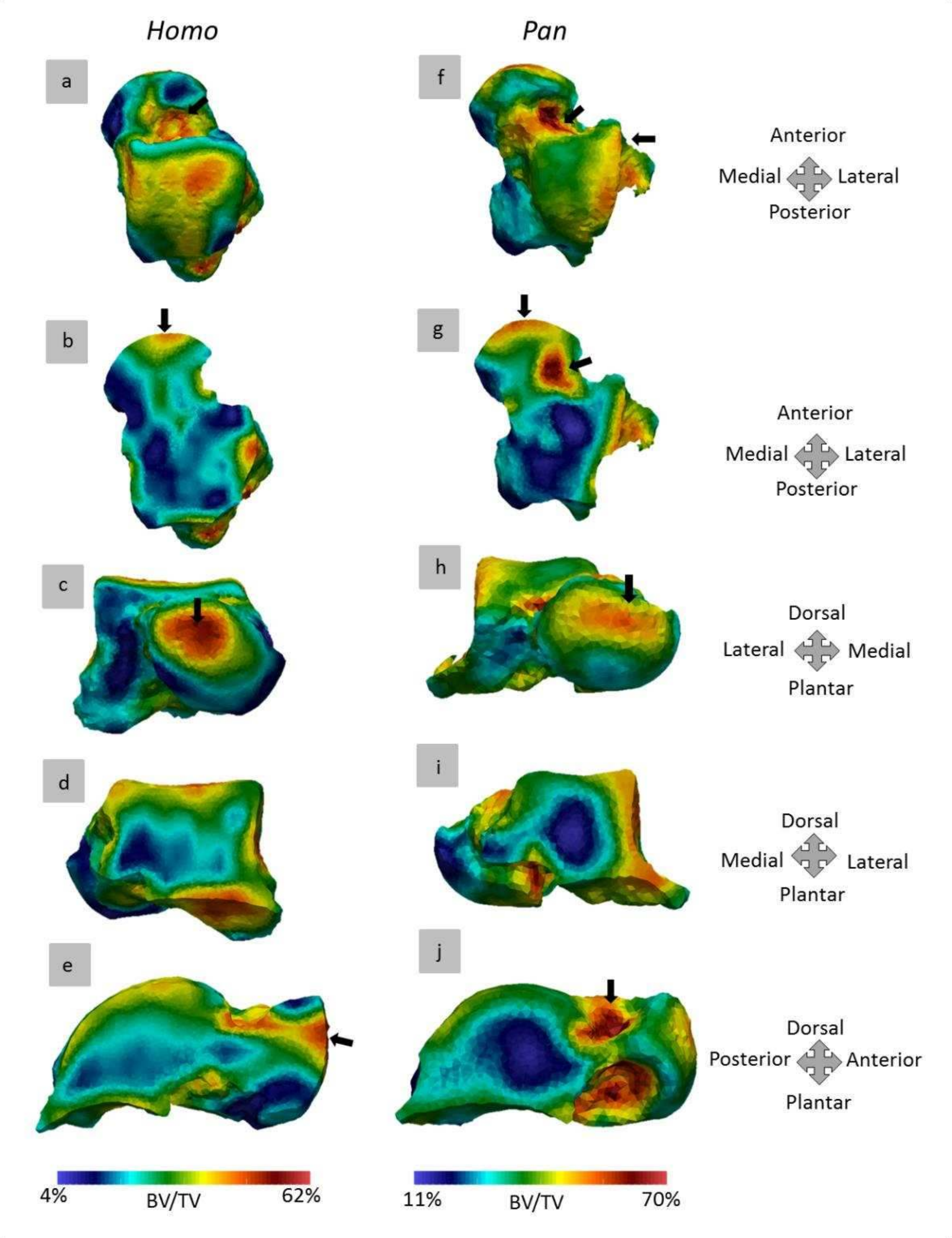

Fig 8. Morphometric maps of BV/TV in the tibia in one individual of Homo (a-e) and Pan (f-j) in (from top to bottom) distal view, mid-sagittal plane of distal tibia, anterior view and mid-coronal plane of distal tibia. Each specimen is scaled to its own data range, as shown in the scale bars. Black arrows indicate regions described in the text.

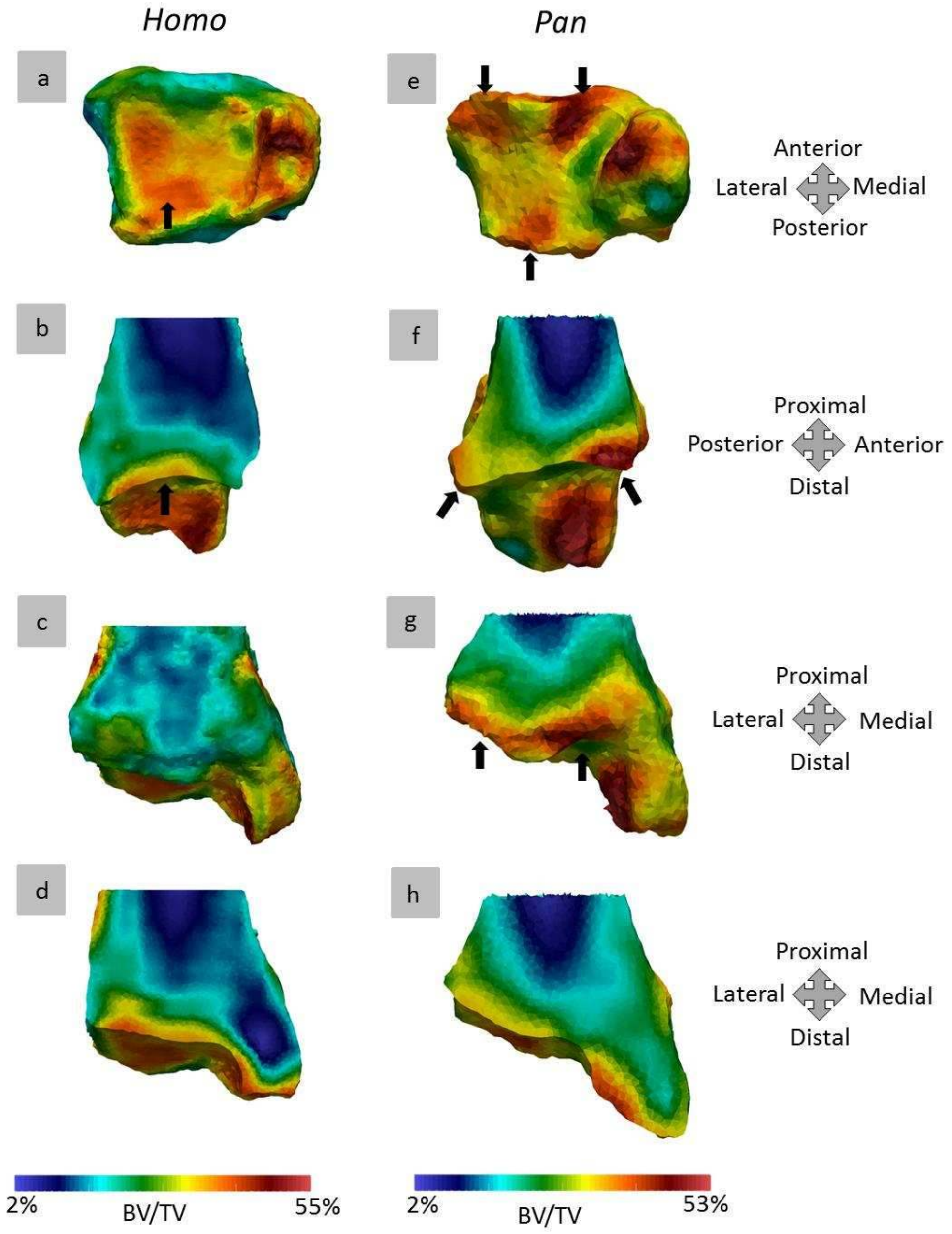

Fig 9. Morphometric maps of mean relative cortical thickness on the canonical talus in Homo (a) and Pan (b) in (from left to right) anterior, dorsal and plantar views. Red indicates thick regions and blue indicates thin regions. (c) Differences between the species are shown as the difference in Pan compared to Homo with positive values (red) indicating thicker bone and negative values (blue) indicating thinner bone. (d) Regions of significant differences between the species at vertices and clusters (red-yellow) and at clusters (blue) of the surface mesh.

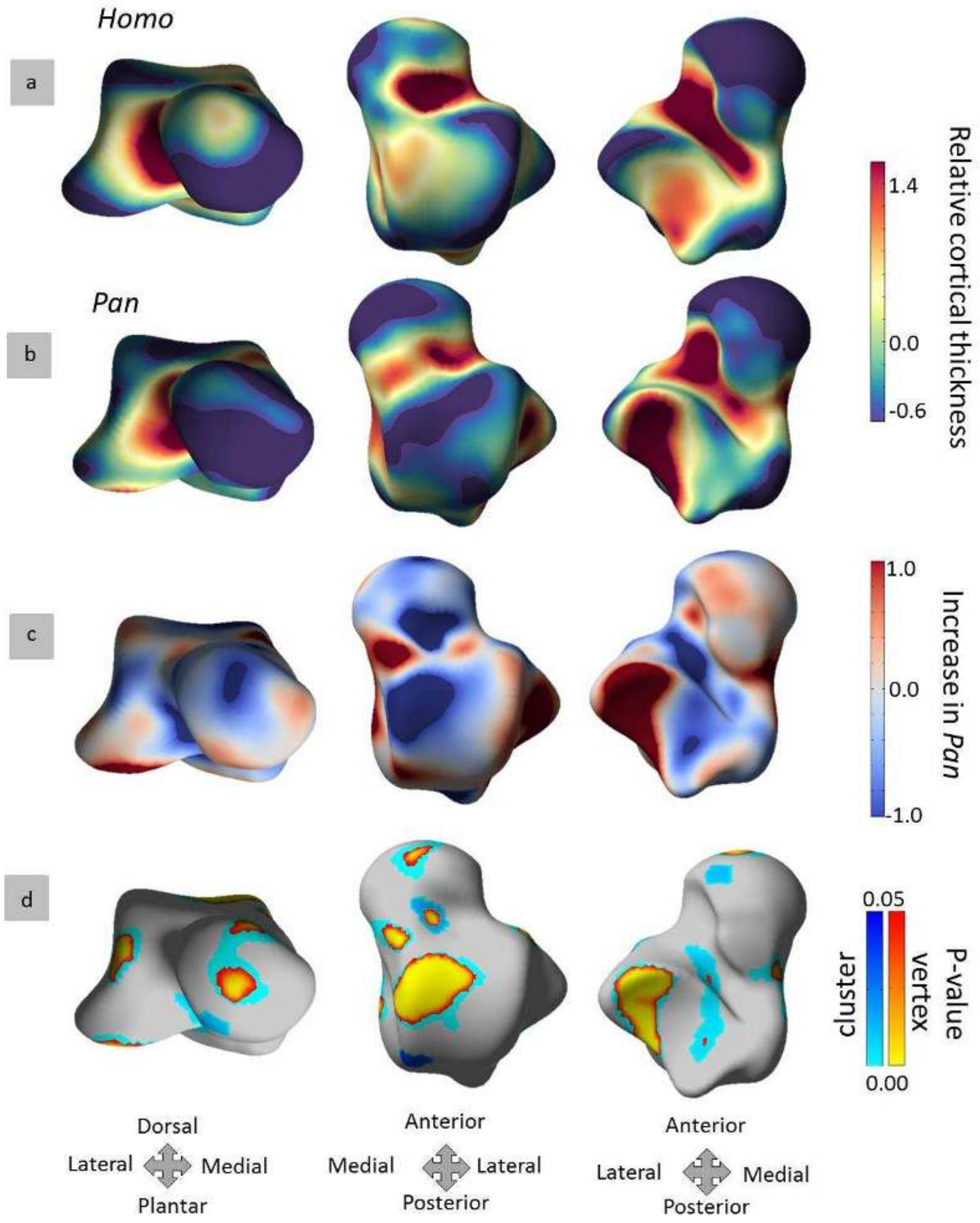

Fig 10. Morphometric maps of mean relative cortical thickness on the canonical tibia in (a) Homo and (b) Pan in (from left to right) lateral, distal and anterior views. Red indicates thick regions and blue indicates thin regions. (c) Differences between the species are shown as the difference in Pan compared to Homo with positive values (red) indicating thicker bone and negative values (blue) indicating thinner bone. (d) Regions of significant differences between the species at vertices and clusters (red-yellow) and at clusters (blue) of the surface mesh.