Ontogenetic Structural and Material Variations in Ovine Calcanei: A Model for Interpreting Bone Adaptation

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ABSTRACT

Experimental models are needed for resolving relative influences of genetic, epigenetic, and nonheritable functionally induced (extragenetic) factors in the emergence of developmental adaptations in limb bones of larger mammals. We examined regional/ontogenetic morphologic variations in sheep calcanei, which exhibit marked heterogeneity in structural and material organization by skeletal maturity. Cross-sections and lateral radiographs of an ontogenetic series of domesticated sheep calcanei (fetal to adult) were examined for variations in biomechanically important structural (cortical thickness and trabecular architecture) and material (percent ash and predominant collagen fiber orientation) characteristics. Results showed delayed development of variations in cortical thickness and collagen fiber orientation, which correlate with extragenetic factors, including compression/tension strains of habitual bending in respective dorsal/plantar cortices and load-related thresholds for modeling/remodeling activities. In contrast, the appearance of trabecular arches in utero suggests strong genetic/epigenetic influences. These stark spatial/temporal variations in sheep calcanei provide a compelling model for investigating causal mechanisms that mediate this construction. In view of these findings, it is also suggested that the conventional distinction between genetic and epigenetic factors in limb bone development be expanded into three categories: genetic, epigenetic, and extragenetic factors. © 2007 Wiley-Liss, Inc. Anat Rec, 290:284–300, 2007. © 2007 Wiley-Liss, Inc.

Key words: sheep calcaneus; bone adaptation; collagen fiber orientation; cortical thickness; osteons

The modeling and remodeling processes that produce a bone's ultimate morphology are considered to be strongly influenced by specific strain characteristics, strain thresholds, and/or the repair of microdamage induced by the strain environment (Ehrlich and Lanyon, 2002). In turn, there is evidence that a bone's structural and material organization might reflect its loading history (Riggs et al., 1993; Carter and Beaupré, 2001; Skedros et al., 2001; Currey, 2002). In this context, the characteristics of a bone's hierarchical morphologic organization may indicate relative contributions of a given strain stimulus (Fig. 1) (Martin and Burr, 1989; Skerry et al., 1990; Skedros et al., 1997). However, in the appendicular skeleton, the extent to which stimuli produced by functional loading mediate the emergence of a bone's myriad structural and material characteristics is not known. In addition, it is unclear if temporal changes in the emergence of certain morphologic characteristics are

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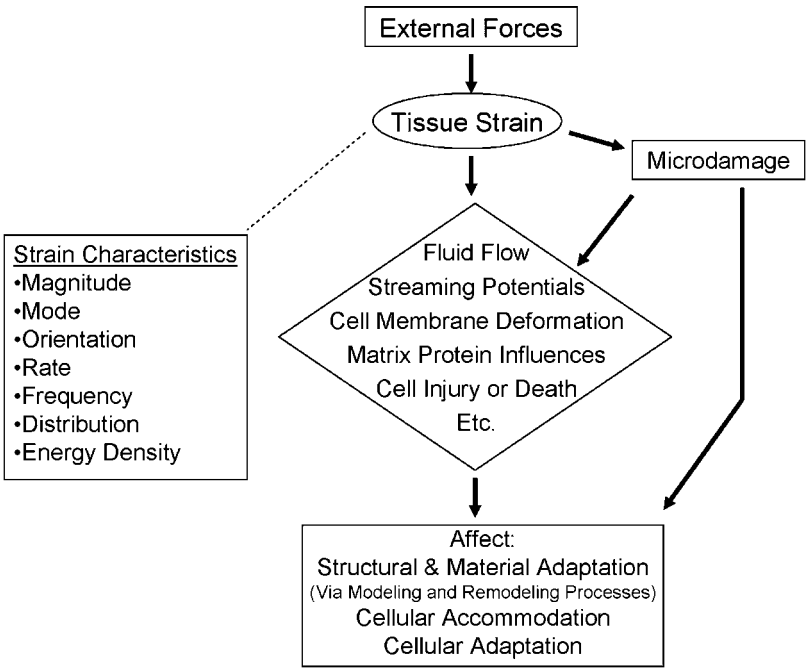

Fig. 1. Proposed bone stimulus-response algorithm. This figure demonstrates that tissue strain may not be as proximate in influencing bone adaptation as are, for example, fluid-flow dynamics and other biomechanical or biochemical stimuli. Cellular accommodation refers to the ability of bone cells to adjust to their physical and biochemical environment. Note that the various mechanisms listed in the central diamond have been proposed as candidate processes mediating bone remodeling (Currey, 2002; Ehrlich and Lanyon, 2002).

produced by extragenetic stimuli (see Appendix for definitions).

Significant regional variations in cortical bone thickness, microstructure (e.g., secondary osteon population densities, osteon cross-sectional areas, and porosity), ultrastructure (e.g., predominant collagen fiber orientation), and mineral content (percent ash) have been reported within transverse cross-sections of calcanei from adult horses, elk, and sheep (Skedros et al., 1997). Developmental changes in the regional distribution or prevalence of these morphologic characteristics have also been reported in an ontogenetic series (fetal to adult) of calcanei from wild mule deer, where most of the significant regional variations (i.e., dorsal vs. plantar cortices) were not apparent until the sub-adult or adult stages (Skedros et al., 2004). This temporal delay may reflect extragenetically induced adaptations (i.e., nonheritable product of the loading environment) aimed at enhancing mechanical properties for prevalent/predominant tension and compression in the respective plantar and dorsal cortices. This is an important hypothesis because it suggests a shift from a dominant role of genetic and epigenetic influences (e.g., positional information and differential growth rates) in earlier appendicular skeletal development to an increasingly important role for extragenetic stimuli (e.g., microdamage events, strain transduction, fluid-flow dynamics) in later development. These ontogenetic morphologic variations, observed in wild mule deer, however, have not been corroborated in a model amenable to experimentation (Table 1). This confirmation is important since examining developmental spatial-temporal variations in bones with well-characterized strain environments may have broad implications for clarifying the mechanisms that produce structure-function relationships in other mammalian limb bones, including those with complex and/or nonquantifiable strain histories (e.g., fossilized bones, or in bones where strain measurements are difficult or impossible to perform in vivo, such as the human proximal femur). Additionally, such investigations are broadly applicable since habitual bending is not only highly conserved in many appendicular long bone diaphyses, but bending also produces the majority (> 70%) of longitudinal strains occurring during peak loading of controlled in vivo activity (Biewener et al., 1986; Biewener and Bertram, 1993), and since structural and material adaptations, such as those shown in artiodactyl and perissodactyl calcanei, are expected in a bending environment because the mechanical properties of cortical bone differ substantially in tension, compression, and shear (Reilly and Burstein, 1975; Carter and Hayes, 1977; Reilly and Currey, 2000).

In order to better establish the artiodactyl calcaneus as an experimental model for studying bone adaptation, two specific hypotheses were considered in this study: one, the data from an ontogenetic series of domesticated sheep calcanei will corroborate structural and material data reported in an ontogenetic series of wild mule deer calcanei (Skedros et al., 2004), and two, the developmental emergence of the regional structural and material differences correlate with a habitual nonuniform strain distribution that begins in utero.

In order to demonstrate broader applications of artiodactyl calcanei as models for examining basic issues in bone adaptation, the following questions are also consid-

TABLE 1. Predicted developmental structural and material changes of the sheep calcaneus from fetus to adult

	Predicted changes	
	Newborn	Adults
Structural characteristics		
Cross-sectional shape	Circular	Elliptical (with long axis in dorsal-plantar direction)
Cortical thickness	Regionally uniform[a]	Thickest cortex: dorsal; Equal medial and lateral cortices
Cortical Inertia (mm^4)		Increased % of total cross-sectional area (i.e., increased robusticity)
Trabecular bone	Lack of arched patterns in lateral view (fetal bones)	Presence of arched patterns in lateral view
Material characteristics		
Mineral content (% ash)	Generally lower[b] Regionally uniform	Generally higher Highest in dorsal cortex Lowest in plantar cortex Intermediate in medial/lateral cortices
Predominant CFO[c]	Regionally uniform No apparent strain-mode relationship[c]	Oblique-to-transverse in dorsal cortex Longitudinal in plantar cortex

[a]"Regionally uniform" means roughly equivalent in the dorsal, plantar, medial, and lateral cortices.
[b]"Generally" refers to the entire cross section (i.e., all regions analyzed).
[c]CFO, collagen fiber orientation; strain mode, tension, compression, shear.

ered in the perspective of the results of this study showing developmental emergence of regional variations in material organization. Is the progressive reorganization of material characteristics possibly a response to regional differences in various components of a bone's habitual strain history (e.g., strain magnitudes, modes, and directions)? What are the likely mechanical consequences of the regional variations in material organization? How are the regional variations in collagen fiber orientation produced? What alternative explanations, other than strain transduction, might be offered to explain the mechanisms that mediate these material variations?

MATERIALS AND METHODS

Artiodactyl Calcaneus Model and Sheep Calcaneus Specimens

In vivo strain gauge data suggest that, during functional loading, the artiodactyl (sheep, deer) calcaneus (Fig. 2) behaves like a short-cantilevered beam with longitudinal compression and tension strains predominating in opposing dorsal and plantar cortices, respectively (Lanyon, 1974; Su et al., 1999). Corroborative ex vivo analyses in deer calcanei reported in detail in recent studies (Su, 1998; Su et al., 1999) include a variety of loading regimes to estimate strain distributions during walking, running, and jumping. These data were obtained from calcanei from sub-adult and adult deer, with each bone being instrumented with up to seven rosette strain gauges. One of these gauges was also placed on the plantar cortex beneath the plantar ligament. In order to estimate affects of turning during ambulation, extreme off-axis loading conditions (with the Achilles tendon loaded 5° medially to the sagittal plane of the bone and 5° lateral to the sagittal plane of the bone) have also been conducted on these bones (Su, 1998). These results of on-axis and extreme off-axis loading demonstrate that in > 80% of stance phase there is a highly consistent distribution of net compression in the dorsal cortex and net tension in the plantar cortex.

One calcaneus was randomly obtained from each of 22 domesticated sheep (*Ovis aries*; breed is crossed Suffolk/Hampshire and Rambouillet) ranging from near-term fetus to adult. Although it is not known if left-right differences in morphology occur in these bones, the random selection process served to minimize this potential variability. The specimens were taken from animals kept in large pastures, and in open range during the majority of the year, near Kemmerer, in Wyoming. Animals were stratified into the following groups: fetal (n = 2); less than 1-month-old or neonate (n = 7, including one stillborn); 6- to 8-month-old (sub-adult; n = 8); and 1.5- to 2-year-old (adults; n = 5). Mean ± standard deviation shaft lengths (mm; Fig. 2) of the bones in each group are as follows: fetal, 22.8 ± 0.7; newborn, 23.0 ± 2.8; sub-adult, 39.1 ± 1.8; and adult, 47.1 ± 2.3. Data reported in the present study are compared to published data from an ontogenetic series (fetal to adult) of calcanei from wild Rocky Mountain mule deer (*Odocoileus hemionus hemionus*) (Skedros et al., 2004).

Radiographic Analysis

Medial-to-lateral radiographs of each bone were examined for the presence of arched trabecular patterns that have been described as adaptations for tension and compression stress trajectories (Lanyon, 1974; Skedros et al., 2001). In this study, no attempt was made to distinguish further the trabecular architecture between fetal, neonate, and sub-adult animals, which span the transition from the earliest primary spongiosa to the highly remodeled/modeled secondary spongiosa.

Shaft Length and Sectioning

The diaphyseal length of each bone was measured using a digital vernier caliper (Mitutoyo, Kanagawa,

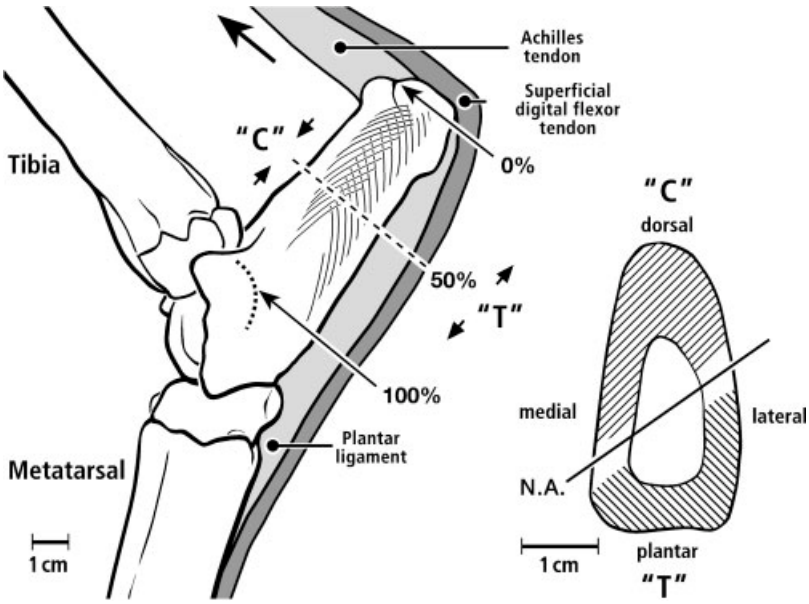

Fig. 2. Lateral-to-medial view of the tarsus region of a skeletally mature mule deer showing the calcaneus, shaft length, and other associated bones, ligaments, and tendons. The trabecular patterns are stylized and are based on a medial-to-lateral roentgenogram. The dotted line at the tip of the 100% arrow indicates the projected location of the contour formed by the talus-calcaneus articular surfaces. The large dorsal-directed arrow indicates the direction of force imparted by the Achilles tendon during mid-stance, loading the dorsal cortex in compression ("C") and plantar cortex in tension ("T") (Su et al., 1999). The cross-section is from a 70% section and shows the location from which the microscopic images were taken and the approximate location of the neutral axis (NA).

Japan), and each bone was cut transversely into three 3 to 6 mm thick segments at the 50%, 60%, and 70% locations (Fig. 2). As described below, measurements of cortical thickness, cross-sectional geometry, and percent ash were made on the 60% segments. The 50% and 70% segments were embedded in polymethyl methacrylate (PMMA) according to published methods (Skedros et al., 1996) and prepared for microscopic analysis.

Cortical Thickness

The cortical thickness of dorsal, plantar, medial, and lateral cortices of each 60% segment were measured to within + 0.01 mm using the digital caliper. Subperiosteal dorsal/plantar heights and medial-lateral widths of the 60% segments were also measured; medial-lateral width was measured midway between the dorsal and plantar endosteal margins of the medullary cavity.

Mineral Content (Percent Ash)

One 3 to 4 mm fragment from dorsal, plantar, medial, and lateral aspects of each 60% cross-sectional segment was analyzed for mineral content by ashing. Mineral content (percent ash) was calculated by dividing the weight of the ashed bone (W_{AB}) by the weight of the dried defatted bone (W_{DB}) prior to ashing, and multiplying this quotient by 100 [$(W_{AB}/W_{DB}) \times 100$].

Circularly Polarized Light Analysis

A 1 mm thick section was obtained from the PMMA-embedded 50% and 70% segments of a subset (n = 20) of bones representing an age range from fetus to adult. These sections were ultramilled to 100 ± 5 microns and analyzed for predominant collagen fiber orientation (CFO) using circularly polarized light (Skedros et al., 1996). Regional differences in CFO were quantified in terms of corresponding differences in the transmitted light intensity, where darker gray levels (lower numerical values) represent relatively more longitudinal CFO and brighter gray levels (higher numerical values) represent relatively more oblique-to-transverse CFO. Transmitted light intensity is referred to as weighted mean gray level (WMGL). The methods used to express regional CFO differences in cortical bone as differences in gray levels (Skedros et al., 1996) have produced relative differences that are similar to the longitudinal structure index used by others (Martin et al., 1996b). Relative differences in CFO between dorsal (D) and plantar (P) cortices are expressed as the ratio of corresponding D/P WMGL differences; in each section, the WMGL obtained from the dorsal cortex was divided by the WMGL obtained from the plantar cortex.

Cross-Sectional Geometric Analyses

An algorithm created for public domain NIH image (v1.61) software (http://rsb.info.nih.gov/nih-image/) was used to obtain the following measurements from scanned tracings of each 60% segment: total subperiosteal area (TA), cortical area (CA), and major axis (Imax) and orthogonal minor axis (Imin) of the second moment of area (inertia, I), polar moment of inertia (J = Imax + Imin, in mm^4), and the CA:TA ratio. According to engineering principles: the bone cross-sectional area (excluding the medullary cavity) in beam-like structures provides an estimate of axial compressive or tensile strength; CA:TA ratio provides an estimate of robusticity; and the Imax:

Imin ratio provides information about the cross-sectional shape and distribution of the material, indicating the degree of deviation from circularity.

Statistical Analysis

The statistical significance ($P \leq 0.05$) of paired comparisons was assessed using a one-way analysis of variance (ANOVA) for multiple comparisons with Fisher's PLSD posthoc test. Comparisons were evaluated using the sheep calcaneus data in the present study and in the mule deer calcaneus data from our previous investigation (Skedros et al., 2004). Least-squares linear and nonlinear regression analyses were used to evaluate growth-related changes. Pearson correlations were used to evaluate comparisons between structural and material characteristics.

RESULTS

Material Characteristics

Mineral content (percent ash; Table 2). The dorsal cortex shows the highest percent ash in all age groups except for the fetal bones, where the plantar cortex had higher percent ash compared to all other cortices. These results and the relatively highly mineralized plantar cortices of several newborns (data not shown) can be attributed to residual calcified cartilage from the resorbing anlagen, as previously described in fetal deer calcanei (Skedros et al., 2004). In contrast, the plantar cortex showed a lower average percent ash compared to all other cortices in the sub-adult ($P < 0.05$) and adult ($P < 0.05$) age groups, although the plantar-medial difference is not statistically significant in adult bones ($P = 0.47$). The sub-adults exhibited statistically significant percent ash differences between medial and lateral cortices, but this average absolute difference was only 1.2%. The ratio of mean dorsal/plantar percent ash remains fairly constant from fetus to adult.

Predominant CFO (Tables 2–4). Although, fetal, newborn, and sub-adult bones showed relative inconsistency in regional CFO variations, young sheep specimens generally showed a dorsal/plantar WMGL ratio greater than 1.0 when compared with mule deer (Fig. 3). Most of the newborn sheep show a dorsal/plantar CFO difference greater than 1.0 as was seen in adult mule deer; this dorsal/plantar difference in the sheep calcanei persists throughout the ontogenetic range studied and is present in all but one adult specimen. In both sheep and deer, low positive correlations exist between bone length and dorsal/plantar CFO ($r = 0.296$ and 0.036, respectively; $P > 0.2$ in both); this can be attributed to the data scatter noted in the younger age groups. Predominant CFO becomes progressively more longitudinal in both dorsal and plantar cortices (Fig. 4, Table 4). Additional correlations are shown in Table 3, and the following are the most notable differences in comparisons between all bones vs. only the sub-adult and adult bones, respectively: CFO vs. percent ash, $r = -0.435$, -0.050; J vs. CFO, $r = -0.564$, -0.224; and J vs. cortical thickness, $r = 0.704$, 0.301.

Structural Characteristics

Cross-sectional shape and cortical thickness (Tables 3 and 4). Throughout ontogeny, both sheep and deer calcanei exhibit greater expansion in the dorsal-plantar direction than in the medial-lateral direction, with the height:width ratio increasing linearly (i.e., along the dorsal-plantar bending axis; $r^2 = 0.85$; $P < 0.001$). Imax:Imin (Fig. 5A) reveals a steep linear age-related increase, which is more dramatic in the mule deer than in the sheep. In contrast, polar moment of inertia (J), which correlates with torsional rigidity, exhibits a nonlinear increase that is more rapid in the sheep bones (Fig. 5B). Both cortical area (CA) and total area (TA) progressively increase in both species, with a linear increase in CA:TA ratio (Fig. 6). As in mule deer, sheep calcanei also exhibit greater increase in the dorsal cortex relative to the other cortices (Fig. 5C). Thus, in cross-section, the calcaneus is quasicircular in the late fetal stages and becomes steadily more elliptical with growth, primarily because of greater bone appositional growth in the dorsal direction (Fig. 7).

Trabecular bone patterns. Examination of lateral radiographs of fetal bones revealed the presence of arched trabecular patterns in both species. These patterns are easily recognizable and are grossly similar to those seen in bones of all other age groups.

DISCUSSION

In support of the first hypothesis, the spatial/temporal structural and material variations that we found in our sample of domesticated sheep calcanei generally closely corroborate those previously reported in calcanei of wild mule deer (Skedros et al., 2004). Consistent with results in wild deer calcanei, statistically significant differences in predominant collagen fiber orientation (CFO) in dorsal vs. plantar cortices also emerged by the sub-adult stage. These differences correlate well with the corresponding compression/tension strain distribution described in vivo in sheep calcanei (Lanyon, 1974) and ex vivo in deer calcanei (Su et al., 1999). Our sheep calcanei also exhibited relatively more rapid increase in J (polar moment of inertia, an index of torsional rigidity) relative to bone shaft length (Fig. 5B). This may reflect the existence of relatively more prevalent shear as a consequence of torsional loading in sheep calcanei than in deer calcanei. Support for this possibility, however, presumes that behaviors producing shear/torsion are relatively more prevalent/predominant in immature sheep, which seems unlikely in view of available data on sheep behavior (Banks, 1964; Grubb, 1974). Furthermore, habitual torsion, which produces prevalent shear stresses, is also highly correlated with annular cross-sectional geometry, in contrast to a more oval cross-section typically seen in habitual bending (Fig. 7). A more probable explanation for the more rapid increase in J in sheep calcanei relative to deer calcanei reflects their relatively more rapid increase in body mass, which is expected since they are raised for consumption. This idea is supported by results of studies in growing humans, showing that body mass was the strongest single predictor of femoral cross-sectional geometry (van der Meulen et al., 1996).

Since it is probable that dorsal/plantar bending is consistent throughout the development of sheep and deer calcanei (including in utero), hypothesis 2 suggests that

TABLE 2. Percent ash and collagen fiber orientation (CFO) (mean values ± standard deviations) stratified into age categories. Sheep data are from the current study and deer data are from a previous study (Skedros et al., 2004)

SHEEP					DEER (from Skedros et al., 2004)				
Cortical Region	% Ash Mean S.D.	D:P or M:L % ash ratio	CFO Mean S.D.	D:P or M:L CFO ratio	Cortical Region	% Ash Mean S.D.	D:P or M:L % ash ratio	CFO Mean S.D.	D:P or M:L CFO ratio
Fetal Sheep (n = 2)					Young Fawn (n = 9)				
Dorsal	60.9 ± 5.2	1.0	152.6 ± 0.4	1.1	Dorsal	64.6 ± 3.8	1.0	126.1 ± 19.0	1.1
Plantar	62.3 ± 1.4		143.2 ± 55.0		Plantar	62.1 ± 4.8		114.3 ± 32.6	
Medial	57.2 ± 1.0	0.9	155.2 ± 4.5	1.1	Medial	62.2 ± 5.5	1.0	128.3 ± 16.9[c]	1.4[c]
Lateral	60.8 ± 3.1		138.5 ± 3.9		Lateral	64.9 ± 4.7		89.1 ± 19.0	
All regions	60.3 ± 2.7		147.4 ± 16.0		All regions	63.4 ± 4.7		114.4 ± 32.4	
Newborn Sheep (n = 7)					Older Fawn (n = 6)				
Dorsal	62.1 ± 3.4[b]	1.1[b]	161.1 ± 40.1[†]	1.2[†]	Dorsal	69.6 ± 2.0[a]	1.1[a]	104.4 ± 25.6	0.9
Plantar	55.2 ± 6.2		129.0 ± 23.3		Plantar	65.3 ± 3.6		116.5 ± 33.9	
Medial	55.1 ± 3.8	1.0	138.8 ± 32.1	1.1	Medial	67.7 ± 2.0	1.0	115.8 ± 26.5	0.9
Lateral	53.1 ± 7.0		126.3 ± 15.3		Lateral	66.6 ± 3.1		125.8 ± 26.5	
All regions	56.4 ± 5.7		138.8 ± 23.6		All regions	67.3 ± 2.7		115.6 ± 25.4	
Sub-adult Sheep (n = 8)					Sub-adult Deer (n = 11)				
Dorsal	69.9 ± 0.7[a]	1.1[a]	110.7 ± 16.0[b]	1.3[b]	Dorsal	69.7 ± 4.6[a]	1.1[a]	98.3 ± 23.9[†]	1.2[†]
Plantar	66.3 ± 0.9		86.7 ± 14.6		Plantar	65.4 ± 3.6		81.1 ± 12.9	
Medial	67.5 ± 0.9[c]	1.0[c]	119.3 ± 13.2	1.0	Medial	67.8 ± 5.4	1.0	119.5 ± 23.0	1.1
Lateral	68.7 ± 1.2		123.4 ± 20.1		Lateral	67.7 ± 4.9		106.1 ± 36.8	
All regions	68.1 ± 1.1		110.0 ± 16.0		All regions	67.7 ± 4.6		101.2 ± 27.8	
Adult Sheep (n = 5)					Adult Deer (n = 10)				
Dorsal	73.5 ± 0.6[a]	1.0[a]	94.8 ± 13.6[a]	1.5[a]	Dorsal	71.5 ± 2.3[a]	1.1[a]	88.4 ± 18.7[a]	1.4[a]
Plantar	70.7 ± 0.9		63.0 ± 12.2		Plantar	67.1 ± 4.9		63.3 ± 10.6	
Medial	71.1 ± 1.0	1.0	126.0 ± 14.4	1.2	Medial	70.5 ± 2.0	1.0	110.7 ± 24.9	1.1
Lateral	72.3 ± 1.0		107.4 ± 15.9		Lateral	71.1 ± 2.0		102.1 ± 23.1	
All regions	71.9 ± 1.0		97.8 ± 14.0		All regions	70.1 ± 2.8		91.1 ± 26.2	

D = Dorsal; P = Plantar; M = Medial; L = Lateral
[a]= statistically significant dorsal-plantar difference ($P \leq 0.01$).
[b]= statistically significant dorsal-plantar difference ($P \leq 0.05$).
[c]= statistically significant medial-lateral difference ($P \leq 0.05$).
[†]= statistical trend dorsal-plantar difference ($0.05 < P \leq 0.09$).

TABLE 3. Correlation coefficients (r values) for all bones (at left) and sub-adult and adult bones (at right) showing comparisons of structural vs. material characteristics in all cortices combined

		All Cortices							
		% ash	CFO	Cort. Th	Ht (D-P)	Wd (M-L)	J	Lng	
All Bones	% ash	1.000	−0.050	0.410[a]	0.730[a]	0.480[a]	0.695[a]	0.769[a]	Sub-adults and Adults
	CFO	−0.435[a]	1.000	−0.503[a]	−0.285[b]	−0.165	−0.224	−0.267[c]	
	Cort. Th	0.667[a]	−0.601[a]	1.000	0.295[b]	0.244[c]	0.301[b]	0.262[c]	
	Ht (D-P)	0.841[a]	−0.620[a]	0.720[a]	1.000	0.779[a]	0.941[a]	0.939[a]	
	Wd (M-L)	0.814[a]	−0.597[a]	0.687[a]	0.968[a]	1.000	0.888[a]	0.766[a]	
	J	0.800[a]	−0.564[a]	0.704[a]	0.963[a]	0.938[a]	1.000	0.953[a]	
	Lng	0.835[a]	−0.624[a]	0.710[a]	0.990[a]	0.964[a]	0.968[a]	1.000	

[a]$P \leq 0.01$, [b]$P < 0.05$, [c]$P \leq 0.05 < P < 0.09$.
Lng = shaft 'length'; Ht = 'height' (D-P, diameter of entire bone cross-section); Wd = 'width' (M-L, diameter of entire bone cross section); Cort. Th = cortical thickness; D = Dorsal; P = Plantar; M = Medial; L = Lateral.
r values on the bottom left half of the table are data from all the sheep age groups; r values in the upper right half of the table are from data from only the sub-adult and adult groups.

the developmental emergence of morphologic differences between these opposing cortices might represent adaptations that are causally linked to the history of a habitual nonuniform strain-mode (compression/tension/shear) distribution. This hypothesis is based on data showing that the mechanical properties, microdamage/fracture mechanics, and fatigue behavior of cortical bone differ substantially in compression, tension, and shear (Carter and Hayes, 1977; Reilly and Currey, 1999; Hiller et al., 2003), and that cortical bone tissue has the capacity to respond to these prevalent/predominant strain modes or related stimuli by adjusting microstructure, ultrastructure, cross-sectional geometry, and/or cell physiologic responses (Skerry et al., 1988; Takano et al., 1999). Therefore, there are natural selective advantages for limb bones habitually loaded in bending to undergo structural/material reorganization during development in order to accommodate regional variations in prevalent strain modes.

It is plausible that the temporal variations in the emergence of the regionally nonuniform structural and material organization reported herein in sheep calcanei and in our prior studies of deer calcanei reflect shifts in the relative influences of genetic, epigenetic, and extragenetic factors as diagrammatically illustrated in Figure 8. For example, the temporal delay in the emergence of statistically significant dorsal/plantar CFO differences (Table 2), as well as the observed association of CFO with osteon formation, is consistent with the idea that these variations are adaptations to extragenetic consequences, or stimuli, of functional loading. This interpretation does not preclude the possibility that the delay revealed by the CFO data is simply a reflection of the early dominance of factors such as growth rates. In this case, the CFO data for fetal and young sheep calcanei showing high gray levels are consistent with the bright birefringence of woven bone tissue, which composes fast-growing young bone. If the osteonal remodeling that subsequently occurs reflects functionally produced extragenetic stimuli, then these stimuli could include strain-related remodeling/modeling thresholds (Skedros et al., 2001; Lovejoy et al., 2002), and/or the formation and repair of fatigue microdamage, the incidence and morphology of which are known to differ significantly in prevalent/predominant compression, tension, and shear.

TABLE 4. Correlation coefficients (r values) for all bones (at left) and sub-adult and adult bones (at right) showing comparisons of structural vs material characteristics in only dorsal (top) or plantar (bottom) cortices

	Dorsal Cortex	% ash	CFO	Cort. Th	Lng	
All Bones	% ash	1.000	−0.533[c]	0.524[c]	0.903[a]	Sub-adults and Adults
	CFO	−0.791[a]	1.000	−0.270	−0.612[b]	
	Cort. Th	0.864[a]	−0.732[a]	1.000	0.534[c]	
	Lng	0.933[a]	−0.821[a]	0.928[a]	1.000	
	Plantar Cortex					
All Bones	% ash	1.000	−0.613[b]	0.495[c]	0.900[a]	Sub-adults and Adults
	CFO	−0.605[a]	1.000	−0.340	−0.548[b]	
	Cort. Th	0.793[a]	−0.772[a]	1.000	0.350	
	Lng	0.783[a]	−0.835[a]	0.882[a]	1.000	

See Footnote of Table 3 for abbreviations and notes.

For example, forming secondary osteons could reorient collagen during microdamage repair by changing their migration direction as they follow microcracks, which can have different morphologies and/or orientations in tension vs. compression vs. shear environments (Dempster and Liddicoat, 1952; Boyce et al., 1998).

In sheep and deer calcanei and other appendicular bones, there is evidence supporting the possibility that microdamage-directed repair that occurs during development and after maturity is an important mechanism in driving the remodeling activities that ultimately enhance bone tissue-level mechanical properties by removing old bone, repopulating osteocytes, reorienting CFO, introducing secondary osteonal interfaces, etc. (Martin et al., 1998; Currey, 2002). We hypothesize that microdamage and functionally induced remodeling/modeling thresholds are important extragenetic stimuli in evoking bone structural and material enhancements during normal development, and these cause-effect relationships might persist throughout development and into maturity well beyond the time that genetic/epigenetic-derived morphologic modifications are established.

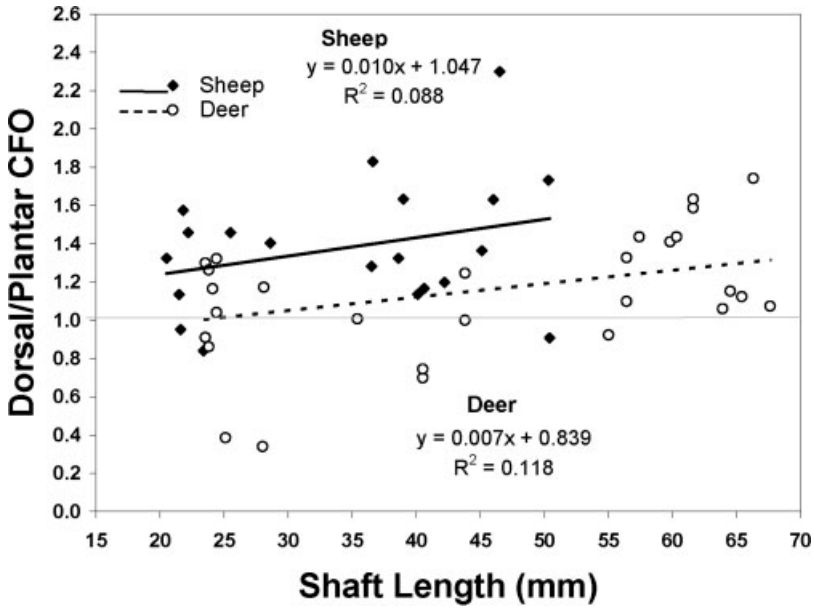

Fig. 3. Ratio of dorsal-to-plantar (D/P) collagen fiber orientation (CFO) versus calcaneal shaft length in mule deer and sheep. Both species follow a similar pattern of increase in dorsal/plantar CFO ratio. The ratios in the sheep are about 20% higher than ratios in deer. Among sub-adult and adult sheep bones, one adult bone had a D/P CFO ratio < 1.

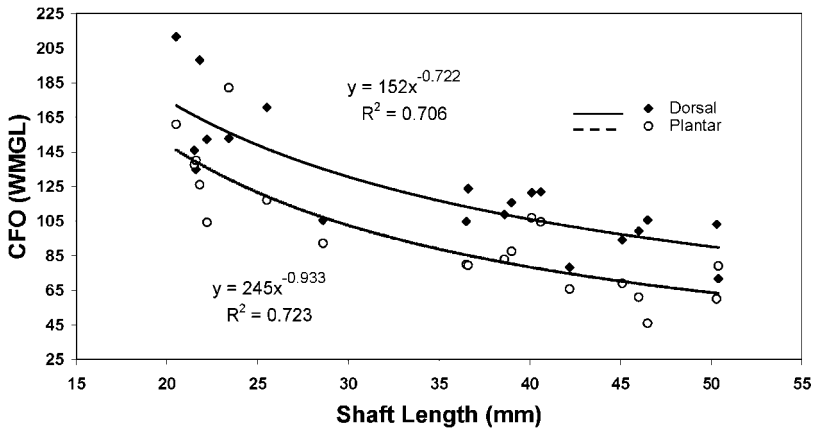

Fig. 4. Predominant collagen fiber orientation (CFO) in dorsal and plantar cortices of sheep calcanei. Note that there is a nonlinear decrease in CFO in both cortices with increasing calcaneal shaft length (length correlates positively with increasing age). Both cortices follow the same trend. The plantar cortex was consistently 20–25 gray levels lower than the dorsal cortex representing more longitudinal collagen in plantar cortices and more oblique-to-transverse collagen in dorsal cortices.

Beyond Intrinsic-Extrinsic, Genetic-Epigenetic Distinctions

The idea that the growth of cartilage anlage of limb bones is generally under intrinsic (i.e., strictly genetic) control, while the deposition of mineral and resulting bone growth is under extrinsic (i.e., nongenetic) control (Murray, 1936), is now considered conventional wisdom by some (Hall, 1985; Wong, 1992; Carter and Beaupré, 2001) despite other studies reporting a more predominant role of extrinsic factors in guiding skeletal morphogenesis (Rodriguez et al., 1992, 2003). In the former context, nongenetic influences of functional loading would be considered as having a dominant role in affecting the postnatal emergence of the morphologic changes shown in this study (Fig. 8). For example, since the Imax:Imin ratio is ~ 1.5 at mid-diaphyseal cross-sections of neonatal deer and sheep calcanei, the steeper curve of the deer might represent more dramatic bending adaptations for more rigorous loading during the early postnatal life of these wild animals. Alternatively, these differences might reflect genetically/epigenetically mediated growth trajectories that are less significantly influenced by extragenetic modifications for loading demands. Compared to extragenetic stimuli, genetic influences are thought to dominate in the early phase of

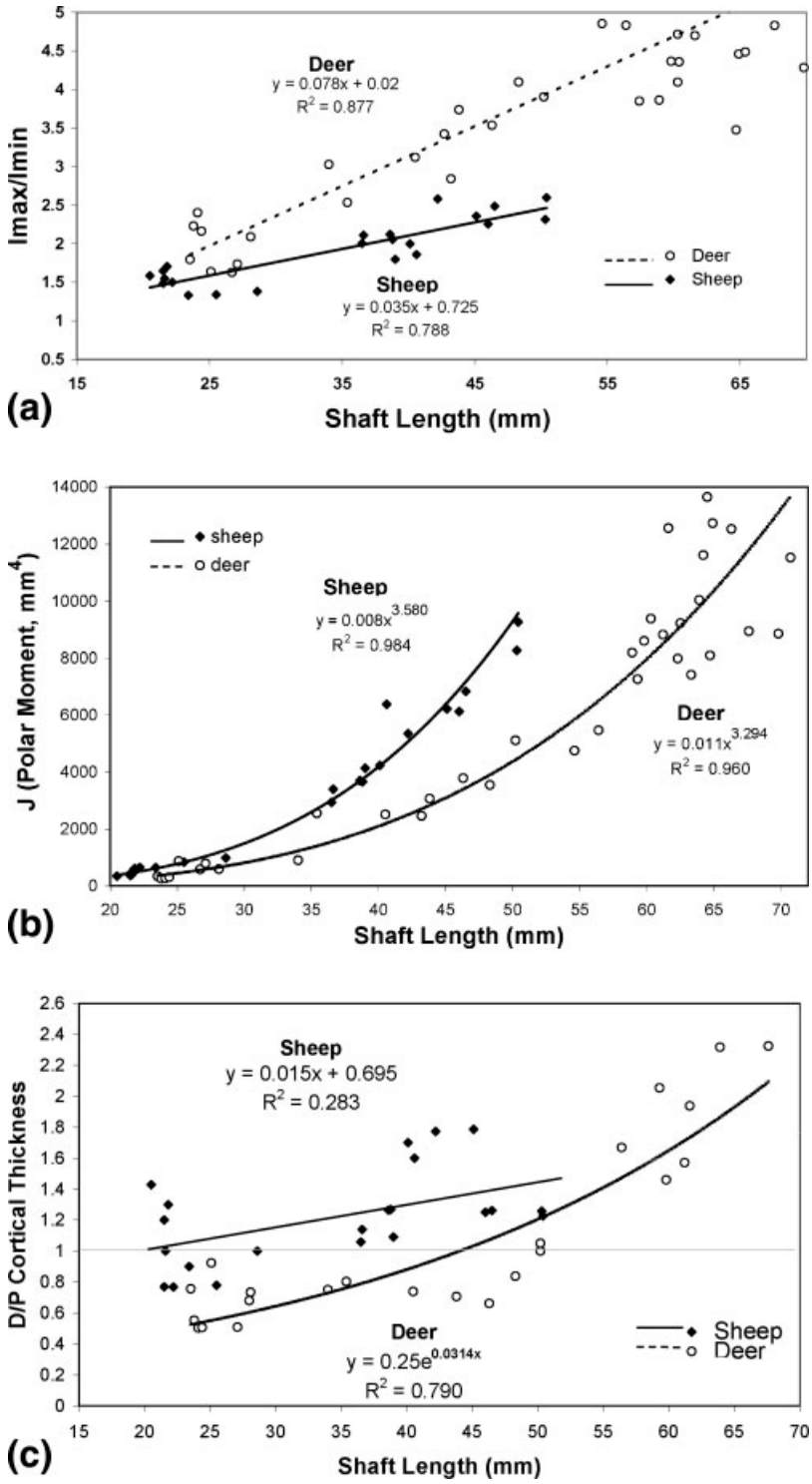

Fig. 5. Results of geometric analyses showing (**A**) Imax/Imin ratio vs. bone shaft length and (**B**) polar moment of inertia (J) vs. shaft length. It is important to note that both the sheep and deer show J scaling with negative allometry to shaft length (exponent of 4 yields isometry). This suggests that J is proportionally higher in the juveniles than it is in the adults (this is shown in Fig. 8). **C:** Dorsal/plantar cortical thickness vs. shaft length.

skeletal mass accumulation and limb-bone development, whereas some of the more obvious epigenetic influences subsequently become important when muscle contractions and weight-bearing stresses become more prominent (Carter and Beaupré, 2001; Henderson and Carter, 2002; Lovejoy et al., 2002). For example, arched trabecu-

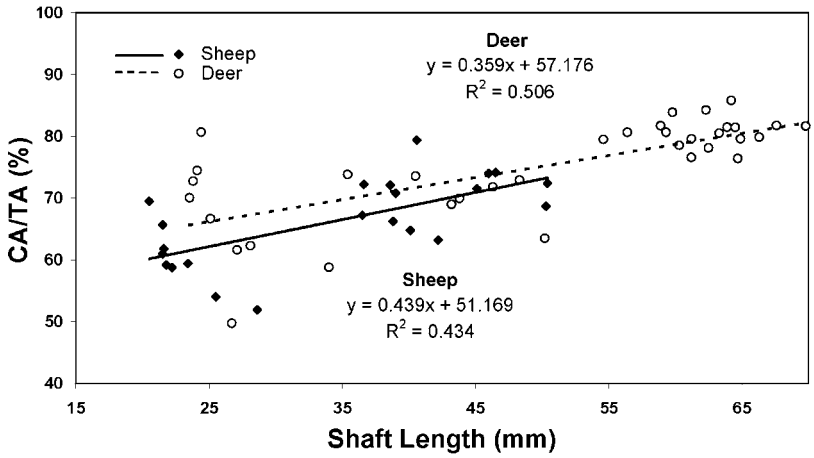

Fig. 6. Cortical area/total area (%) vs. calcaneal shaft length. Both mule deer and sheep exhibit similar linearity in increased CA/TA % with age.

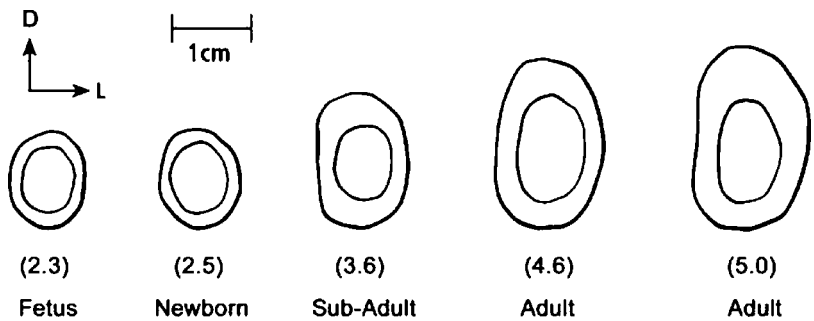

Fig. 7. Ontogenetic changes in cross-sectional geometry of the sheep calcaneus from fetus to adult at 60% diaphyseal length. Numbers in parentheses indicate bone shaft length in cm.

lar patterns found in the human proximal femur, which have traditionally been compared to arched patterns of stress in accordance with beam models, are considered adaptations for tension/compression stress trajectories associated with the onset of unassisted bipedalism (Ryan and Krovitz, 2006). Similar arched trabecular patterns that we observed in all bones (including fetal) examined in the present study have also been shown to correlate strongly with tension/compression stress trajectories (Lanyon, 1974). These observations and the "unmistakable presence of arched trabecular patterns" in lateral radiographs of fetal deer calcanei (Skedros et al., 2004) raise questions as to the relative influence of genetic stimuli on the emergence of these patterns (Skedros and Baucom, 2007).

While the presence of arched trabecular patterns in fetal sheep and deer calcanei suggests a strong genetic basis for their development, little is known about the influence of in utero muscle contractions. There is indirect evidence that the epigenetic influences of in utero muscular loading can produce trabecular and cortical morphologies such as those that we describe herein in the developing sheep calcaneus (Wong, 1992; Carter et al., 1996). For example, Skerry (2000) describes the unpublished qualitative observation of Lanyon and Goodship, who transected the Achilles tendon of a fetal lamb and noted that subsequent prenatal growth produced disorganized trabeculae in the experimental calcaneus compared to the contralateral calcaneus. Surgical tenotomy, however, could significantly perturb vascular, neural, and other nonmechanical aspects of a bone's "functional matrix" (Moss, 1997), hence impairing normal cell-cell interactions and forces that are essential in establishing morphology through positional address. This view is supported by observations that challenge the simple assumption that the forces a bone experiences are primary, or even secondary, in guiding its morphological formation (Biewener and Bertram, 1993). This view is also consistent with computational studies of Carter and colleagues (Carter and Wong, 1988; Carter and Beaupré, 2001), suggesting that positional information is a dominant influence in *early* skeletal development. However, these investigators suggest that positional information becomes less important once the bone anlage is established. In contrast, a growing body of data supports the possibility that pattern formation continues to dominate through a broad range of development, and that the proposed early shift from pattern formation to mechanobiologic regulation probably does not occur (Lovejoy et al., 2000); mechanical forces remain perpetually secondary influences on the emergence of bone structure. This minimizes the traditional importance of mechanical stimuli

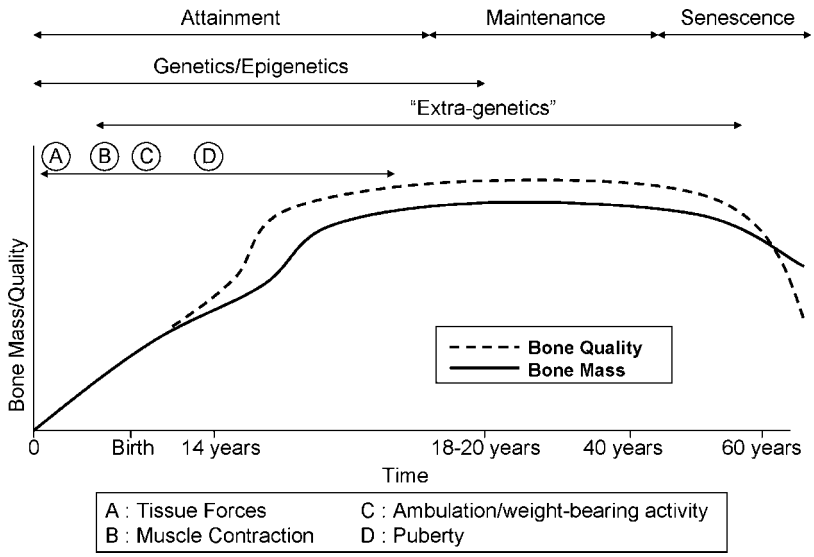

Fig. 8. This stylized depiction of ontogenetic changes in bone mass and quality (e.g., bone tissue-level mechanical properties) in humans is adapted from several sources (Biewener and Bertram, 1993; Kassem et al., 1996; Carter and Beaupré, 2001). With the exception of the pubescent and senescent phases, mule deer and domesticated sheep generally follow analogous paths of mass accumulation (with the exception that sheep accumulate body mass at a relatively faster rate) (Anderson et al., 1974, and personal communication with Truman Julian). This figure helps to conceptualize the putative shifts that we propose exist in the temporal importance of these influences, especially with respect to varying histocompositional characteristics within or between bones. In this context, the regional material variations that emerge during mid-to-late phases of growth of deer and sheep calcanei may serve to enhance tissue-level mechanical properties that accommodate the habitual nonuniform strain environment. It is also interesting to note that the second bone mass growth spurt that appears to occur in humans has not been demonstrated in any other amniote.

in the emergence of morphology in accordance with Wolff's law of the functional adaptation of bone. Additional studies are needed to reconcile the extreme views represented by this pattern formation vs. mechanobiology dichotomy, and how stimuli associated with each might interact. In these studies, regression analyses supporting the early vs. delayed (genetic/epigenetic vs. extragenetic) distinction must not only show a great deal of unexplained variance between bone size and material properties, but also that some of that variance can be explained by genetic or epigenetic influences (e.g., longitudinal and radial growth rates, respectively). We speculate that the mechanisms that produce the spatial/temporal morphologic heterogeneities in the growing sheep calcaneus will reflect relative influences of genetic, epigenetic, and extragenetic factors. Consequently, this bone appears to be a good model for controlled experimental investigations of these possibilities and controversies.

Material Reorganization Might Be Strongly Influenced by Components of Load History

In ex vivo studies of calcanei of wild mule deer using up to seven rosette strain gauges on each bone, Su et al. (1999) demonstrated consistent regional differences in strain modes (dorsal cortex primarily in compression; plantar cortex primarily in tension), strain magnitudes (highest in the dorsal cortex), and principal strain directions (most oblique with respect to the bone's long axis in the medial and lateral cortices). We have speculated that this distribution of these strain parameters and/or other strain-related stimuli (Fig. 1) are important in the emergence of the corresponding regional morphologic variations in sheep and deer calcanei, including predominant CFO (Table 2), secondary osteon population densities (highest in dorsal cortex), and secondary osteon orientation (most oblique in medial and lateral cortices) (Su et al., 1999; Skedros et al., 2004). These explanations have implications for interpreting appendicular bone adaptation across a broad range of terrestrial species because in vivo measurements have shown that directionally consistent bending is a common loading condition (Biewener et al., 1986; Lieberman et al., 2003). However, these interpretations for the ontogenetic changes that occur in sheep and deer calcanei must be considered provisional until they can be confirmed by detailed in vivo strain data.

Confounding Issues and Limitations

An important weakness of this study is that we could not rigorously account for the effects of cortical drift. For example, the use of fluorochrome labels is a method that is commonly used for quantifying differences in regional appositional growth rates. Estimation of regional variations in mean tissue age is also an important consideration because the rate and extent of osteonal remodeling could differ significantly between cortical regions. In addition to changes in cross-sectional shape and cortical thickness of the sheep calcaneus (Fig. 7), our observations demonstrating that cortical drift occurs primarily in the dorsal direction also include the more prevalent circumferential lamellae along the periosteal aspect of

the developing dorsal cortex of sub-adults when compared to the medial, lateral, and plantar cortices. We have also reported similar observations in our previous study of deer calcanei (Skedros et al., 2001). Consequently, without the occurrence of extensive remodeling, the oldest endochondrally derived bony tissue in dorsal/plantar comparisons would be in the plantar cortex. If true, this could confound our interpretations of dorsal/plantar differences in CFO and mineralization data. For example, in Figure 7, it is clear that the representative cross-section of a sub-adult calcaneum overlaps with that of an adult. If the time interval between the sub-adult and adult stage that these sections depict is insufficient to allow for extensive remodeling, then this could result in cross-sections of adults being composed of primary bone that was deposited as a sub-adult as well as an adult. One way to circumvent the influence of this possibility and minimize its influence on statistical dependence would be to restrict the CFO analysis to nonoverlapping bone laminae and treating CFO of primary osteons separate from that of secondary osteons. An alternative method, employed in the present study and in our previous study of deer calcanei, is to allow sufficient time for osteonal remodeling to occur between the growth stages. Histological observations of our specimens demonstrated that the 10–18 months that separate the sub-adult and adult animals allowed for extensive remodeling of the primary bone cortex, which minimizes the potential influence of overlapping regions on statistical dependence. Nevertheless, more rigorous measures of growth rates and mean tissue age would be needed in studies that examine narrower growth intervals. Another important weakness of this study is that aged sheep were not studied, which precluded our ability to evaluate the magnitude of age-related trends.

Regional Material Variations Probably Enhance Toughness and Fatigue Resistance

It is clear that limb bones undergo developmental modifications in structural or material organization that correlate well with mechanical demands of their loading environment (Carrier, 1983; Heinrich et al., 1999; Currey, 2002). In contrast to the present study, however, studies of adaptation in limb bones often focus on structural/geometric variations with little or no consideration of variations in material organization within and between bones of the same animal (Carter and Beaupré, 2001; Currey, 2002, 2003; Ruff et al., 2006). Even when material variations are quantified, the mechanical relevance can be evasive if stiffness and strength criteria are emphasized while failing to consider other aspects of mechanical behavior (e.g., fatigue resistance, toughness, and impact strength) (Martin et al., 1997; Reilly and Currey, 1999; Skedros et al., 2006a). Thus, it seems redundant, in the traditional focus on stiffness and strength criteria, that the dorsal compression cortex of sheep calcanei, in which the bone is naturally more adept at resisting compression and failure in bending, was shown in the present study to be further modified with increased cortical thickness (structural) and the highest percent ash (material). But as demonstrated by results of strain-mode-specific mechanical testing (i.e., compression testing of the habitually compressed dorsal cortex, and tension testing of the habitually tensed plantar cortex) in deer calcanei (Dayton et al., 1997; Skedros et al., 2003), this redundancy, and the additional dorsal/plantar material differences (e.g., differences in porosity, and population densities of secondary osteons and osteocyte lacunae) might be enhancements that accommodate disparities in toughness and fatigue resistance in compression vs. tension (Skedros et al., 2006a). In addition to variations in predominant CFO and percent ash shown in the present study, regional and ontogenetic enhancements of these mechanical properties might also be achievable by adjusting additional material characteristics including osteon morphologies (e.g., size and shape), the degree of collagen crosslinking, and preferred collagen/mineral alignments, all of which can exhibit significant dorsal/plantar differences in these calcanei (Gunasekaran et al., 1991; Skedros et al., 2004, 2006b,c).

Osteonal Remodeling Appears to Mediate Emergence of Regional CFO Variations

Correlations of secondary osteon formation with strain-mode-related ontogenetic changes in regional CFO patterns have been reported in "tension" and "compression" cortices of horse radii (Riggs et al., 1993), sheep radii (Skedros and Kuo, 1999), and deer calcanei (Skedros et al., 2004). Observations of our circularly polarized light images in sheep calcanei also suggest that the developmental reorganization of the collagen matrix in the plantar tension cortex is produced by the formation of secondary osteons with relatively more longitudinal collagen compared to osteons with more oblique-to-transverse collagen in the dorsal compression cortex (Fig. 9). Additionally, although the dorsal/plantar CFO ratio increases with age in deer and sheep (Fig. 3), predominant CFO generally becomes reoriented toward more longitudinal in both the dorsal and plantar cortices, reflecting the fact that woven bone is not retained in either cortex in the analyzed regions. It has been suggested that prevalent shear stresses and/or oblique principal strains might also be important in producing the relatively more oblique-to-transverse CFO observed in the medial and lateral (neutral axis or shear) cortices (compared to the dorsal compression cortex) of sub-adult and adult deer (Su et al., 1999). This organization was also seen in the sheep calcanei examined in the present study. The CFO in these medial/lateral cortices might also be influenced by what appear to be obliquely oriented secondary osteons (seen as oval cross-sectional shapes) as well as the highly transverse CFO in nonosteonal bone (Fig. 9) (Skedros et al., 1997, 2004). Additionally, the observance of bright (lamellar or alternating) osteons in the dorsal cortex and prevalent dark (parallel-fibered) osteons in the plantar cortex might represent mechanically adaptive plasticity of osteonal organization (Hiller et al., 2003; Skedros et al., 2006b) that cannot be simply attributed to variations in their three-dimensional orientation (Riggs et al., 1993; Martin et al., 1996a). Candidates for the upstream cellular mechanisms that mediate regional differences in the formation of different osteon morphotypes include functionally induced, and strain-mode-related, fluid-flow dynamics or electrical potentials associated with osteonal resorption cavities (Martin et al., 1998; Burger et al., 2003).

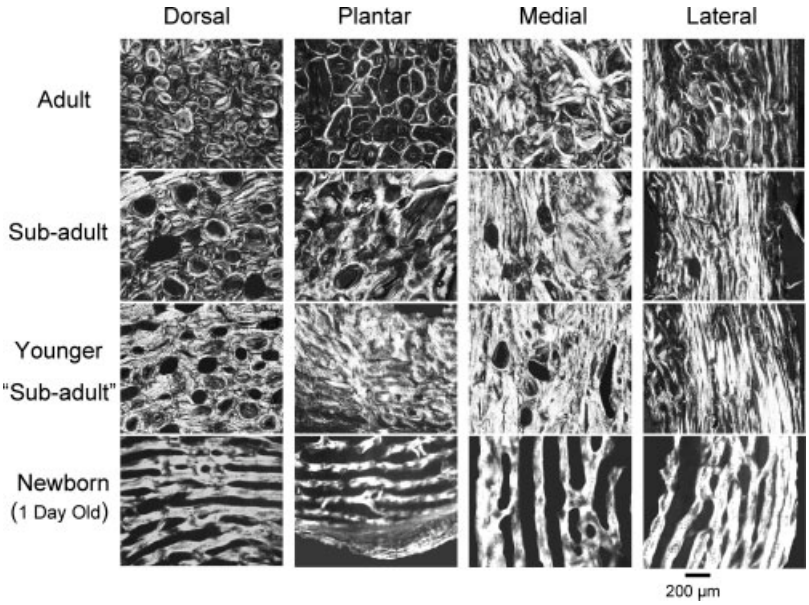

Fig. 9. Representative circularly polarized light images showing prevalent lamellar osteons (i.e., with patterns of alternating birefringence) in dorsal cortices at 70% section locations from the bones of an adult, older sub-adult, younger sub-adult, and 1-day-old newborn. Hoop/parallel-fibered osteons (i.e., with more longitudinal collagen than found in lamellar osteons) begin to appear in sub-adult bones and are prevalent in the plantar cortices of adult bones. Hoop refers to the highly transverse (circumferential) collagen at the osteon periphery (Martin et al., 1996a; Skedros et al., 2006b). Note also that the secondary osteons in the dorsal cortex are smaller than those in the plantar cortex; secondary osteons are absent in the plantar cortex of the younger sub-adult shown here. In all images, dorsal is toward the top edge of the image and lateral is toward the right edge of the image.

Distinguishing Modeling vs. Remodeling, and Confounding Influences of Bone Formation Rate

In contrast to remodeling-mediated adaptations (i.e., those that are produced by secondary osteon formation), modeling events are relatively more important for enhancing whole-bone structural performance (e.g., load predictability) in weight-bearing limb bones (Fig. 10) (Bertram and Biewener, 1988). However, in the artiodactyl calcaneus, the morphology of the talocalcaneal joint also highly constrains loading to the sagittal plane, and the increased thickness of the dorsal cortex simply augments the rigidity of this short cantilever. Consequently, it is unclear if the functional importance of the relative temporal delay in the emergence of dorsal/plantar cortical thickness differences is linked to the emergence of their histocompositional differences. This possibility should be considered since, for example, regional variations in appositional bone formation rates that are typical of normal development can produce corresponding differences in tissue-level mechanical properties, demonstrating that growth rate can confound interpretations of mechanical adaptation (de Margerie et al., 2004). This issue, however, is less important when the bone is remodeled with osteons (Ferretti et al., 1999; Currey, 2003), suggesting that regional differences in growth rates might not be an important confounding variable in the developmental stages when deer and sheep calcanei develop significant differences in dorsal/plantar cortical thickness. The sheep calcaneus may be most useful for comparative studies since, other than availability for testing, its bone remodeling dynamics are similar to humans (Newman et al., 1995; Les et al., 2004).

Alternative Explanations to Strain Transduction

In contrast to the above suggestions supporting further investigation of the idea that microdamage is an important extragenetic stimulus for osteon formation, it has been suggested that less than 30% of osteon remodeling is targeted to microdamage repair (Burr, 2002). Consequently, it is possible that other mechanotransduction pathways might predominate, leading to the nonuniform remodeling/modeling activities in the bones of the present study. These include innervation of bone and changes in the dendritic processes of osteocytes within canaliculi (Colopy et al., 2004; Szczesniak et al., 2005). The strong hypothesis that the development of cortical bone is governed by mechanical stimuli also does not preclude other alternative explanations, such as those that assume that patterning results from the presence of threshold values for signaling molecules (Lovejoy et al., 2002; Tanck et al., 2006). For example, important interactions between mechanics and the mechanisms that mediate patterning seem to occur. This is suggested by studies supporting the idea that mechanical loading is not the direct stimulus for bone remodeling and modeling activities, but rather the stimulus that determines the expression of biochemical signaling molecules (Lovejoy et al., 2002). The local concentration(s) of this putative biochemical factor(s) is subsequently compared to a set point value at the bone surface. Upon exceeding

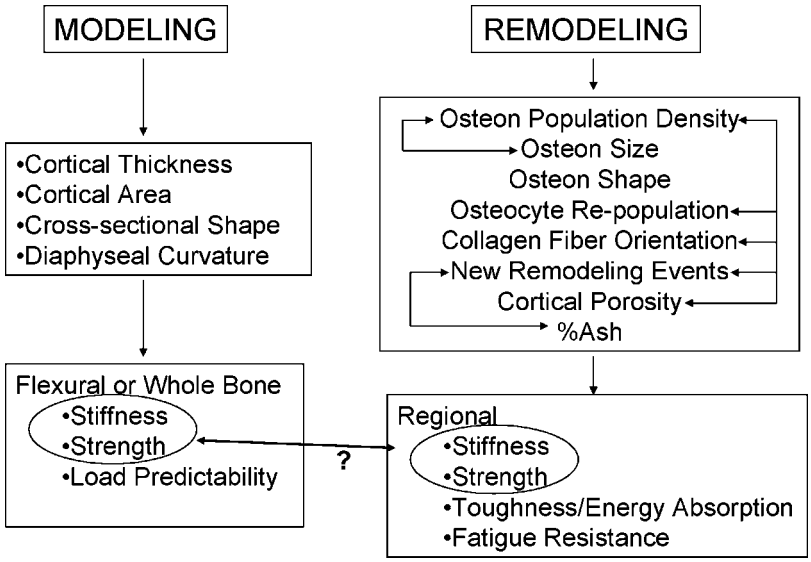

Fig. 10. A structural vs. material division of labor is suggested, respectively, between modeling and remodeling processes in the development and adaptation of a limb bone. In this context, modeling processes seem sufficient for achieving requisite stiffness, strength, and load predictability in a discrete region (e.g., a plantar cortex vs. a dorsal cortex in the same cross-section) and/or in a whole bone (Bertram and Biewener, 1988; Skedros et al., 1996), and remodeling processes influence regional tissue-level mechanical properties primarily in the context of fatigue resistance and toughness. Interrelationships between the material characteristics are shown with arrows and in the context of biomechanically relevant adaptation. Some of these interrelationships might reflect epiphenomena. The question mark indicates that it is controversial whether or not necessary adjustments in regional stiffness and strength in a bone diaphysis are actually achieved by the remodeling process in natural conditions since these adjustments can be satisfied by the modeling process during growth.

a certain threshold, local bone apposition/resorption occurs. The sheep calcaneus has been promoted as a model for examining these issues (Skedros et al., 2001), and experimental protocols for controlling this bone's loading have been described (Skerry and Lanyon, 1995; Thomas et al., 1996).

Our results in sheep calcanei corroborate the emergence of spatial/temporal structural and material variations reported previously in an ontogenetic series of wild deer calcanei. It is hypothesized that these developmental modifications might be differentially influenced by genetic, epigenetic, and extragenetic stimuli, the relative importance of which may also change as development progresses. We also hypothesize that extragenetic strain-related stimuli (e.g., microdamage-targeted remodeling, and/or the establishment or changes in remodeling/modeling thresholds) are important causal influences in the subsequent regional material reorganization of the sheep calcaneus and other mammalian limb bones that exhibit secondary osteon formation. This reorganization may enhance regional tissue mechanical properties that are not strictly defined by stiffness and strength criteria, including fatigue resistance, toughness, and impact strength. An important testable hypothesis is that this process begins early in ontogeny and becomes more strongly correlated with putative extragenetic stimuli as postnatal development progresses. But it is difficult to reconcile this view with the possibility that pattern formation dominates over mechanobiological factors through a broad range of development. In order to examine these possibilities and controversies (e.g., interactions between mechanics and patterning) in the sheep calcaneus, strain distributions measured in vivo throughout ontogeny are needed since these data are crucial for clarifying the mechanisms at work in producing the marked spatial/temporal variations exhibited by this bone. An understanding of this regulation will help elucidate the mechanisms involved in the attainment, maintenance, and objectives of the ultimate structural and material organization of these and other bones. These studies may also help explain how overly restrictive developmental and genetic constraints on postnatal (and even fetal) organ/tissue growth and maturation can constrain or preclude adaptive plasticity and/or accommodation of environmental stimuli.

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APPENDIX

Adaptation

Adaptation in cortical bone commonly refers either to changes in bone structure and/or material organization in response to loading conditions outside a normal physiologic stress/strain range, distribution, and/or duration (Schaffler et al., 1985; Martin and Burr, 1989), or to the presence of regional differences in structural and/or material organization that are strongly influenced by normal functional stimuli occurring during normal development within or between bones (Martin and Burr, 1989; Bertram and Swartz, 1991; Riggs et al., 1993). In the present investigation, the use of the term adaptation is not meant to imply an evolutionary adaptation within a species, but rather a specific adaptation within the lifetime of an individual. In other words, adaptations are considered to be biomechanically relevant regional variations and temporal changes in cortical bone structural and material organization that are produced by the modeling and remodeling processes during normal skeletal development. In addition to being mediated by genetic and epigenetic influences, which are heritable, these processes can be influenced by nonheritable (extragenetic) stimuli such as regional variations in microdamage incidence.

The present study deals with identifying correlations between structure, function, and load history. These correlations might be produced by adaptation via natural selection, and accommodation via epigenetic events. This study suggests that the nonuniform strain distribution experienced in the early development of the ovine calca-

neus is proximate to the historical origin (i.e., mechanistic/causal) of the structure-function relationship. Therefore, the word "adaptation" in this context is deemed appropriate sensu stricto. We avoid the use of the word "accommodation" since we did not attempt to alter the epigenetic signals that influence bone development (like Starck and Chinsamy, 2002).

Epigenetic and Extragenetic Influences in Bone Adaptation

There is an evolving neology for the traditional use, and present polysemy, of the terms "genetic" and "epigenetic." For this reason, we also avoid using the terms "intrinsic" (i.e., "genetic") vs. "extrinsic" (i.e., "epigenetic") influences in skeletal development and adaptation. This is because in the present study, we have expanded the concept of extrinsic factors to include nonheritable extragenetic stimuli. This usage differs from conventional use of "genetic" and "epigenetic" (e.g., see Pearson and Lieberman, 2004).

An epigenetic influence is one in which some environmental factor (e.g., functional ambulation or muscle contraction) allows changes in the full expression of some heritable genetic factor (e.g., some material or structural characteristic encoded in the DNA that would not be similarly expressed without the same proper environmental stimuli). In this sense, it is only correct to use the term "epigenetic" if the changes that occur in bone material and structural characteristics imposed by mechanical loading are heritable (Holliday, 1987; Jones and Baylin, 2002). Hypothesized examples of epigenetic information that can influence gene expression, and that can be transmitted from one generation to the next (i.e., are heritable), include DNA methylation and genomic imprinting (Alberts et al., 2002; Suzuki et al., 2005), transcription regulation (e.g., histone modifications) (Moehrle and Paro, 1994), and positional information (Wolpert et al., 2002; Lovejoy et al., 2003). The field of epigenetics is the study of heritable changes in gene expression and regulation. Epigenetic inheritance is the transmission of non-DNA sequence information. Therefore, epigenetic inheritance provides a rapid and sensitive mechanism by which an organism can respond to its environment without having to change its hardware, because it is simply easier for a genome to pick up a methyl group than to alter a DNA sequence.

Extragenetic (environmental) influences that affect bone adaptation include microdamage events and strains that exceed hypothesized thresholds (Skedros et al., 2001). These influences or stimuli are not heritable (in contrast to genetic and epigenetic influences). It is suggested that extragenetic influences can have important influences on bone's structural and material organization by modifying bone modeling and remodeling processes. However, it is important to consider that while extragenetic stimuli may not be heritable, they do act on genetic and cellular machinery that is heritable. So, as long as the external stimulus is habitual, the effect on bone formation and maintenance can be heritable and produce general trends rather than idiosyncrasies.

Mechanical Strain

Mechanical strain is the change in length of a loaded structure as a percentage of its initial (unloaded) length. This unitless ratio is a measure of material or tissue deformation. In vivo strain data from a variety of animals suggest that physiologically normal strains are generally between 200 and 3,000 microstrain (i.e., between 0.02% and 0.30% change in length) in compression. The upper limit may be only 1,500 microstrain in tension. For an isotropic material loaded axially, stress and strain are related by Hooke's law, which says that they are proportional to one another. Available data suggest that strain is the mechanical parameter most directly involved in mediating bone adaptation (Martin et al., 1998; Skedros et al., 2001; Ehrlich and Lanyon, 2002). The complete record of forces imposed on a bone over a period of time is called the loading history of the bone (Carter and Beaupré, 2001: p. 49). The functional adaptation of a bone to its loading history is best explained or understood by considering the strains and stresses that are created by the applied loads.