

Biomechanical Implications of Mineral Content and Microstructural Variations in Cortical Bone of Horse, Elk, and Sheep Calcanei

JOHN G. SKEDROS, STEVE C. SU, AND ROY D. BLOEBAUM*
Bone and Joint Research Laboratory, VA Medical Center, Salt Lake City, Utah

ABSTRACT *Background:* Artiodactyl and perissodactyl calcanei have been recently introduced as models for examining bone for mechanically mediated adaptation. We have reported substantial regional variations in cortical bone microstructure and mineral content *within the same cross-section* of mule deer calcanei. In part, these variations may be adaptations accommodating the customary presence of predominantly tension, compression, and shear strain modes in mutually exclusive cortical locations. Calcanei from skeletally mature horses, elk, and sheep were examined in order to corroborate these previous findings.

Methods: From each species, one calcaneus was obtained from each of 13 animals. Each bone was cut transversely near mid-shaft into two segments and examined for mineral (ash) content. From each species, an additional segment obtained from each of 7 of the original 13 bones was examined for microstructure using 50 \times backscattered electron images. Regions examined included the compression (cranial), tension (caudal), and medial and lateral (shear) cortices. Periosteal (P), middle (M), and endosteal (E) regions were also examined separately within the compression and tension cortices. Quantified microstructural parameters included: (1) secondary osteon population density (OPD), (2) fractional area of secondary bone (FASB), (3) porosity, (4) population density of new remodeling events (NRE = resorption spaces and newly forming secondary osteons), and (5) secondary osteon diameter and minimum-to-maximum chord ratio.

Results: Results in each species showed variations that are considered to be mechanically important and are similar to those reported in mule deer calcanei. Mineral content data suggest that remodeling activity in the compression, medial, and lateral cortices was occurring at a slower rate than remodeling in the tension cortex. In comparison to the tension cortices, the compression cortices have approximately 6.0% higher mineral content ($P < 0.007$) and 35% higher OPD ($P < 0.01$). Additionally, the compression cortices have more nearly perfectly round osteons and lower FASB, porosity, NRE, and osteon diameter ($P < 0.05$; except for FASB in horse where $P = 0.087$ and NRE in sheep where $P = 0.520$). However, patterns of microstructural variations between intracortical regions (P, M, E) are inconsistent when compared to data reported in mule deer calcanei. Microstructural characteristics between the medial and lateral cortices were similar although some significant differences were identified. In general, the microstructure of the medial and lateral cortices differ from the neighboring compression and tension cortices.

Conclusions: Differences in mineral content and microstructure between opposing compression and tension cortices of these three species resemble differences previously reported in mule deer calcanei. The majority of the microstructural variations can be explained in the context

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*Correspondence to: Roy D. Bloebaum, Ph.D., Bone and Joint Research Laboratory, VA Medical Center (151F), 500 Foothill Blvd., Salt Lake City, UT 84148.

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of strain-magnitude-based rules of Frost's Mechanostat Theory of mechanically induced bone adaptation. These variations may also be strongly influenced by the strain mode predominating in each cortical location. The hypothesis that intracortical material adaptations are correlated with progressive transcortical strain magnitude variations is not supported by the inconsistent transcortical variations in material organization. These interpretations do not preclude the possibility that other specific strain features may contribute to a complex adaptive signal. Anat. Rec. 249:297-316, 1997. © 1997 Wiley-Liss, Inc.†

Key words: bone adaptation; osteons; bone strain; artiodactyl calcaneus; horse calcaneus

In recent studies, we have described the artiodactyl calcaneus as a cantilevered "tension/compression system" (Skedros, 1995; Skedros and Bloebaum, 1991; Skedros et al., 1994a,b, 1995a). This description was based on in vivo strain gauge measurements demonstrating that this bone receives habitual cranial-caudal bending during normal controlled gaits (Lanyon, 1974) (Fig. 1). We also examined the hierarchical structural and material organization of cortical bone in mature deer calcanei (Skedros and Bloebaum, 1991; Skedros et al., 1994a,b). The aim of these studies was to document any evidence of structural/material variation that may represent adaptations that are correlated with differences in specific features of the physiologic strain environment. These specific strain features include the habitually prevailing longitudinal strain polarities or modes (e.g., tension and compression strains) and their associated strain magnitude differences, where the highest strains are invariably in the cranial cortex of this bone during functional end-loading.

We reported structural and material differences between the opposing tension (caudal) and compression (cranial) cortices: the compression cortex has statistically greater cortical thickness, mineral content (percent ash), secondary osteon population density, and cross-linked collagen (Gunasekaran et al., 1991; Skedros et al., 1994a,b, 1995a). There were conspicuous regional patterns of matrix ultrastructural anisotropies (i.e., preferred collagen fiber and/or mineral crystalite orientations) between the compression and tension cortices (Skedros, 1994). Additionally, the compression cortex has significantly lower porosity, lower fractional area of secondary osteon bone, and smaller diameter secondary osteons when compared to the tension cortex (Skedros et al., 1994b). We hypothesized that these differences represent interrelated adaptations that accommodate the coexisting strain-magnitude- and strain-mode-dominated mechanical environments. Furthermore, Skedros et al. (1994a,b) suggested that these differences may be linked to the marked disparity in the mechanical properties of bone when loaded in longitudinal tension vs. compression: cortical bone is typically significantly stronger, stiffer, and more fatigue resistant in compression.

In contrast to tension/compression cortices, no significant variations in mineral content or cortical thickness were found between the medial and lateral cortices. It was argued that mechanically relevant differences would not be expected in these opposing regions because their strain milieu is likely to be similar during

the majority of physiologic loading conditions since they lie along a theoretical neutral axis of bending. Although the mineral content of the medial and lateral cortices approximated those of the tension cortex, Skedros et al. (1994b) speculated that ambient shear strains (which likely represent the major strain mode component in the medial and lateral cortices) would be accommodated by specific microstructural adaptations. Microstructural analyses required to investigate such adaptation were not conducted in the mule deer calcanei.

Possible strain-related microstructural differences were also noted to occur between the endosteal region and the middle and periosteal regions within the tension and compression cortices (Skedros et al., 1994b). The endosteal regions have secondary osteons with larger cross-sectional areas, and also have significantly lower secondary osteon population density and fractional area of secondary bone.

Recognizing that small adjustments in a bone's structural and/or material organization can significantly affect its structural and material mechanical properties (Currey, 1984a, pp. 88-97; Martin and Burr, 1989, pp. 57-89), it has been concluded that the conspicuous non-uniform morphologic constructions of the opposing cortices of the mule deer calcaneus could result in important differences in their mechanical properties (Gunasekaran et al., 1991; Skedros et al., 1994a,b, 1995a). We also agreed with previous investigators who have suggested that the relatively different microstructure in the endosteal regions of appendicular bones, if mechanically relevant, may be related to their close proximity to the neutral axis of bending, where the lowest tensile and compressive strain magnitudes occur (Amprino and Godina, 1947; Atkinson and Woodhead, 1973; Currey, 1984a, pp. 265-268; Frost, 1990b; Martin and Burr, 1989, p. 184) (Fig. 1).

In general, the regional structural and material variations in mule deer calcanei were remarkably consistent with predictions of Frost's Mechanostat Theory (of mechanically mediated bone adaptation) in a bone habitually loaded in bending (Frost, 1988a,b, 1990a,b). What appeared particularly intriguing were data suggesting that the tension cortex was habitually stress-shielded, and hence was in a *chronic state of relative disuse* (Skedros et al., 1994a,b, 1995a). If this interpretation is correct, then this bone may provide a model for examining the regulation and kinetics of remodeling during both physiologic use (compression cortex) and relative disuse (tension cortex) within the same bone cross-section (Skedros et al., 1995a). Addi-

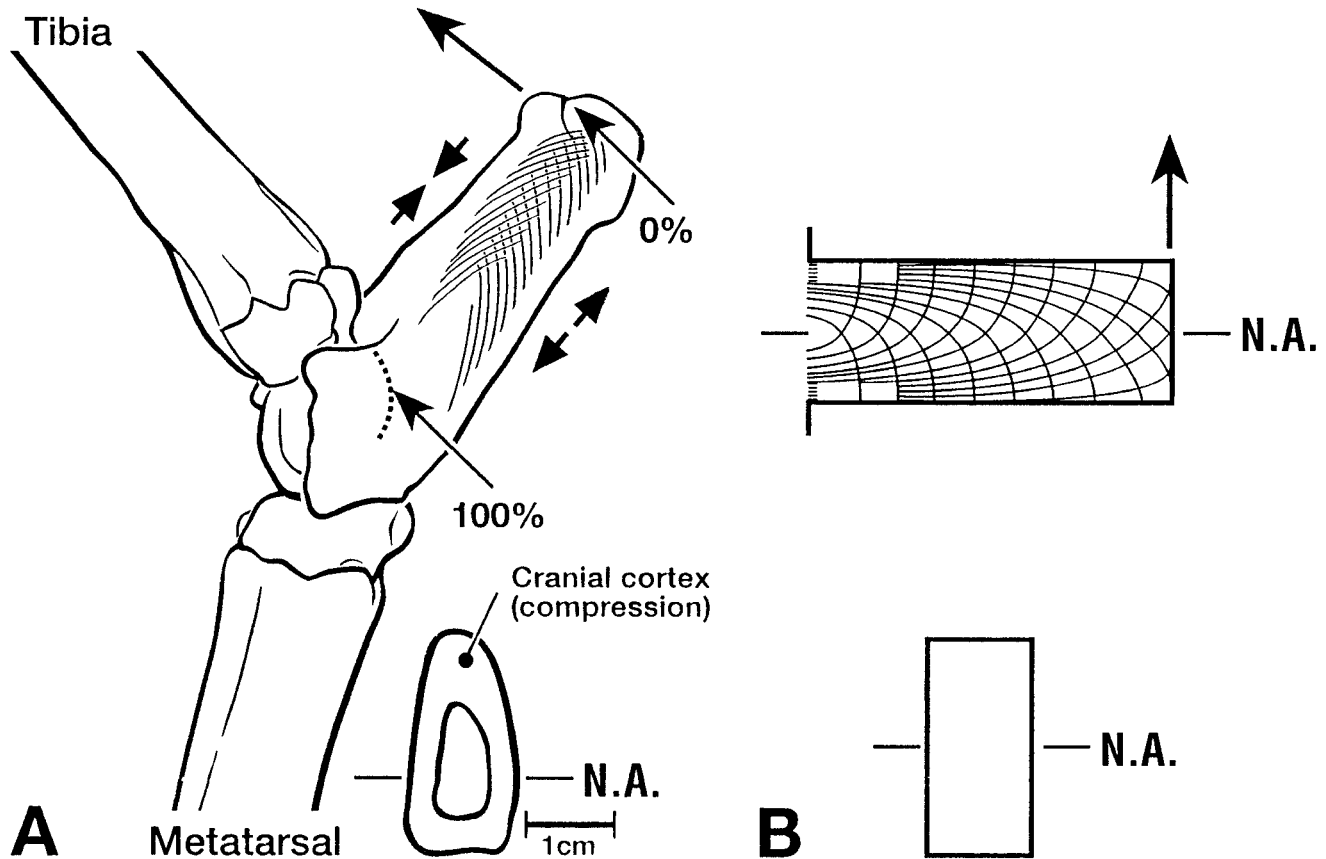


Fig. 1. **A**) Lateral-to-medial view of the bones of the left ankle of a skeletally mature mule deer. The stylized arched trabecular patterns shown are similar to patterns seen in lateral-to-medial roentgenograms of the same specimen. The large arrow toward the distal end (near the 0% location) of the calcaneus shows the general cranial-directed force imparted through the Achilles tendon during mid-flexion in stance phase; this places the cranial cortex in compression (converging arrows). The dotted line at the tip of the 100% arrow indicates the projected location of the contour formed by the interface of the astragalus-calcaneus articular surfaces. The transverse section is from the 60% location. An approximate location of the theoretical

neutral axis (NA) of bending is shown. Although not shown, the plantar ligament and tendon of the superficial digital flexor muscle are adjacent to the tension cortex (Skedros et al., 1994a). **B**) The trabecular patterns of the artiodactyl calcaneus resemble stress trajectories of an idealized, homogeneous, isotropic cantilever subject to bending from a force applied at the free (distal) end (arrow) (redrawn from Currey, 1984a, p. 140). The principal stress trajectories are diagrammatically represented. The more crowded the trajectories, the greater the stress. The trajectories at the base of the cantilever have been omitted for clarity. The beam and cross section show the location of the neutral axis (NA).

tionally, this interpretation favors the relative importance of strain-magnitude-based remodeling thresholds; Frost's Mechanostat Theory is relatively reticent to an important role for strain mode in influencing remodeling activity (Frost, 1990a,b; Skedros et al., 1994a,b; Turner, 1992).

To further examine this possibility, and the possibilities that the myriad of unusual findings mentioned above represent the manifestation of local bone material adaptations, perhaps to specific strain features, corroborative data is needed in homologous bones of similar large cursorial animals.

This study tests the hypothesis that the tension and compression cortices of calcanei of various artiodactyl and perissodactyl species will exhibit mineral content and microstructure variations similar to the patterns of those that have been demonstrated in the mule deer calcaneus. In contrast to our previous studies of the mule deer calcaneus, the present investigation also quantifies mineral content in each intracortical region (periosteal, middle, endosteal), and mineral content

and microstructure in the medial and lateral cortices. The intracortical regions were examined since differences in their mineral content and various microstructural features may reflect local strain-magnitude-related differences in remodeling rates. It also was hypothesized that no significant differences in mineral content and/or microstructure will be found between the medial and lateral cortices.

MATERIALS AND METHODS

Model

The artiodactyl calcaneus typically receives relatively simple, directionally constrained loading regimes during controlled activities (Alexander and Bennett, 1987; Schaeffer, 1947; Skedros et al., 1994a,b). In sheep this bone has also been documented as experiencing habitual in vivo bending (tension/compression) loads during controlled gaits (Lanyon, 1974). Perissodactyl (horse) calcanei are also similarly loaded during functional activities (Badoux, 1987; Skedros et al., 1993c;

Vander Sloten and Van der Perre, 1989). Similar functional morphologies of the calcanei and ankle regions of artiodactyl and perissodactyl calcanei are demonstrated in their similar osseous, musculo-tendinous, and ligamentous structures (Badoux, 1987; Getty, 1975; Lawrence, 1951; Schmid, 1972). The plantar ligament of these bones is firmly adherent along the caudal cortex (Skedros et al., 1994a; McMahon et al., 1995; Alexander and Bennett, 1987). The superficial digital flexor tendon courses across the distal end of the calcaneus (where it forms an insertion on the tuber) and along the caudal aspect of the plantar ligament (Getty, 1975; Skedros et al., 1994a; McMahon et al., 1995). These fibroelastic structures have been described as tension members, which may serve to shift the neutral axis of bending in the caudal direction during functional end-loading (Skedros et al., 1994a).

Specimens and Image Acquisition

One calcaneus was obtained from each of 13 standard breed horses, 13 wild North American elk (*Cervus elaphus*), and 13 domestic sheep (*Ovis aries*). The elk were males and were obtained from their natural habitat (Utah). The horse and sheep calcanei were from mixed sexes that had been set to pasture. None of the animals had evidence of skeletal disease and all were sacrificed for reasons other than limb lameness. Skeletal maturity was confirmed in all bones by gross examination showing coossification of the distal calcaneal growth plate (near the insertion of the Achilles tendon). At the time of specimen collection, the periosteal ("velvet") covering of the elk antlers had been shed and antler growth was complete, showing that calcium was no longer in demand for antler growth (Goss, 1983). The sheep were females, and each was approximately 2 years old and weighed between 60 and 70 kg. Although specific ages and weights of elk and horses could not be determined, animals with relatively advanced age were avoided by the examination of dental wear and/or antler morphology. Additionally, using criteria of Skedros et al. (1996) and Mason et al. (1995), mineral content and microstructure variations were initially examined to minimize the possibility that the bones were from animals of relatively advanced age.

For the purpose of clarity when comparing the results of this study in the context of our past work, the cranial cortex will be referred to as the compression cortex, and the caudal cortex as the tension cortex (Fig. 1). In contrast to past studies, the intracortical "envelopes" (pericortical, middle, endocortical) will be referred to as "regions" (periosteal, middle, endosteal) (Mason et al., 1995; Skedros et al., 1994b).

Shaft length of each calcaneus was measured using described methods (Skedros et al., 1994a). Transverse segments (4- to 5-mm-thick) were cut from each calcaneus at 50, 60, and 70% of shaft length (Figs. 1 and 2). Cortical thickness of the compression, tension, medial, and lateral cortices was measured at the 60% sections (Skedros et al., 1994a). The 50 and 70% segments were used for mineral content analyses. Segments (60%) used for microscopic analyses were randomly selected from the contralateral calcaneus of seven of the 13 animals. These seven segments were embedded in polymethyl methacrylate (PMMA) using conventional methods (Emmanuel et al., 1987). The proximal face

(toward the ankle joint) of each of the embedded segments was ground, polished, and prepared for imaging in the backscattered electron (BSE) mode of a scanning electron microscope (JSM-T330A, JEOL Inc., Peabody, MA) (Bloebaum et al., 1990; Skedros et al., 1994b). Brightness and contrast adjustments were made for each image in order to provide a broad range of graylevel contrast.

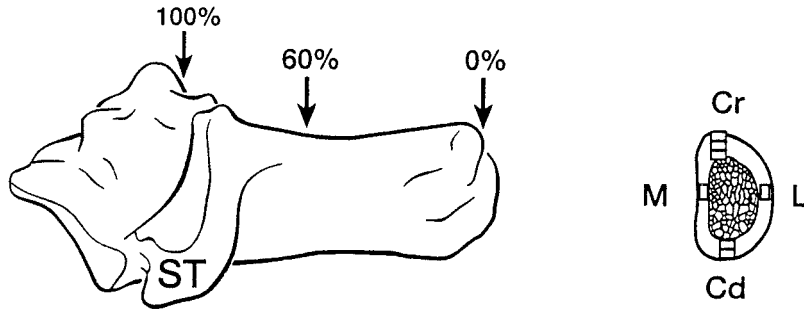
One 50 \times BSE image representing 2.23 \times 1.53 mm (3.41 mm²) was taken within each cortical region (periosteal, middle, endosteal) of the compression and tension cortices (Fig. 2). These images excluded circumferential lamellar bone. This was done for several reasons, including: (1) circumferential lamellar bone may have different mechanical properties when compared to osteon-remodeled bone (Currey, 1984a; Martin and Burr, 1989), and therefore comparisons of these two distinct bone types would be inappropriate in the context of the present study, and (2) circumferential lamellar bone was not present along the compression cortex of any of the horse calcanei, but was present in six of seven sheep calcanei (average thickness 0.44 mm), and was present in five of seven elk calcanei (average thickness 0.86 mm). Without exception, the images of the cortical regions did not overlap and did not contain trabecular bone of the microscopic porous transition zone between the cortical and trabecular bone types (Skedros et al., 1994b). However, in some cases the tension cortices of the sheep calcanei were so thin that three non-overlapping BSE images could not be obtained (Fig. 2). Fewer images were used when this occurred; these images were then taped together and the resulting cortical breadth of imaged bone area was equally subdivided to sample intracortical regions. Total grid hits used in point counting (described below) were also adjusted to accommodate the proportional reductions in area. One 50 \times BSE image was also taken within each medial and lateral cortex (of each bone of each species) at the location midway between the caudal and cranial macroscopic cortical-trabecular margins of the medullary canal (Fig. 2).

Microstructure

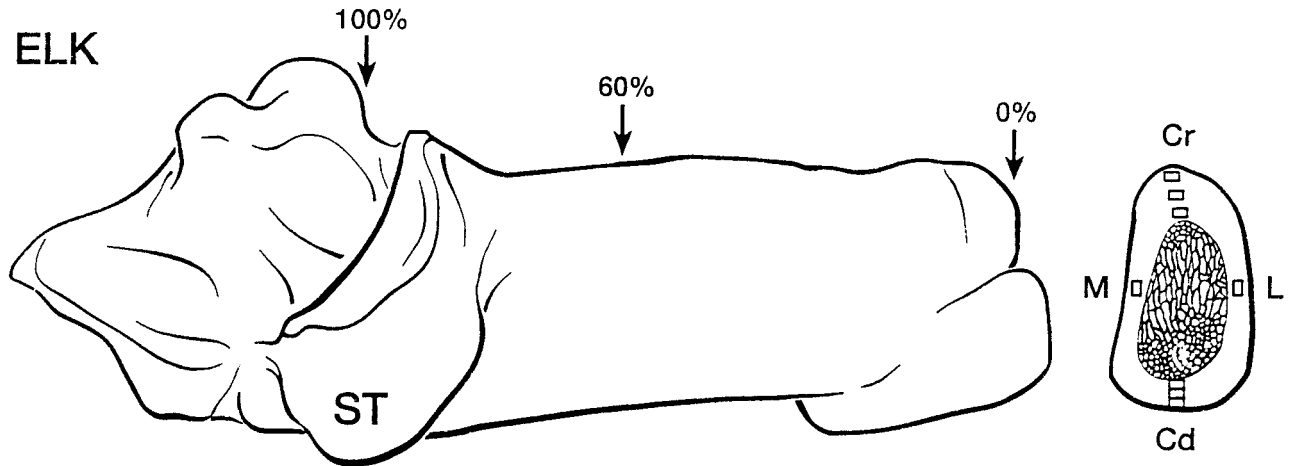
Using conventional point counting techniques (Skedros et al., 1994b), a 8.0 \times 8.0 mm grid was overlaid with a random orientation onto each BSE image and the fractional area of secondary osteonal bone (FASB) was determined. The FASB of an image is defined as the total area of secondary bone (S) divided by total area of secondary bone plus interstitial bone (I), [S/(S + I)]. By definition interstitial bone did not include secondary bone or central canals of secondary osteons. Secondary osteons were distinguished from primary osteons or unremodeled primary bone using conventional histologic criteria described by Skedros et al. (1994b, 1996).

In each image the area occupied by porous (unmineralized tissue) spaces—seen as central canals, resorption spaces, and large vascular spaces—was quantified using a 2.5 \times 2.5 mm grid. Correction for porosity was made by subtracting the porosity of central canals from the fractional area of secondary bone. This correction allowed the exclusion of the influence of potentially larger canals in the tension cortex (Skedros et al., 1994b). Unmineralized areas represented by lacunae

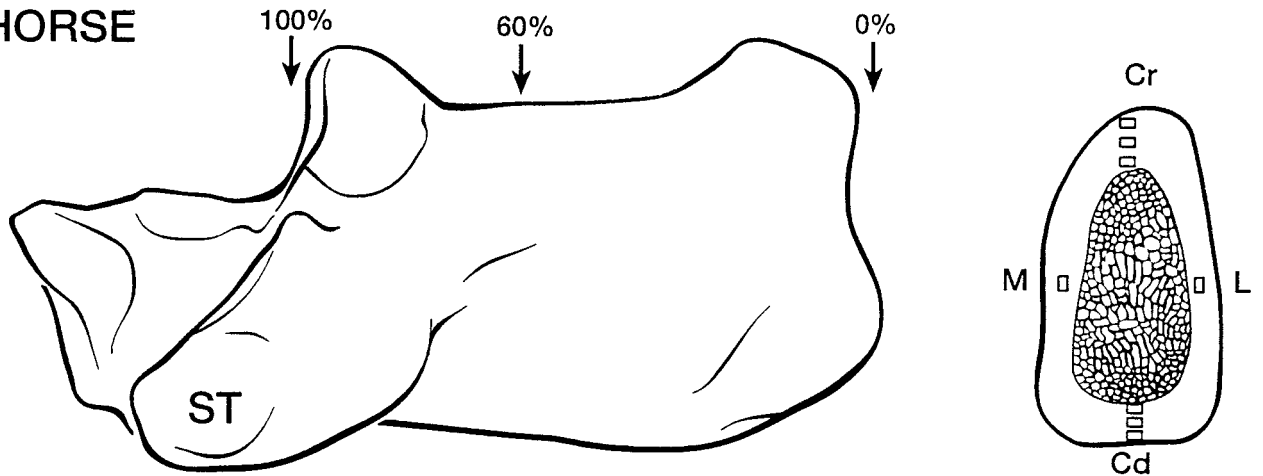
SHEEP



ELK



HORSE



1cm

Fig. 2. Medial-to-lateral views of a skeletally mature sheep calcaneus (top), elk calcaneus (middle), and horse calcaneus (bottom). Arrows indicate bone 'length' (0% to 100%) and the 60% section location. Each representative drawing of a 60% section shows the locations (rectangles) of the images used in the microstructural

analyses. Cortical pieces used for mineral content analyses were obtained from the adjacent 50% and 70% segments. All drawings are scaled proportionally. ST = sustentaculum talus; Cr = cranial; Cd = caudal; M = medial; L = lateral.

and canaliculi were not quantified, and are not considered to contribute significantly to the mechanical properties of cortical bone (Martin, 1984).

Secondary osteons were counted using the following modification of the criteria described by Skedros et al. (1994b). If a secondary osteon crossed the image boundary, but more than 80% of its area was present within the image, it was considered to be a complete secondary osteon and was included in the secondary osteon population density (OPD). If 20% or more of the area of a complete secondary osteon was beyond the image boundary, then, depending on the estimated fraction of the osteon remaining in the image, the secondary osteon was placed in one of three area fraction categories: 0.25, 0.50, or 0.75. For each image these fractional areas were summed and the resulting number, obtained from the secondary osteons crossing the image boundary, was added to the OPD of each image.

Incomplete secondary osteons (i.e., $\geq 20\%$ of their area missing) that are present within an image, but did not cross image boundaries, were considered to be fragments of secondary osteons, and were included in the FASB, but not in the OPD. In this study, incomplete secondary osteons within an image were invariably those that had been partially replaced by another secondary osteon.

Population densities of new remodeling events (NRE), which include resorption spaces and newly forming secondary osteons, were also determined. Newly forming secondary osteons are defined as those exhibiting both relatively poorly mineralized bone, seen as relatively darker gray levels in the BSE images (Skedros et al., 1993a,b), and less than one-half of their radial closure. By definition, resorption spaces did not have observable mineralized bone in the BSE images. In addition to regional differences in mineral content, regional differences in NRE can be used to infer the presence of corresponding differences in intracortical remodeling rates (i.e., formation frequency of new secondary osteons) (Currey, 1984a, pp. 264–269; Martin and Burr, 1989, pp. 105–122, 214–217; Martin, 1993).

A mean diameter was estimated for the secondary osteons within each image. This was accomplished by averaging the minimum and maximum chord lengths of each of 5 secondary osteons that were randomly selected in each image (Jowsey, 1966).

Mineral Content (Percent Ash)

Four pieces were cut from each 50 and 70% segment. These pieces were obtained from the tension, compression, medial, and lateral cortices at locations where the BSE images were taken in the adjacent 60% segment (Fig. 2). Each of these pieces was 4 to 5 mm in transverse breadth and spanned the entire cortical thickness. The segments were meticulously manually cleaned of adherent soft tissue. Furthermore, to avoid circumferential lamellar bone (compression and tension cortices) and/or residual fibrous tissue (all cortices), 0.25 to 0.80 mm of bone was removed from the periosteal and endosteal margins of the cortex. Using an osteotome, the pieces from the compression and tension cortices were then each cut into three smaller fragments corresponding to the three intracortical regions. Since the tension cortices are relatively thin in the 50% sections of all bones of each species, only one or

two pieces could be obtained from these locations. In these cases, mineral content results were tabulated into separate groupings. A $4 \times 4 \times 4$ mm cube of bone was also obtained from the floor of the trochlea of the sustentaculum talus. Since bone in this region is likely habitually loaded in compression, it has been used for comparing mineral content adaptations in compression vs. other strain modes (Skedros et al., 1994a).

The method used for determining mineral content by ashing is described in a previous study (Skedros et al., 1993a). Mineral content is expressed by dividing the weight of the ashed bone (AW) by the weight of the dried, defatted bone (DW), and multiplying this quotient by 100 [(AW/DW)*100].

Data and Statistical Analysis

Data are expressed in terms of means \pm one standard deviation. Unless otherwise stated, percent differences in microstructural parameters and mineral content that are reported in the text are calculated with reference to the tension cortex, lateral cortex, or combined data from medial and lateral cortices (see footnote in Table 2). Data were transformed to achieve normality so that standard parametric statistical tests could be used. Statistical comparisons were conducted using a one-way analysis of variance (ANOVA) with Fisher's Least Significant Difference Test for evaluating pairwise comparisons. Alpha levels of <0.05 are considered statistically significant, 0.05 to 0.09 are trends, and >0.09 are not statistically significant. Pearson correlation coefficients were determined for various paired comparisons.

RESULTS

In Figure 3 appear representative BSE images from all cortical locations of one elk calcaneus. Mineral content and microstructure data are presented in Table 1, *P* values for most pairwise comparisons in Table 2, Pearson correlation coefficients in Table 3, and graphic depictions of selected data appear in Figures 4 and 5. Comparisons of the results of the present study with comparable data reported previously in mature mule deer calcanei appear in Table 4 (Skedros et al., 1994a,b).

Cross-Sectional Shape and Cortical Thickness

The transverse sections of the calcanei of each species (Fig. 2) resemble the cross-sectional shapes and regional cortical thickness differences reported previously in mule deer calcanei (Skedros et al., 1994a) (Fig. 1). In each species the thickness of the compression cortex was approximately two (sheep) to three (elk and horse) times greater than the opposing tension cortex ($P < 0.0001$). In each species the medial and lateral cortices had similar thickness ($P > 0.1$) (Fig. 2).

Mineral Content, Tension, and Compression Cortices (50 + 70% data)

In each species the mineral content of the compression cortex is on the order of 6% greater than the opposing tension cortex (horse 6.0%, $P < 0.0001$; elk 6.5%, $P < 0.0001$; sheep 5.5%, $P < 0.0001$).

Within the compression cortex of each species the mineral contents of the regions are nearly equivalent. Horse calcanei demonstrated the greatest difference

between adjacent regions (2.4%, periosteal < middle region). In contrast, nearly all comparisons between regions of the tension cortex of each species show that the periosteal region has lower mineral content than each of the other two regions (percent that periosteal is less than middle and endosteal, by species: horse 3.2 and 2.3%; elk 4.8 and 3.8%; sheep 6.8 and 4.4%). However, when considering all of these three regions, no *progressive* transcortical decrease or increase in mineral content was found (i.e., no statistically significant difference between middle and endosteal regions) in either the compression or tension cortices of any of the species (Table 2; Fig. 4).

Mineral Content, Medial and Lateral Cortices (50 + 70% data), and Sustentaculum Tali

In each species, mineral content differences do not exceed 2.5% between the medial and lateral cortices (horse 2.5%, $P < 0.0001$; elk 0.3%, $P = 0.62$; sheep 1.9%, $P = 0.07$).

In each species, mineral content of the compression cortex is nearly equivalent to, or slightly greater than, the mean of combined mineral content data of the medial and lateral cortices (horse 0.9%, $P = 0.011$; elk 2.2%, $P < 0.0001$; sheep 1.4%, $P = 0.062$). In contrast, the mean of combined mineral content data of the medial and lateral cortices is *greater* than the mineral content of the tension cortex in each species (horse 5.5%, $P < 0.0001$; elk 4.2%, $P < 0.0001$; sheep 4.0%, $P < 0.0001$).

In each species, the mineral content of the compression cortex is considerably greater than the mineral content of the bone from beneath the trochlea of the sustentaculum talus (horse 5.1%, $P < 0.001$; elk 6.2%, $P < 0.001$; sheep 6.7%, $P < 0.001$). In contrast, in each species mineral content of the sustentaculum tali and tension cortices are nearly equivalent (horse 0.8%, $P = 0.12$; elk 0.3%, $P = 0.67$; sheep 1.3%, $P = 0.2$). Similar to results in mule deer calcanei (Skedros et al., 1994a), these data question the use of bone from sustentaculum tali as a basis for making comparisons of mineral content adaptation to compression loads.

Osteon Population Density (OPD) and Fractional Area of Secondary Bone (FASB) (60% Section Data)

In each species the compression cortex has greater OPD than the opposing tension cortex, and these differences are statistically significant (horse 54.0%, $P < 0.0001$; elk 26.2%, $P = 0.003$; sheep 25.1%, $P = 0.007$).

Although means of OPD data exhibit a distinct tendency of a progressive decrease from the periosteal to endosteal regions of the compression cortex of each of the species (Fig. 4), this tends to be statistically significant only in the elk calcanei (periosteal vs. middle, $P = 0.003$; middle vs. endosteal, $P = 0.094$; periosteal vs. endosteal, $P < 0.0001$) (Table 2). In the other two species, only the sheep calcanei show a statistically significant difference in OPD between adjacent regions of the compression cortex; OPD of the middle region is 42.8% greater than the OPD of the endosteal region ($P = 0.001$).

Although data in Figure 4 suggest a progressive decrease in OPD from the periosteal to endosteal re-

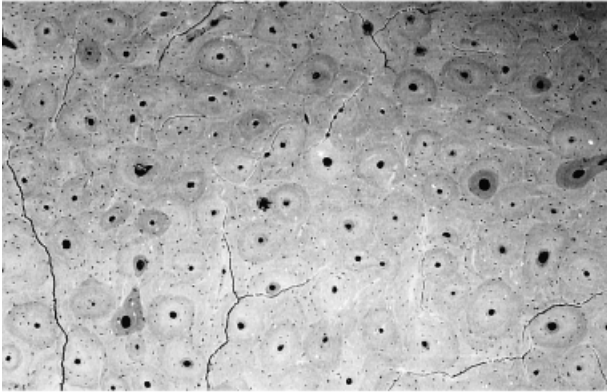
gions of the tension cortices in each species, this pattern is not statistically significant in any of the species.

Since osteon remodeling of a previously unremodeled cortical region can result in a progressive increase in both OPD and FASB, it would be predicted that OPD and FASB data would be strongly positively correlated (Burr et al., 1990). However, this correlation is typically negative and its strength is less than anticipated in each species (Table 3A). These negative correlations may be attributed to the observed regional variations in secondary osteon size and in the number of secondary osteon fragments. For example, regions with relatively large secondary osteons will tend to have higher FASB but relatively lower OPD. Additionally, secondary osteon fragments (i.e., secondary osteons within an image, but with <20% area present) may influence this correlation since they are not included in OPD but are still included in FASB, thus increasing FASB relative to OPD. Retrospective examination of the BSE images of elk and sheep bones suggests that the combined influences of secondary osteon fragments and secondary osteon cross-sectional size account for the two instances where the pattern of transcortical variations in the FASB data do not parallel the pattern of transcortical variation in means of the OPD data (i.e., tension cortices of elk and sheep calcanei) (Table 1, Fig. 4). Retrospective examination of all BSE images of tension and compression cortices also demonstrated that in the elk and sheep bones the secondary osteons appear conspicuously smaller in the periosteal regions of the tension cortices (compared to the middle and periosteal regions). This increased variation would contribute to the increased OPD while not affecting the corresponding FASB in the tension cortices of these two species.

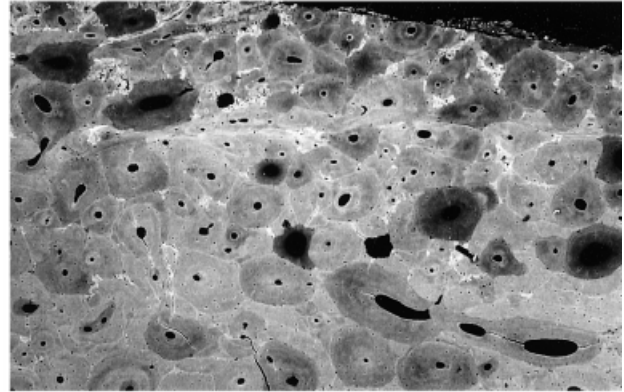
In the medial and lateral cortices of all species, no consistent patterns of similarities or differences were seen in the OPD and FASB data (Fig. 4). No statistically significant differences in OPD were found between the medial and lateral cortices of horse and elk calcanei. In sheep calcanei, OPD of the medial cortex is considerably greater than the lateral cortex (49.6%; $P = 0.028$). However, there were no statistically significant medial vs. lateral differences in FASB in sheep calcanei ($P = 0.760$). Observations of BSE images of the sheep calcanei suggest that this result can be attributed to relatively larger secondary osteons in the lateral cortex compared to the medial cortex. In horse calcanei, FASB is significantly greater in the lateral cortex (-21%; $P < 0.001$) and in elk calcanei there is a trend for greater FASB in the lateral cortex (-10.4%; $P = 0.06$). Examination of the BSE images suggests that these results may be influenced by variations in osteon sizes between the medial and lateral cortices of these two species.

In each species, OPD of the compression cortex is nearly equivalent to (elk), or greater than (horse and sheep), the mean of combined OPD data of the medial and lateral cortices (horse 37.4%, $P = 0.0019$; elk 0.5%, $P = 0.947$; sheep 49.0%, $P < 0.0001$). In contrast, the mean of combined OPD data of the medial and lateral cortices is not statistically different than the OPD of the tension cortex in any of the species (horse -10.6%,

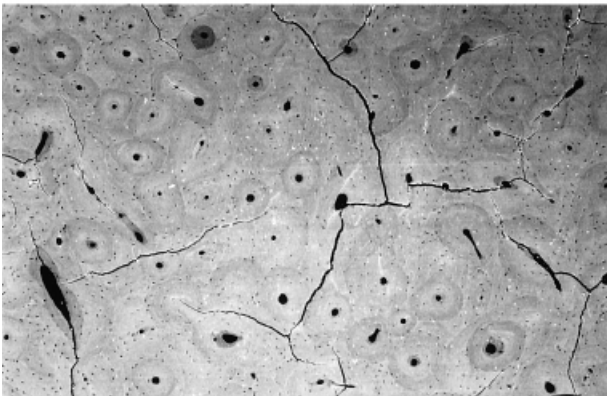
A. Compression Periosteal



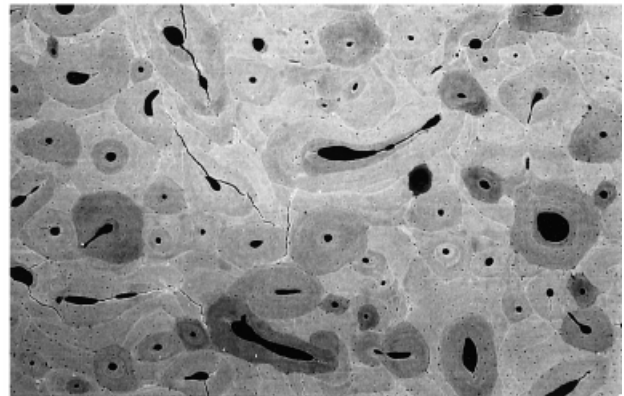
D. Tension Periosteal



B. Compression Middle



E. Tension Middle



C. Compression Endosteal



F. Tension Endosteal

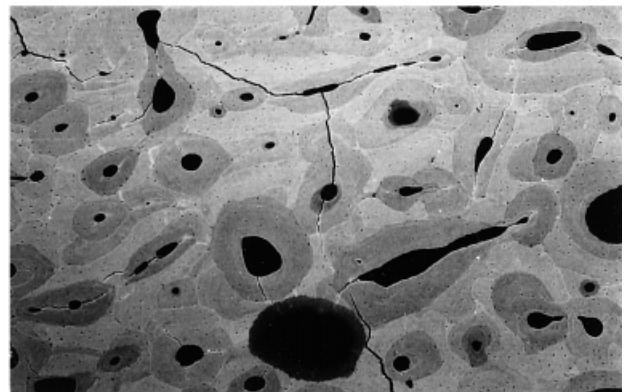


Fig. 3. Backscattered electron images showing representative fields of the compression and tension intracortical regions. Images are from a skeletally mature elk calcaneus sectioned transversely at 60% of length. Cracks within the images were unavoidable artifacts of

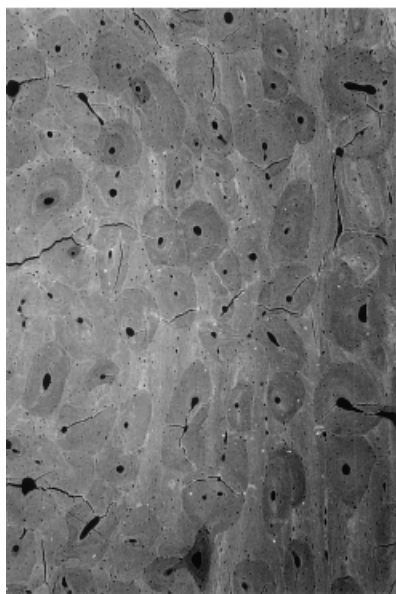
processing. The black space in the upper right hand corner of the tension periosteal region is not part of the specimen. The top edge of each image is directed towards the periosteal surface.

$P = 0.356$; elk -20.5% , $P = 0.10$; sheep 18.9% , $P = 0.108$).

In each species FASB of the compression cortex is nearly equivalent to the mean of combined FASB data of the medial and lateral cortices (horse 2.5% , $P = 0.500$;

elk -4.9% , $P = 0.207$; sheep 0.3% , $P = 0.955$). In contrast, the mean of combined FASB data of the medial and lateral cortices is *less* than the FASB of the tension cortex in two of the species (horse 8.1% , $P = 0.029$; elk 3.9% , $P = 0.331$; sheep 20.1% , $P < 0.001$).

G. Lateral



H. Medial

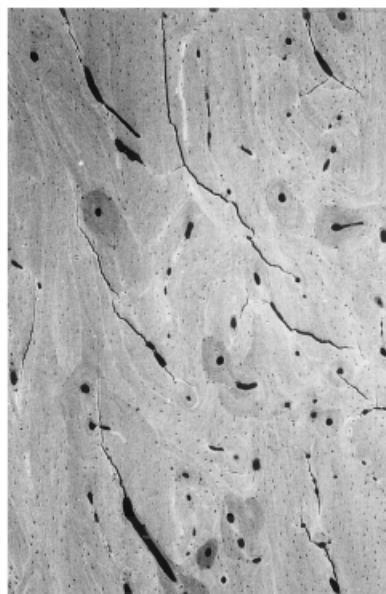


Fig. 3 (continued) Backscattered electron images showing representative fields of the lateral and medial regions of the same section used in figure 3.1. The right edge of each image is directed towards the periosteal surface.

New Remodeling Events (NRE)

New remodeling events, defined as resorption spaces and newly forming osteons, are significantly more prevalent in the tension cortex, compared to the opposing compression cortex in the horse (69.4%, $P = 0.009$) and elk (50.4%, $P = 0.036$) calcanei (Fig. 5). In contrast, sheep calcanei show no difference in NRE between the compression and tension cortices ($P = 0.520$).

No significant transcortical increase or decrease in NRE is seen in the compression or tension cortices of any of the species (Table 2). No significant differences in NRE data were found between the medial and lateral cortices of any of the species (horse, $P = 0.59$; elk, $P = 0.608$; sheep, $P = 0.160$).

In each species, the prevalence of NRE in the compression cortex appears to exceed that shown by the mean of combined NRE data of the medial and lateral cortices, but these differences are not statistically significant (horse 14.0%, $P = 0.647$; elk 37.8%, $P = 0.27$; sheep 79.6%, $P = 0.244$). Means of NRE data of the tension cortices is statistically greater than the NRE of the combined medial and lateral cortices in only the horse and elk calcanei (horse 99.4%, $P = 0.005$; elk 106.7%, $P = 0.004$; sheep 19.8%, $P = 0.700$).

Porosity

In each species the compression cortex has lower porosity than the opposing tension cortex, and these differences are statistically significant (horse -56.3%, $P < 0.0001$; elk -64.7%, $P < 0.0001$; sheep -34.5%, $P = 0.04$) (Tables 1 and 2, Fig. 5).

No statistically significant porosity differences were found between adjacent regions of the compression cortices of any of the species. Similarly, no significant

differences in porosity were found between the adjacent regions of the tension cortices of each species (Table 2).

No significant differences in porosity were found between the medial and lateral cortices of the elk ($P = 0.24$) and horse ($P = 0.29$) calcanei. In sheep calcanei the medial cortex had greater porosity than the lateral cortex, and this difference is statistically significant (161.2%, $P = 0.004$).

In each species, porosity of the compression cortex is not statistically different from the combined porosity data of the medial and lateral cortices (horse 43.8%, $P = 0.274$; elk 39.2%, $P = 0.338$; sheep -13.1%, $P = 0.583$). In contrast, combined porosity data of the medial and lateral cortices is less than the porosity of the tension cortex in each species, but in the sheep the difference is not statistically significant (horse 227.5%, $P < 0.0001$; elk 295.0%, $P < 0.0001$; sheep 33.7%, $P = 0.164$).

Secondary Osteons: Area, Diameter, Min.-to-Max. Chord Ratio, and Observed Cross-Sectional Shape

In each species, secondary osteons in the compression cortex have smaller mean diameter than secondary osteons in the opposing tension cortex, and these differences are statistically significant (sheep -21.7%, $P = 0.002$; elk -20.7%, $P < 0.0001$; horse -14.0%, $P = 0.002$) (Tables 1 and 2; Fig. 5).

With one exception, no statistically significant differences in secondary osteon diameter were found between adjacent regions of the compression cortices of all species (Table 2). The only exception was the periosteal region of the elk calcaneus where the secondary osteons are smaller than those in the middle (17.3%, $P = 0.008$) and endosteal (17.3%, $P = 0.007$) regions (Fig. 4).

TABLE 1. Means and standard deviations: Mineral content and microstructure¹

Animal (cortex or regions)	Mineral content (% ash)	OPD (no./mm ²)	FASBX100 (%)	Porosity (%)	NRE (no./mm ²)	Osteon diameter (mm)	Minimum-maximum chord ratio
Horse calcaneus							
Compression (all regions)	65.7 (1.1)	25.2 (7.7)	80.0 (7.1)	5.4 (3.1)	1.2 (1.0)	0.19 (0.03)	0.82 (0.08)
Tension (all regions)	62.0 (1.8)	16.4 (5.6)	84.4 (7.7)	12.3 (6.8)	2.1 (1.2)	0.22 (0.04)	0.78 (0.07)
Compression periosteal	64.6 (0.9)	27.1 (10.3)	80.0 (8.8)	6.9 (4.2)	1.6 (1.2)	0.18 (0.03)	0.82 (0.11)
Compression middle	66.1 (0.8)	27.1 (6.4)	81.1 (5.0)	4.9 (2.3)	1.1 (1.1)	0.19 (0.03)	0.83 (0.07)
Compression endosteal	66.3 (0.7)	21.4 (5.0)	79.0 (8.0)	4.4 (2.0)	1.0 (0.9)	0.12 (0.02)	0.80 (0.06)
Tension periosteal	61.0 (1.3)	19.4 (5.1)	88.1 (5.6)	9.4 (4.8)	2.0 (1.1)	0.23 (0.05)	0.75 (0.06)
Tension middle	62.9 (1.4)	16.7 (5.1)	86.4 (3.9)	10.8 (3.8)	2.2 (1.1)	0.21 (0.03)	0.82 (0.09)
Tension endosteal	62.3 (2.0)	13.1 (5.3)	78.7 (9.7)	16.7 (9.1)	1.9 (1.4)	0.21 (0.04)	0.76 (0.04)
Medial	65.8 (0.9)	16.5 (5.9)	68.9 (5.8)	3.2 (1.2)	0.9 (1.0)	0.22 (0.02)	0.75 (0.06)
Lateral	64.3 (0.8)	20.2 (3.8)	87.2 (7.3)	4.3 (1.7)	1.2 (0.6)	0.20 (0.03)	0.77 (0.07)
Sustentaculum	62.5 (1.7)	—	—	—	—	—	—
Elk calcaneus							
Compression (all regions)	69.6 (1.3)	24.0 (6.6)	76.5 (12.1)	3.7 (2.1)	1.2 (1.0)	0.18 (0.03)	0.84 (0.06)
Tension (all regions)	65.4 (2.2)	19.0 (5.3)	83.6 (8.4)	10.5 (5.2)	1.8 (1.0)	0.23 (0.04)	0.73 (0.11)
Compression periosteal	69.2 (1.2)	30.7 (2.0)	85.9 (4.2)	4.2 (2.9)	1.6 (1.2)	0.16 (0.02)	0.86 (0.05)
Compression middle	69.9 (1.6)	22.7 (4.4)	78.7 (7.0)	3.6 (1.9)	1.0 (0.8)	0.20 (0.03)	0.84 (0.05)
Compression endosteal	69.8 (0.9)	18.6 (5.9)	64.8 (12.3)	3.3 (1.6)	1.0 (0.7)	0.20 (0.01)	0.84 (0.08)
Tension periosteal	63.8 (1.8)	22.2 (7.4)	84.9 (10.5)	10.5 (6.3)	2.4 (1.0)	0.22 (0.06)	0.76 (0.15)
Tension middle	66.9 (1.3)	19.3 (3.1)	87.5 (5.0)	8.8 (4.7)	1.5 (0.9)	0.23 (0.03)	0.72 (0.10)
Tension endosteal	66.2 (1.9)	15.6 (1.9)	78.2 (6.8)	12.3 (4.5)	1.5 (1.0)	0.25 (0.03)	0.70 (0.08)
Medial	68.2 (1.3)	22.7 (3.7)	76.1 (5.5)	2.1 (0.3)	0.7 (0.4)	0.17 (0.02)	0.75 (0.09)
Lateral	68.0 (1.7)	25.1 (4.2)	84.9 (6.0)	3.2 (1.6)	1.0 (1.0)	0.19 (0.01)	0.72 (0.02)
Sustentaculum	65.6 (1.3)	—	—	—	—	—	—
Sheep calcaneus							
Compression (all regions)	72.1 (2.9)	32.2 (7.4)	76.9 (14.9)	2.6 (1.3)	1.2 (1.7)	0.14 (0.02)	0.80 (0.08)
Tension (all regions)	68.4 (3.7)	25.7 (6.3)	92.1 (3.5)	4.0 (2.7)	0.8 (1.0)	0.18 (0.04)	0.78 (0.07)
Compression periosteal	71.5 (2.7)	37.6 (3.9)	85.9 (5.6)	2.5 (1.8)	1.6 (2.6)	0.14 (0.02)	0.80 (0.06)
Compression middle	73.0 (2.4)	35.0 (3.8)	81.3 (9.7)	2.3 (0.9)	0.8 (1.0)	0.13 (0.02)	0.84 (0.05)
Compression endosteal	72.1 (3.2)	24.5 (5.9)	64.1 (16.9)	3.0 (1.2)	1.0 (1.3)	0.15 (0.03)	0.77 (0.10)
Tension periosteal	66.2 (4.0)	30.6 (6.0)	92.7 (2.8)	2.8 (0.6)	0.9 (1.1)	0.17 (0.04)	0.81 (0.05)
Tension middle	70.7 (2.0)	24.1 (6.1)	94.8 (3.0)	2.5 (0.7)	0.2 (0.4)	0.18 (0.03)	0.74 (0.10)
Tension endosteal	69.1 (3.0)	21.9 (3.6)	90.0 (3.5)	6.0 (3.5)	1.1 (1.1)	0.19 (0.04)	0.78 (0.06)
Medial	70.4 (2.3)	25.9 (8.0)	77.2 (10.5)	4.3 (2.0)	1.1 (1.0)	0.16 (0.04)	0.68 (0.10)
Lateral	71.8 (2.2)	17.3 (5.4)	76.1 (8.2)	1.7 (0.8)	0.2 (0.3)	0.19 (0.05)	0.71 (0.14)
Sustentaculum	67.5 (1.8)	—	—	—	—	—	—

¹Mineral content data taken from 50 and 70% segments. Microstructure data taken from 60% segments. OPD = secondary osteon population density; FASB = fractional area of secondary bone; NRE = new remodeling events; Sustentaculum = bone from sustentaculum talus.

Although observations of BSE images of the periosteal regions of the tension cortices suggest that osteons in these regions are smaller than those in the neighboring middle and endosteal regions, no statistically significant differences in secondary osteon diameter were found between adjacent regions of the tension cortices of the three species (Table 2).

In horse and elk bones, mean secondary osteon diameters are roughly similar in the medial and lateral cortices (<9.5% difference in means, $P > 0.17$). In the sheep calcanei, secondary osteons in the lateral cortex appear to have larger mean diameters than those in the medial cortex, but this difference is not statistically significant (difference in lateral mean vs. medial mean: -14.6%, $P = 0.172$).

Inspection of all BSE images of each species showed that the secondary osteons in the medial cortices and in the lateral cortices have cross-sectional shapes that are more oblong than those in the compression cortices. Minimum-to-maximum chord ratios support this observation by showing that in most cases the secondary osteons in the medial and lateral cortices have statistically more oblong cross-sectional shapes than the os-

teons in the compression cortices (all P values <0.04 except for compression vs. lateral of the horse bones where $P = 0.13$). In contrast, in all species the mean minimum-to-maximum chord ratio of osteons in the tension cortices are not significantly different compared to the osteons in the medial and in the lateral cortices. However, examination of BSE images clearly demonstrates a broad range of irregularly shaped osteons in the tension cortex or each species. Consequently, this similarity in "mean values" obscures the notably irregular osteon shapes in the tension cortex.

Circumferential Lamellae in Compression and Tension Cortices

Microscopic examination of peripheral margins of all bone sections showed that circumferential lamellar bone was absent in the compression or tension cortices of the horse calcanei and in the tension cortices of all bones from the three species. Circumferential lamellar bone was present in only the compression cortex in 6 of 7 sheep (average thickness 0.86 ± 0.33 mm) and 5 of 7 elk (average thickness 0.44 ± 0.05 mm). In the sections where circumferential lamellae are absent or indis-

TABLE 2. One-way ANOVA *P* values of paired comparisons: microstructure and mineral content data¹

Parameter	Compression (cranial) regions		Tension (caudal) regions		Compression vs. tension (%) ³	Medial vs. lateral (%) ³	
	P vs. M	M vs. E	P vs. M	M vs. E			
Horse	Mineral content (50 and 70% Sections) ²	<.0001	0.608	0.025	0.880	<.0001 (6.0)	<.0001 (2.45)
	Osteon population density	0.984	0.175	0.345	0.208	<.0001 (53.9)	0.270 (-18.1)
	Fractional area of secondary bone	0.936	0.678	0.653	0.050	0.087 (-5.2)	<.0001 (-21.0)
	Porosity	0.353	0.772	0.491	0.192	<.0001 (-56.3)	0.294 (-25.9)
	New remodeling events	0.388	0.933	0.745	0.584	0.009 (-40.9)	0.589 (-24.3)
	Osteon diameter	0.344	0.702	0.552	0.990	0.002 (-14.0)	0.262 (9.36)
Elk	Mineral content (50 and 70% Sections) ²	0.065	0.819	<.0001	0.589	<.0001 (6.5)	0.624 (0.3)
	Osteon population density	0.003	0.094	0.268	0.159	0.003 (26.2)	0.398 (-9.6)
	Fractional area of secondary bone	0.133	0.007	0.535	0.039	0.015 (-8.5)	0.059 (-10.4)
	Porosity	0.587	0.831	0.547	0.224	<.0001 (-64.7)	0.236 (-35.3)
	New remodeling events	0.244	0.904	0.093	0.993	0.036 (-33.1)	0.608 (-25.1)
	Osteon diameter	0.008	0.980	0.605	0.400	<.0001 (-20.7)	0.167 (-8.5)
Sheep	Mineral content (50 and 70% Sections) ²	0.077	0.279	0.002	0.554	<.0001 (5.5)	0.069 (-1.9)
	Osteon population density	0.331	0.001	0.066	0.518	0.007 (25.1)	0.028 (49.6)
	Fractional area of secondary bone	0.382	0.020	0.298	0.028	<.0001 (-16.5)	0.760 (1.4)
	Porosity	0.739	0.330	0.529	0.070	0.042 (-34.5)	0.004 (161.2)
	New remodeling events	0.645	0.789	0.249	0.171	0.520 (39.5)	0.106 (446.8)
	Osteon diameter	0.465	0.333	0.631	0.686	0.002 (-21.7)	0.172 (-14.6)

¹P = periosteal; M = middle; E = endosteal.

²Comparisons of mineral content in tension regions (i.e., P vs. M and M vs. E) used data only from 70% sections.

³The numbers in parentheses below each *P* value are the percent differences of the paired comparison. Percent differences are calculated as follows: [(compression - tension)/(tension)] × 100; [(medial - lateral)/(lateral)] × 100.

tinct, the subperiosteal bone was extensively remodeled with secondary osteons.

DISCUSSION

Mineral Content, Porosity, and Microstructural Variations: Tension and Compression Cortices

In each species, mineral content of the compression cortex consistently exceeded the mineral content of the opposing tension cortex. These differences approximate the pattern (compression > tension) and magnitude of those reported at the same locations of skeletally mature mule deer calcanei (6.6% average difference, $P < 0.0001$) (Skedros et al., 1994a) (Table 4). Similar to mule deer calcanei (Skedros et al., 1994b), results show that in each species the compression cortex has significantly greater OPD, lower porosity and FASB, and smaller secondary osteon diameters, than the opposing tension cortex (Table 4). Mineral content data suggest that in each species the remodeling activity of the

compression, medial, and lateral cortices is occurring at a slower rate than remodeling in the tension cortex.

Experimental data in recent (Dayton et al., 1997) and previous (Currey, 1969, 1975, 1988; Vose, 1962; Vose and Kubala, 1959; Bonfield and Clark, 1973) studies demonstrate that the mineral content, porosity, and microstructural variations reported in this study are of sufficient magnitude to affect mechanical properties. Important changes in mechanical properties of cortical bone will occur when ash content differences reach approximately 3 to 4% (Currey, 1969, 1984a pp. 88-97; Skedros et al., 1994a). Bone material density (g/cm³), which is positively correlated with mineral (ash) content (Skedros et al., 1993a,b), is also correlated with enhanced fatigue life (Carter and Hayes, 1976, 1977b; Carter et al., 1976; Wright and Hayes, 1977). Porosity has also been shown to be inversely correlated with compressive strength (Carter and Hayes, 1977a) and elastic modulus in specimens of cortical bone (Carter

TABLE 3. Pearson correlation coefficients for selected comparisons¹

Parameter	Region(s)	Horse	Elk	Sheep
A. Fractional area of secondary bone				
Osteon Population Density	All	-0.391***	-0.157	-0.259
	Cr, Cd	-0.341**	-0.229	-0.312
	Cr	-0.341**	-0.136	-0.111
	Cd	-0.074	0.214	-0.082
	M, L	-0.604**	0.528*	0.069
B. Mineral content at 70% section				
New Remodeling Events	All	-0.427***	-0.476****	-0.223
	Cr, Cd	-0.446***	-0.481***	-0.066
	Cr	-0.446***	-0.640***	-0.260
	Cd	-0.442*	-0.396*	0.341
	M, L	-0.058	-0.465	-0.576*
C. Porosity				
Fractional area of secondary bone	All	-0.102	-0.030	-0.070
	Cr, Cd	-0.306**	-0.070	-0.197
	Cr	-0.419*	-0.042	-0.530**
	Cd	-0.927****	-0.746****	-0.798****
	M, L	0.014	-0.112	0.118
D. Osteon diameter				
Osteon Population Density	All	-0.595****	-0.648****	-0.501****
	Cr, Cd	-0.590****	-0.680****	-0.510****
	Cr	-0.669****	-0.523**	-0.145
	Cd	-0.310	-0.740****	-0.555**
	M, L	-0.632**	-0.067	-0.473

¹All = cranial (Cr); caudal (Cd); medial (M); lateral (L) regions.

*0.05 < P < 0.09 (statistical trend); **0.01 < P < 0.05; ***P < 0.01; ****P < 0.001; No symbol = nonsignificant (P > 0.09).

and Hayes, 1977a; Currey, 1988; Schaffler and Burr, 1988).

In recent studies, we suggested that differences in material adaptation between cortical regions of the same cross-section of a limb bone, if present, would be most conspicuous on opposing sides of the neutral axis in a habitual bending environment (Skedros et al., 1994a,b) (Fig. 1). Disparities in the mechanical behavior of bone in tension vs. compression favor this hypothesis. For example, both yield strength (Cezayirlioglu et al., 1985) and ultimate strength (Burstein et al., 1976; Cezayirlioglu et al., 1985; Yamada, 1970) are approximately 30% greater in compressive loading than in tensile loading.

But if cortical bone is stronger in compression, then the magnitude of the increased mineral content and reduced porosity in this region, compared to the tension region, appears excessive if these function solely for adapting elastic- and early yield-related mechanical properties. Alternatively, these variations may be primarily aimed at enhancing other failure-related mechanical properties. For example, in mechanically tested bones, Currey (1969) reported a level of mineral content where impact strength was greatest but yield strength was relatively reduced. Therefore, mineral content differences in the opposing cortices of the calcanei may enhance other mechanical properties such as work-of-fracture and/or the energy of failure in their characteristic loading mode (Currey, 1969, 1984a, pp. 88-97; Martin and Burr, 1989).

A previous study (Skedros et al., 1995b) has shown that the mineral content in the compression cortex of mature deer calcanei is not unusually high, but approxi-

mates mineral contents of other long limb bones in the mature deer skeleton. Rigidity of the calcaneal shaft may therefore be achieved primarily through structural adaptations including increased cortical thickness along the highly stressed compression side and enhancement of the cross-sectional moment of inertia in the sagittal plane. In contrast to the compression cortex, the tension cortex has *unusually low* mineral when compared to other long bones from the appendicular skeleton of this deer species (Skedros et al., 1995b). As discussed below, this may be a consequence of increased remodeling activity in the tension cortex.

Some Correlations and Associations May Reflect Interdependent Adaptations

In general, correlation coefficients of selected comparisons (Table 3) demonstrate the *absence* of clear and consistent relationships among many of the measured parameters. However, some moderate-to-strong correlations help to clarify how some parameters may be linked: (1) the unanticipated typically *negative* correlations of FASB and OPD (Table 3A) suggest that differences in osteon size and shape, and number of secondary osteon fragments, strongly influence FASB; (2) negative correlation of mineral content and NRE (Table 3B) suggests that mineral content variations in the tension and compression cortices are partially related to the presence of active remodeling. (Yet, the positive correlation between these parameters in the tension cortices of sheep calcanei suggests that increased remodeling can not entirely explain the relatively lower mineral content of this region.); (3) in most cases, FASB and porosity are consistently negatively correlated

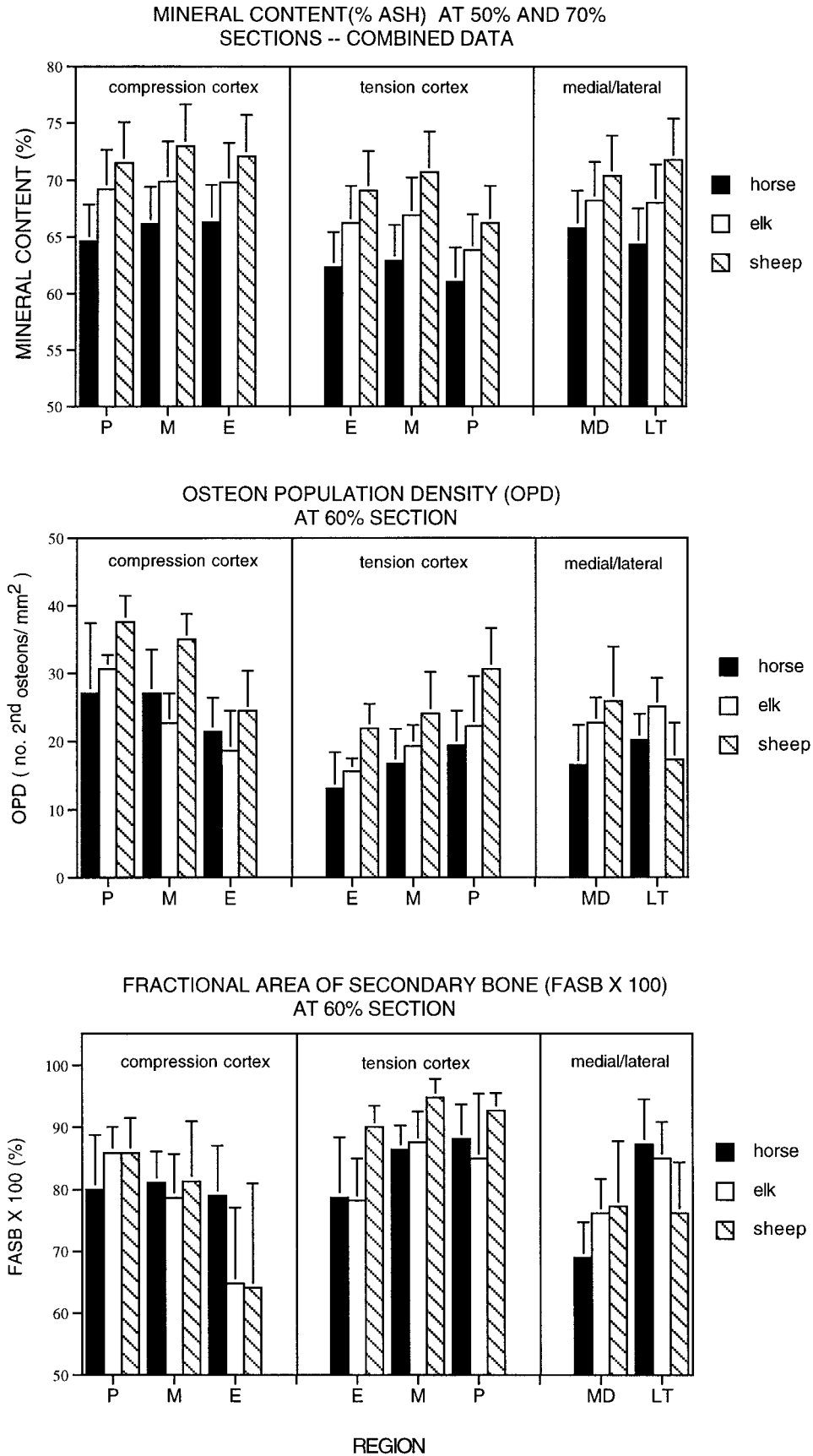


Fig. 4. Means and standard deviations of mineral content (percent ash), secondary osteon population density (OPD) and fractional area of secondary bone (FASB) data. See Table 2 and text for statistical probabilities of paired comparisons. FASB is expressed as a percentage. (Regions: P = periosteal, M = middle, E = endosteal, MD = medial, LT = lateral.)

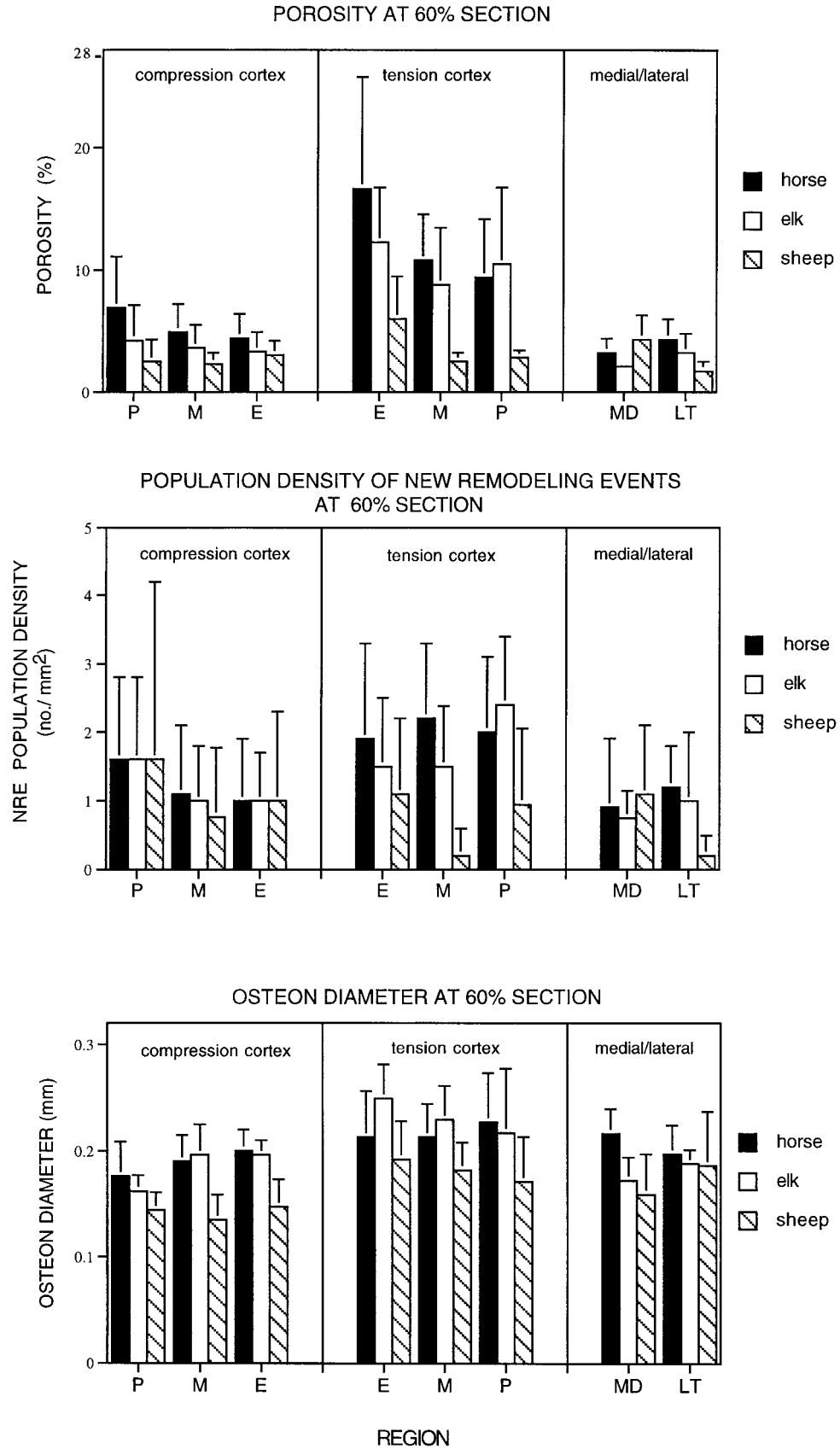


Fig. 5. Means and standard deviations of porosity, new remodeling events (NRE), and secondary osteon diameter data. See Table 2 and text for statistical probabilities of paired comparisons. (Regions: P = periosteal, M = middle, E = endosteal, MD = medial, LT = lateral.)

TABLE 4. Comparisons of mineral content and microstructure data with results in deer calcanei¹

Parameter	Cortex	Deer	Horse	Elk	Sheep
Mineral content (% ash)	Cranial vs. caudal	Cr > Cd****	—****	—****	—****
	Medial vs. lateral	Md = Lt (<2.5% diff.)	—****	—	—*
Osteon population density	Cranial	P = M	—	P > M***	—
		P > E***	P = E	—****	—****
	M > E***	M = E	M = E	—****	
	Caudal	P = M	—	—	P > E*
Fractional area of secondary bone	Cranial vs. caudal	P > E***	—***	—**	—***
		M > E***	M = E	M = E	M = E
	Cranial	Cr > Cd***	—****	—***	—****
	Cranial	P = M	—	—	—
		P > E***	P = E	—****	—***
	Caudal	M > E***	M = E	—***	—**
		P = M	—	—	—
	Cranial vs. caudal	P > E***	—**	P = E	P = M
M > E***		—*	—**	—**	
Porosity	Cranial vs. caudal	Cr < Cd***	—*	—***	—****
		Cranial	P = M	—	—
	Caudal	P = E	—	—	—
		M = E	—	—	—
Osteon diameter	Cranial vs. caudal	P = M	—	—	—
		P < E***	—*	P = E	P = E
	Cranial	M < E***	M = E	M = E	—*
		Cr < Cd***	—****	—****	—**
	Caudal	P = M	—	P < M***	—
		P < E****	P = E	P < E***	P = E
		M < E****	M = E	M = E	M = E
		P = M	—	—	—
New remodeling events	Cranial vs. caudal	P < E****	P = E	P = E	P = E
		M < E****	M = E	M = E	M = E
	Cranial	Cr < Cd****	—***	—****	—***
	Cranial	P = M	—	—	—
		P = E	—	—	—
	Caudal	M = E	—	—	—
		P = M	—	—	—
	Cranial vs. caudal	P = E	—	—	—
M = E		—	—	—	
		Cr < Cd****	—***	—**	Cr = Cd

¹Cr = cranial; Cd = caudal; Md = medial; Lt = lateral; P = periosteal; M = middle; E = endosteal. —, same relationship with deer calcaneus. Mineral content data of horse, elk, and sheep were obtained from 50 and 70% segments, mineral content data of deer were obtained from 60% segment. Microstructure data of horse, elk, and sheep were obtained from 60% segment, microstructure data of deer were obtained from 50 and 70% segments. *0.05 < P < 0.09; **0.01 < P < 0.05; ***P < 0.01; ****P < 0.001; No symbol = nonsignificant difference (P > 0.09); In the case of medial vs. lateral mineral content, there are statistically significant differences in the horse and sheep. Since these differences are relatively small (<2.5%), they are considered similar to the elk and deer (where no statistical differences were detected).

(Table 3C), which is contrary to expectations that the increased percentage of secondary bone would result in increased porosity (Schaffler and Burr, 1988; Burr et al., 1990); and (4) in all cases, negative correlation of OPD and osteon diameter suggests that increases in OPD may be coupled with reductions in osteon diameter (Table 3D). Additionally, the increased porosity of the tension cortex is associated with increased remodeling rates, which may produce relatively larger osteons and/or osteons with larger canals (Burr and Martin, 1989; de Ricqles et al., 1991, p. 22).

Matrix Ultrastructural Anisotropies, Not Mineral Content or Microstructure, Are Predictable in Tension/Compression Bones

Although mineral content variations are conspicuous in the bones examined in the present study, this is not a consistent finding between the so-called tension and compression cortices of various bones loaded habitually in bending. For example, mineral content differences are relatively minor between opposing regions of the

following “tension/compression” limb bones: (1) <1.0% mineral (ash) content difference between the cranial (“tension”) and caudal (“compression”) cortices at the midshaft of the both the horse radius (Mason et al., 1995; Riggs et al., 1993b) and midshaft of the sheep tibia (Skedros et al., unpublished data), (2) relatively small mineral content difference (2.6%) between the cranial (tension) and caudal (compression) cortices at the midshaft of the sheep radius (Lanyon et al., 1979), and (3) relatively small mineral content difference (3%) between tension and compression cortices at the midshaft of the horse third metacarpal (MCIII) (Skedros et al., 1996).

Similar to artiodactyl and perissodactyl calcanei, data in horse radii (Mason et al., 1995; Riggs et al., 1993a,b), sheep radii (Lanyon et al., 1979), and horse MCIIIs (Skedros et al., 1996) show increased OPD in the compression cortex, compared to the tension cortex of these bones. However, unlike the findings in the calcanei, this increased OPD is not coupled with alter-

ations in osteon diameter or porosity (Mason et al., 1995; Skedros et al., 1996, unpublished data). In contrast to these data showing inconsistent relationships of mineral content and microstructure with a customary tension/compression milieu, quantitative data demonstrate that conspicuous regional patterns of matrix ultrastructural anisotropies (MUAs) (i.e., preferred collagen fiber orientation and/or mineral crystallite orientations) are highly predictable between the tension and compression regions of these calcanei (Skedros, 1994). Similar predictable regional patterns of MUAs have been reported in other *in vivo* strain-gauge-documented tension/compression limb bones, including horse MCIIIs (Skedros et al., 1996), and horse, sheep, and dog radii (Mason et al., 1995; Riggs et al., 1993a,b; Skedros, 1994; Takano et al., 1996). Based on the hypothesis that a consistent strain history of sufficiently long duration may be reflected in a bone's structural/material organization, these findings strongly suggest that strain modes are important influences in the attainment of the regionally heterogeneous cortical ultrastructural morphology of these limb bone diaphyses (Mason et al., 1995; Riggs et al., 1993a,b; Skedros et al., 1996).

*Medial and Lateral Cortices: Similar Strain Milieu?
Possible Adaptations to Shear?*

Similar to results in mule deer calcanei (Skedros et al., 1994a), medial and lateral cortices of horse, elk, and sheep calcanei show relatively similar mineralization (Tables 1 and 4; Fig. 4). We have suggested that the medial and lateral regions of these bones can be considered "internal controls" where adaptations in these opposing cortices would be similar because they are located along the theoretical neutral axis where the strain environment would be predicted to be uniform (Fig. 1) (Skedros et al., 1994a). We based this strain distribution on the analogy of the artiodactyl calcaneus as a relatively simple, nonprismatic cantilevered beam loaded at its free end, with shear stresses predominating along the neutral axis (Gere and Timoshenko, 1984).

In addition to tension and compression strains, shear strains represent a form of strain mode which may also have a role in mediating bone adaptation (Burr, 1992; Frost, 1990a). Mechanical properties of cortical bone are also markedly different in tension, compression, and shear (Cowin, 1989; Keaveny and Hayes, 1993; Norman et al., 1996). Consequently, if the bone tissue in each major cortical region has adapted in specific ways to specifically enhance mechanical properties in each customary strain mode, then the medial and lateral cortices may exhibit features of material organization that are uniquely different from the tension or compression cortices. However, inconsistent differences in OPD, FASB, and osteon diameter between the medial and lateral cortices in all three species suggest that there may have been important differences in the strain histories of the opposing medial and lateral cortices (Tables 1, 2, and 3). The possibility that the location of the neutral axis may shift, and/or be more obliquely oriented in the transverse plane (vs. the expected medio-lateral orientation) during some loading states (Alexander and Bennett, 1987; McMahan et al., 1995),

may explain some of these unexpected variations. Absence of medial vs. lateral differences in NRE in all three species also suggests that microstructural differences between these cortices can not be explained by differences in active remodeling (renewal).

Frost's Mechanostat Theory and Intracortical Microstructure and Remodeling Activity

The majority of the microstructural variations described in the present study can be explained by Frost's Mechanostat Theory of mechanically induced bone adaptation. According to this theory, as illustrated by Frost's example of an idealized portion of a limb bone loaded in bending (Fig. 6), the mechanical stimulus for modeling/remodeling activities is strongly influenced by strain magnitude thresholds (Frost, 1990a,b, 1997). In our study of mule deer calcanei (Skedros et al., 1994a,b), we concluded that the lower mineral content, higher porosity, and different osteon microstructural features of the tension cortex, compared to the other cortices, were primarily the consequence of locally increased activation of basic multicellular units (BMUs = forming secondary osteons) (Parfitt et al., 1996) in the tension cortex. In accordance with the Mechanostat Theory, we suggested that the increased remodeling activation results from strains that typically are below a putative minimum effective strain (MES) remodeling threshold.

In contrast, we suggested that the compression cortex exists above the MES remodeling threshold, but within a physiologic loading range, where remodeling activity is relatively repressed (Figs. 6 and 7). The idea that tension cortices of these calcanei *chronically* exist below their remodeling MES, has been attributed to stress-reduction caused by the plantar ligament and tendon of the superficial digital flexor muscle, which course along the entire caudal margin of the tension cortex (Skedros et al., 1994b) (Fig. 1). The fact that the plantar ligament forms an enthesis along the entire calcaneal tension cortex of the three species (Fig. 6c) may also, through mechanical and/or metabolic demands (Skedros et al., 1994a,b), influence the production and maintenance of relatively lower mineral content of the periosteal region. Consequently, it may be more appropriate to describe the tension cortex of artiodactyl and perissodactyl calcanei in a functional context as a component of a "fibro-osseous cortex."

Alternatively, the increased remodeling in the tension cortex may be the consequence of increased repair-mediated renewal that is required for a locally increased incidence of microdamage. Differences in fatigue properties and failure characteristics of bone when loaded in tension vs. compression lend some credence to this possibility (Caler and Carter, 1989; Carter, 1982; Carter et al., 1981; Carter and Hayes, 1977c; Currey and Brear, 1974; Evans and Riola, 1970; Keller et al., 1985; Levenston and Carter, 1994; Martin and Burr, 1989; Patten, 1991). However, this possibility contradicts the Mechanostat Theory since increased remodeling for fatigue-related microdamage, although possible, is unlikely in the low strain environment (Schaffler et al., 1990) of the tension cortex. This conclusion is supported by recent data that demonstrate no regional differences in prevalence of *in vivo* microcracks be-

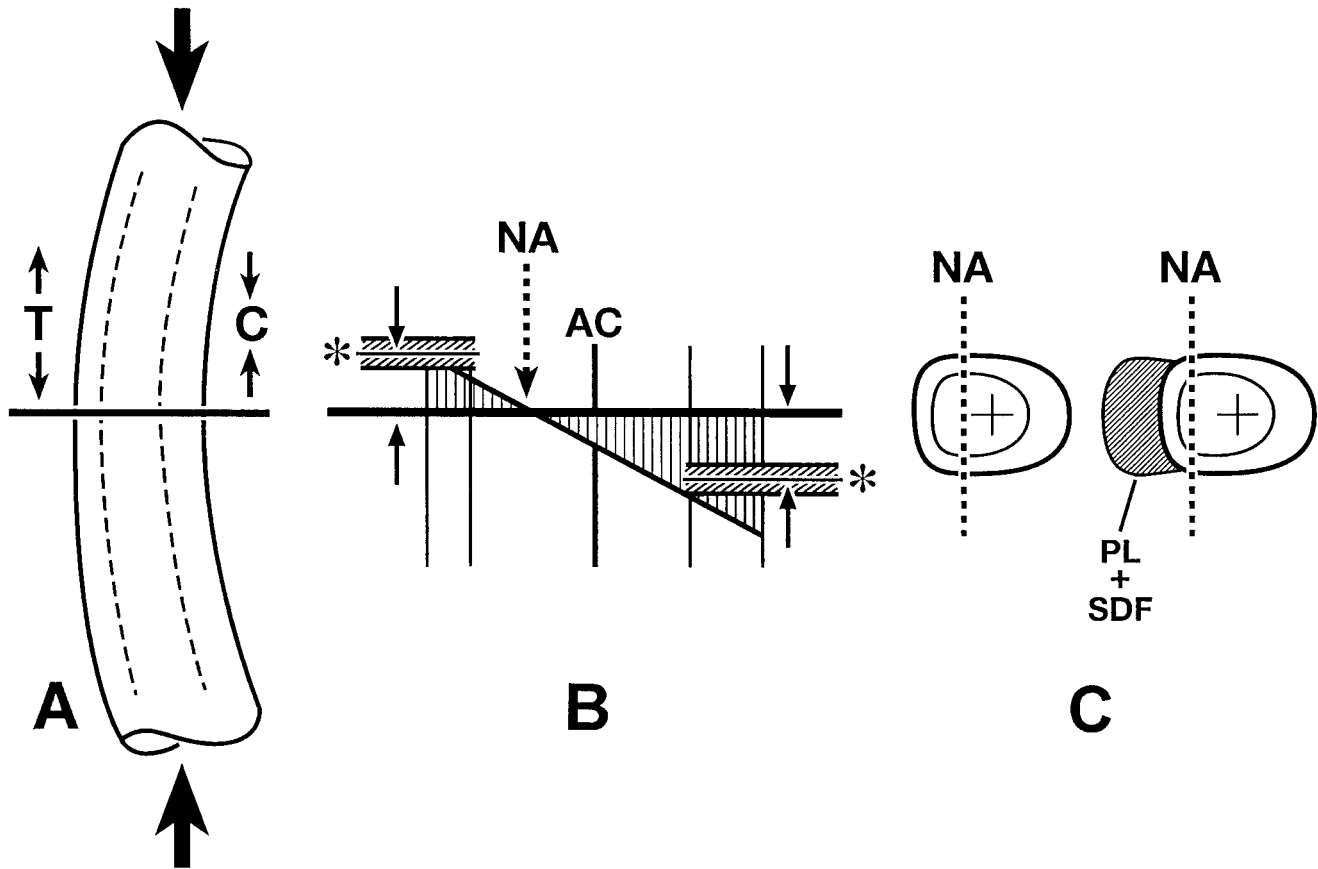


Fig. 6. **A**) This figure illustrates how the remodeling activities are thought to be governed by strain magnitudes, in accordance with the Mechanostat Theory. The drawing on the left shows the combined loading (axial compression and bending) of Frost's idealized diaphyseal segment of a limb bone. **B**) The drawing in the center shows the strain distribution across the horizontal line drawn across the bone segment in (A). Shown are the anatomic center (AC) of the bone, neutral axis (NA), average remodeling MES values (asterisks and paired vertical arrows), and MES ranges (narrow paired parallel horizontal lines with intervening oblique lines). Note that the customary strains (vertical lines) on the compression side (right side of the drawing) of the strain distribution typically exceed the average remodeling MES across the entire breadth of the compression cortex. In contrast, customary tensile strains (left side of drawing) typically are below the average remodeling MES. Accordingly, the entire

tension cortex would be in a state of perceived disuse, and hence would exhibit active remodeling (see Fig. 7), whereas the entire breadth of the compression cortex would have very little remodeling activity since its strain milieu is within the interval above its remodeling MES but below the threshold for high-strain or microdamage-mediated remodeling. **C**) Diagrammatic transverse cross sections of the bone shown in (A) demonstrate that the imposed eccentric end load: at left) shifts the NA toward the tension cortex, which results in a corresponding reduction in strain magnitudes, and at right) when the stress-reducing affect of the plantar ligament (PL) and tendon of the superficial digital flexor (SDF) of the calcanei are considered, the NA would be shifted even farther and strains reduced. This illustrates a theoretically plausible, and purely mechanical, role that the associated fibroelastic tissues have on altering customary strain distributions and magnitudes during functional loading.

tween tension, compression, medial and lateral cortices of elk and sheep calcanei (Skedros et al., 1995a).

According to the Mechanostat Theory, intracortical strain thresholds, which in theory can occur at some distance from the neutral axis (Figs. 1 and 6), influence modeling and remodeling activities on and within the compression and tension cortices of bones that are habitually loaded in bending (Frost, 1988a,b, 1990a,b). We interpreted the periosteal-to-endosteal variations in OPD in both the tension and compression cortices of mule deer calcanei as adaptations in the context of these thresholds (Skedros et al., 1994a,b). However, the inconsistent transcortical microstructural variations reported in the present study challenge this interpretation (Table 4). Additionally, Mason et al. (1995) did not find progressive changes in OPD toward the neutral axis of sections taken at the midshafts of horse radii.

Furthermore, an investigation of the horse MCIII showed no transcortical microstructural variations in association with transcortical variations in strain magnitudes produced during controlled locomotion (Skedros et al., 1996). In view of these conflicting data, we share the opinion of Frost (1990b, 1997) and others (Martin and Burr, 1989, p. 184; Pacifici, 1996) who have suggested that non-mechanical factors related to the proximity of the medullary canal are probably important in influencing remodeling of the adjacent bone.

Alternative Explanations

Insufficient statistical power may also confound interpretations of the relevance of some of the microstructural data. Compared to results of microstructural analyses in mule deer calcanei (Skedros et al., 1994b), the relatively larger standard deviations of some of the

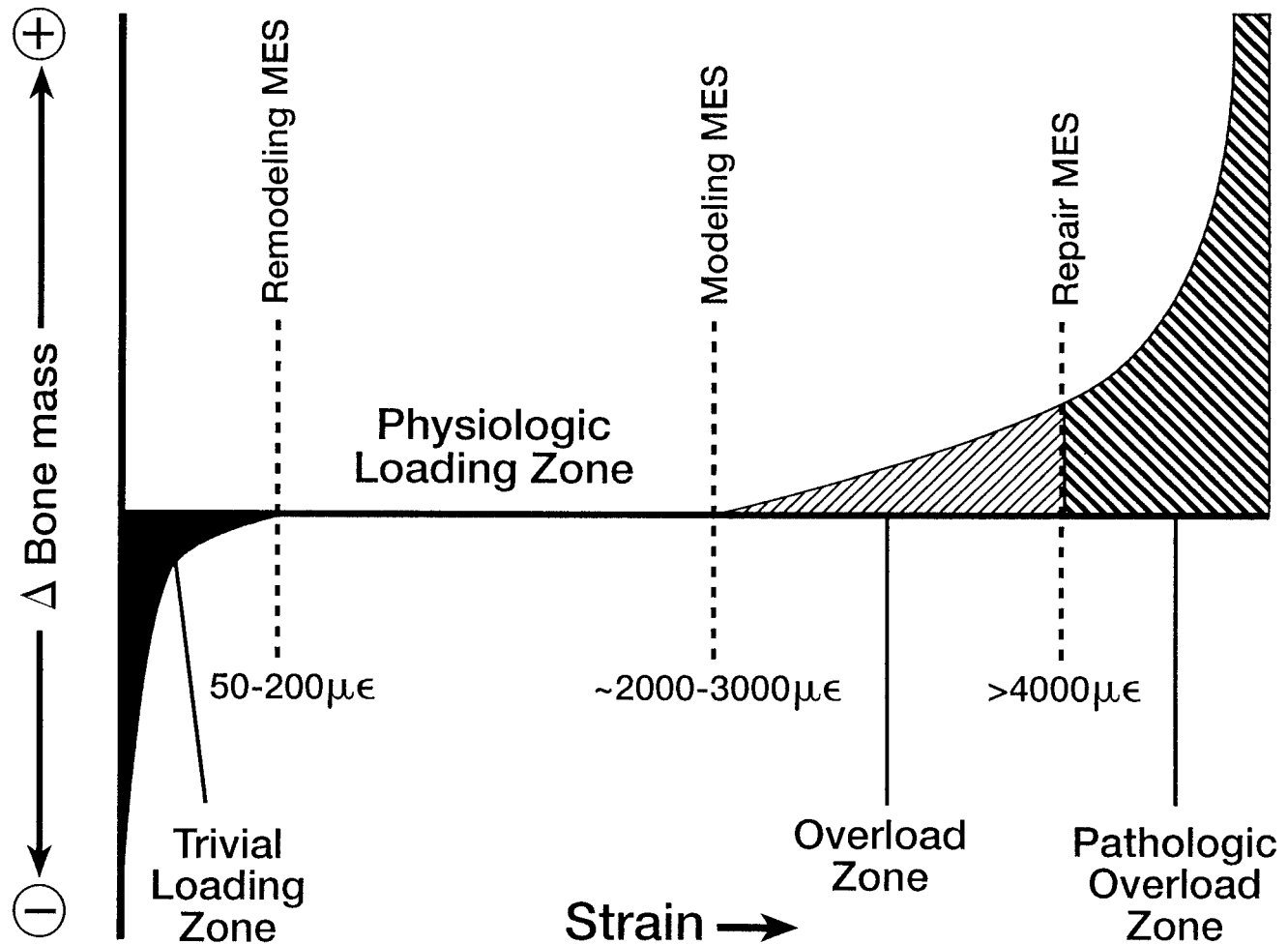


Fig. 7. The four mechanical usage windows or zones, according to the Mechanostat Theory (after figure in Burr 1992). This figure emphasizes that there are differences between thresholds for modeling and remodeling activities. Below the remodeling MES (trivial loading zone), strains are low and bone remodeling is activated. Above the remodeling MES but below the modeling MES (the physiologic loading

zone), remodeling activity is relatively repressed, and is also under the influence of hormonal and metabolic influences. Above the modeling MES, lamellar bone is gained through increased modeling. Above the repair MES (pathologic overload zone), new woven bone is added rapidly to bone surfaces. This is neither modeling nor remodeling, but probably represents a repair reaction.

microstructural data in the present study suggest that increases in sample size may provide increased confidence in differences or similarities between some of the reported comparisons. For example, the apparently anomalous equivalence of NRE in the tension and compression cortices of sheep calcanei may be the result of inadequate statistical power. This conclusion is supported by results of a recent study that clearly demonstrated that in mature sheep calcanei ($n = 11$) the tension cortex had over threefold more NRE than the compression cortex ($P < 0.01$) (Skedros et al., 1995a).

Cortical drift may affect the data by having unexpected influence on mean tissue age, which may affect both mineral content and microstructure. However, measurements in a growth series of mule deer calcanei demonstrate that the antecedent drift, which could have affected the cortical regions sampled in the present study, resulted from bone apposition on the peripheral margins of only the cranial cortex (Skedros et al., unpublished data). Consequently, the influence of corti-

cal drift on the present data is deemed minimal in this context.

Although some of the variations reported in this study may be adaptations to regional disparities in specific strain-related loading environments, other recognized and unrecognized mechanical or nonmechanical factors may also be important (Rubin et al., 1992; Skedros et al., 1994b). It is not clear if the "adaptations" described in the present study are strongly influenced by stresses and/or strains resulting from functional activity (epigenetic), or are primarily the product of the expression of genetic influences. In fact, some investigators have suggested that histologic differences between cortical regions of mature bone may be primarily the product of regional differences in growth and/or growth-related remodeling rates, and hence may not represent local mechanically relevant adaptation (de Ricqles et al., 1991; Enlow, 1962, 1963; Oyen and Russell, 1982; Smith, 1960). Apparent structural and material adaptations in bone must therefore be considered in three

broad contexts (Biewener and Bertram, 1993; Bromage, 1992; Oster et al., 1988; Smith et al., 1985), emphasizing the possibilities that underlying mechanisms that produced the variations may be: (1) unrelated to the strain environment (genetic), (2) related to strain stimuli, but are different from, or are not longer responsive to, the stimuli that exist in later stages of ontogeny (developmentally constrained), and/or (3) are strongly influenced by stimuli associated with functional loads (epigenetic). To examine these possibilities, studies correlating in vivo strain milieus with apparent adaptations in an ontogenetic series of bones are required (Biewener and Bertram, 1993).

In summary, the majority of material variations in these bones can be explained in the context of Frost's Mechanostat Theory of mechanically mediated bone adaptation. If this interpretation is correct, these bones are unusual models for examining the regulation and kinetics of BMU remodeling, since one cortex appears to be actively remodeling as a result of chronic stress-shielding. Consistent regional patterns of MUAs in these calcanei and in other tension/compression bones suggest an important role for strain mode in affecting these variations. This is an important hypothesis since strain mode is a specific variant factor (Rubin et al., 1990; Skedros, 1994) of a bone's internal strain milieu. Interpretations emphasizing preeminent roles for strain magnitude and mode do not preclude the possibility that other specific strain features may contribute to a complex adaptive signal.

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