Ontogenetic changes of tissue compartmentalization and bone type distribution in the humerus of Soay sheep

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Abstract
We studied ontogenetic changes of histomorphological features and bone type distribution in the humeral midshaft region of Soay sheep from three postnatal age classes (13, 25, and 33 months). Our study demonstrated a marked change of bone type distribution in the humeri with age. In the cortical midshaft region of 13-month-old individuals, periosteal fibrolamellar bone was the dominating bone type. This indicates a rapid bone growth during the first year of life, which was only interrupted by a seasonal growth arrest in the animals’ first winter. In individuals from the two older age classes, periosteal lamellar-zonal bone and intermediate fibrolamellar bone had been formed at the periosteal surface, and endosteal lamellar-zonal bone at the endosteal surface. These bone types are indicative of a reduced bone growth rate. A marked reduction in radial growth was already recorded in the 25-month-old individuals. Distribution and extent of secondary bone showed a marked bilateral symmetry in the humeri of individual sheep. The presence of secondary bone was largely restricted to the anterior (cranial) and the medial cortical areas. This characteristic distribution of remodeling activity within the humeral cortex of sheep is consistent with the view that remodeling activity is largely caused by compressive stress. Our study further demonstrated the presence of a considerable cortical drift in the sheep humeri over the study period, with endosteal resorption occurring predominantly in the posterior (caudal) quadrant and formation of a prominent endosteal lamellar pocket in the anterior (cranial) and medial cortical quadrants.

KEYWORDS
bone modeling, bone remodeling, bone resorption, cortical drift, endosteal lamellar pocket, secondary bone

1 INTRODUCTION
Mineralized structures like bone and teeth preserve information about the activity of their forming cells in their microanatomy (Hogg, 2018). Numerous studies demonstrated that the analysis of bone microstructure allows important life-history traits of mammals to be reconstructed such as life-span, growth rate, and age at skeletal maturity (Klevezal, 1996; Bromage et al., 2009; Köhler et al., 2012; Marin-Moratalla et al., 2013; Cambra-Moo et al., 2015; Jordana et al., 2016; Hogg, 2018; Nacarino-Meneses and Köhler, 2018; Calderon et al., 2019). According to the life history theory, bone microstructure is not only affected by internal factors (age, sex, and genetic makeup) but is also shaped by various external factors, including nutrition and mechanical loading (Frost, 1990; Lieberman et al., 2003; Pearson and Lieberman, 2004; Ruff et al., 2006).
Typically, postcranial bones of mammals develop by a combination of two modes of ossification. In endochondral ossification, a cartilaginous precursor structure is replaced by bone, whereas in intramembranous ossification, bone tissue directly differentiates from mesenchymal condensations without an intervening cartilaginous stage (Francillon-Vieillot et al., 1990; Maggiano, 2012; Huttenlocker et al., 2013). Mammalian long bones elongate through endochondral ossification, while their radial growth occurs via intramembranous bone formation. The growth process leading to the formation of the adult shape of an osseous element is called bone modeling. By this, growing bones adapt to changing biomechanical requirements by adjusting amount and distribution of bone tissue (Frost, 1973; Maggiano, 2012; Maggiano et al., 2016). In the course of long bone modeling, the distribution of bone tissue and the position of the medullary cavity undergo changes in a process called modeling drift.

The term remodeling describes the process of bone maintenance and repair in which older bone is replaced by the spatially and temporally coordinated action of bone-resorbing (osteoclasts) and bone-forming (osteoblasts) cells, collectively referred to as basic (or bone) multicellular units (BMUs) (Enlow, 1963; Stout and Crowder, 2012). Remodeling activity is often triggered by mechanical loads impacting the bone (Lieberman et al., 2003; Ruff, 2005; Ruff et al., 2006; Demes, 2007). The typical structural units of remodeled (secondary; Haversian) bone are secondary osteons, also referred to as Haversian systems. As secondary bone replaces previously existing bone, a typical feature of the former is the presence of reversal lines that mark the early stage of bone apposition at resorption surfaces. However, also during the modeling process, bone resorption can take place. Thus, the bone scaffolds that delimit primary vascular spaces are partly resorbed prior to the start of the infilling process that leads to the formation of primary osteons. Such a situation has been described in endosteal lamellar pockets formed in the course of modeling drift (Maggiano et al., 2015, 2016), and in antler bone (Kierdorf et al., 2013b).

The microanatomy of the bone formed by the periosteaum (outer cortex) and the endosteum (inner cortex) can vary widely, depending on the speed of its formation and the number and orientation of the blood vessels included into the newly formed bone (Francillon-Vieillot et al., 1990; Huttenlocker et al., 2013; Maggiano et al., 2016). Amprino (1947) was the first to suggest that the microstructure of primary bone reflects its growth rate, a relationship now referred to as ‘Amprino’s rule’. Formation of lamellar bone, composed of successively formed layers (lamellae), with the collagen fibers largely oriented in parallel within each lamella, is considered indicative of a relatively slow bone growth rate. In contrast, formation of woven bone, with a more random orientation of collagen fibers and no distinct layering, is regarded as reflecting rapid bone growth. A primary bone type combining woven and lamellar bone is known as fibrolamellar bone. It consists of initially formed woven bone scaffolds that surround primary vascular spaces. Concentric layers of more slowly forming lamellar bone subsequently fill in the primary vascular spaces, thereby leading to bone compaction. These lamellar bone structures, which possess one or more vascular canals, are referred to as primary osteons. Based on the orientation of its vascular spaces, primary lamellar or fibrolamellar bone can be classified as longitudinal, laminar, plexiform, reticular or radial (Francillon-Vieillot et al., 1990; de Margerie et al., 2002; Bromage et al., 2009; Huttenlocker et al., 2013; Warshaw et al., 2017).

Mechanical loading of a skeletal element causes an increase in bone mass and strength, whereas reduced mechanical loading is typically associated with a decrease of bone mass and strength (Lieberman et al., 2003). Fully grown bones react to changes in loading by reshaping their microstructure through the process of remodeling (Currey, 2002). In this way, bones adjust their shape and microstructure to the habitual use of the skeleton (Frost, 1990; Lieberman et al., 2003; Pearson and Lieberman, 2004; Ruff et al., 2006). Remodeling activity results in the formation of Haversian bone that is characterized by the presence of secondary osteons and interstitial lamellae, i.e. remnants of secondary osteons resorbed in the remodeling process (Currey, 2002, 2003).

Several explanations for the initiation of bone remodeling have been put forward. Currey (2003) considers three principle causes that can trigger bone remodeling: (a) damage-driven remodeling that replaces dead bone and takes out microcracks from bone tissue, (b) renewal of bone tissue that because of its age may have become hypermineralized and brittle, and (c) adaptation of bone to compressive loading. Evidence for the latter was reported by Riggs et al. (1993a; 1993b) and Mason et al. (1995), who observed a significantly higher density of secondary osteons in cortical regions of the equine radius exposed to compressive loading compared with other cortical regions. Likewise, Zedda et al. (2015) reported a higher secondary osteon density in posterior (caudal) and medial regions of the equine femur where compressive strains are higher than in the anterior (cranial) and lateral regions, where tension strains are dominating. Similar findings were reported for goat and bovine femora by Mayya et al. (2013, 2016). These authors found that the presence of Haversian bone in the studied femora was largely restricted to cortical regions exposed to high compressive stress, whereas cortical regions exposed to low stress, whether tensile or compressive, exhibited a fibrolamellar/plexiform microstructure, i.e. did not undergo remodeling.

The above studies on ungulate long bones demonstrate that cortical bone exposed to tensile stress undergoes no or only little remodeling and, in consequence, keeps its primary, fibrolamellar microstructure. The collagen fibers in this bone are mainly oriented along the long axis of the bone. By contrast, cortical areas of the same bones subjected to high compressive stress show intense remodeling. The secondary osteons present in these regions mainly exhibit transversely oriented collagen fibers, a texture that is better adapted to withstand compressive loading (Currey, 2002).

In quadrupedal terrestrial mammals, the long axis of the humerus is inclined in posterior (caudal) direction against the vertical limb axis, whereas that of the femur shows an anterior (cranial) inclination. Mayya et al. (2013) hypothesized that therefore the loading of the humeri and, in consequence, the distribution of Haversian and non-Haversian bone in the humeral cortical midshaft of quadrupedal mammals should be reversed compared with the situation in their femora. Thus, they assume the presence of Haversian bone in the
anterior (cranial) quadrant and of primary (non-Haversian) bone in the posterior (caudal) quadrant of the cortical midshaft of ovine and bovine humeri. However, thus far, this hypothesis has not been tested.

Only few studies addressed the question of bilateral symmetry in the remodeling of limb bones. Studies in cats and dogs noted a high symmetry in the distribution of remodeled areas between paired limb bones (Marotti, 1963; Currey, 2002; Currey et al., 2017), suggestive of a highly similar loading pattern. Currey et al. (2017) therefore recently hypothesized that remodeling activity should occur in a largely symmetrical fashion in paired limb bones of an individual. However, these authors also stated that supporting evidence for this assumption is still scarce.

The aim of the present study was to reconstruct ontogenetic changes of histomorphological features in humeri of Soay sheep rams (Ovis aries). We addressed both ontogenetic changes in tissue compartmentalization, i.e. in the spatial distribution of (mineralized) bone and non-bone areas, and of bone type distribution. Although ontogenetic changes in the histomorphometry of sheep humeri and tibiae were previously reported by Cambra-Moo et al. (2015), so far data on the symmetry of modeling and remodeling in sheep bone antimeres are missing. Therefore, we paid special attention to the symmetry in modeling and remodeling activities in paired humeri of individual sheep. We further tested whether the predictions of Mayya et al. (2013) about the distribution of Haversian and non-Haversian bone in the cortical midshaft of humeri of quadrupedal mammals hold for sheep.

2 | MATERIALS AND METHODS

The present study was performed on the humeri of six Soay sheep rams (Ovis aries) from the Soay herd of the Tierpark Arche Warder e.V. Soay sheep were selected for this study because they represent an unimproved sheep breed that may potentially deviate in the analyzed parameters from the improved and significantly larger (Merina) sheep breed previously studied by Cambra-Moo et al. (2015). The study animals were earmarked individually and kept under controlled conditions on a pasture with an open stable and additional food from April to October. From late October to April, the animals were kept in a stable.

The studied Soay sheep were born in April or May 2012. Two animals each were slaughtered at 13, 25, and 33 months of age. All animal treatments were performed in accordance with the current animal care regulations in Germany, and with the permission of the responsible veterinary authorities of the federal state of Schleswig-Holstein (Ministerium für Landwirtschaft, Umwelt und ländliche Räume des Landes Schleswig Holstein; Az. V312-72241.123-34, for further information see Kierdorf et al., 2013a).

The bodies of the slaughtered sheep were dismembered and the humeri extracted. The bones were macerated using an enzymatic solution (Enzyrim OSA, Bauer), defatted, and bleached using Supralan UF (Bauer) and sodium-tetraborate (Lach-Ner). The humeri were then photographed and measured following the protocol of von den Driesch (1976) (Table 1). Thereafter, full-diameter cross-sectional slabs of 2 cm thickness were cut from the midshaft (Figure 1) and embedded in epoxy resin (Biodur E 12, Biodur products, Heidelberg, Germany). Midshaft was determined as the midpoint of the proximo-distal extension of the respective humerus. In the case of still unfused epiphyses, only the diaphysis was measured. Ground sections of 50 and 70 µm thickness were prepared from the embedded specimens following an established protocol (Kierdorf et al., 2013a; 2013b, 2017). The 70-µm-thick sections were used for production of the bone type maps, and the 50-µm sections were

| Animal no. | Age at death (months) | side | GL (mm) | MAP (mm) | MML (mm) | CIR (mm) | EF |
|------------|----------------------|------|---------|----------|----------|---------|----|
| 13         | 13                   | Right | 113.6*  | 17.54    | 12.87    | 47.55   | U  |
|            |                      | Left  | 111.4*  | 16.69    | 13.25    | 46.77   | U  |
| 17         | 13                   | Right | 114.1*  | 16.46    | 12.65    | 46.77   | U  |
|            |                      | Left  | 115.3*  | 16.47    | 13.01    | 46.64   | U  |
| 15         | 25                   | Right | 133.7   | 17.54    | 14.18    | 49.00   | P  |
| 18         | 25                   | Right | 126.5   | 17.49    | 14.93    | 49.66   | P  |
|            |                      | Left  | 126.1   | 17.60    | 14.76    | 49.96   | P  |
| 14         | 33                   | Right | 143.9   | 19.81    | 15.93    | 54.80   | F  |
|            |                      | Left  | 143.6   | 20.22    | 15.77    | 54.20   | F  |
| 16         | 33                   | Right | 137.7   | 17.92    | 14.82    | 50.29   | F  |
|            |                      | Left  | 138.1   | 18.06    | 14.98    | 50.76   | F  |

Note: GL: maximum length (mm), *measurements taken following the protocol by von den Driesch, 1976). MAP: maximum diameter along the antero-posterior (cranio-caudal) axis at the midshaft (mm, taken from the cross-section). MML: maximum diameter along the medial-lateral axis at the midshaft (mm, taken from the cross-section). CIR: circumference at the midshaft (mm, data taken from the cross-section). EF (epiphyseal fusion): U, unfused; P, partially fused; F, completely fused. *Only diaphysis measured.
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used for imaging histological details at higher magnifications. In five of the studied sheep, sections of both humeri could be analyzed. In the sixth individual (no. 15, age group 25 months), the left humerus was damaged during processing and therefore only the right humerus was available for study.

Morphometric data of the humeri were obtained with a sliding caliper (maximum length) and measurements on cross-sections (maximum antero-posterior [cranio-caudal] and medio-lateral diameter, and circumference at midshaft) using a Keyence digital microscope (VHX-500 F). Transmitted light microscopy of the sections was performed with a Keyence Biozero 8,000 digital microscope (Keyence) and an Axioskop 2 Plus microscope (Zeiss), using brightfield (plain and phase-contrast illumination) and linearly polarized light imaging (partly with a λ compensator). Inserting a λ compensator in the optical path of a polarized light microscope causes the occurrence of interference colors in thin, birefringent specimens. The hue of the interference colors provides information about the sign of birefringence (additive or subtractive) and can be used to reconstruct the organization of the collagen fiber matrix in bone.

For producing overview images, the sections were photographed with a 4× objective lens (1.54 microns/pixel) in the Keyence Biozero 8,000 microscope, and the individual images were stitched using the Fiji freeware package (http://fiji.sc) with a stitching plugin (Preibisch et al., 2009). The stitched brightfield images were imported into Adobe Photoshop (Adobe Systems Inc.) for a manual tracing of bone types, creating a 'bone type map' following McFarlin et al. (2016) (Figure 2). Images at higher magnifications were acquired using the Zeiss Axioskop 2 Plus microscope.

In the analyzed cross-sections, four histological bone types were distinguished based on the typological classifications by Francillon-Vieillot et al. (1990), de Margerie et al. (2002), Huttenlocker et al. (2013) and Prondvai et al. (2014). Bone types were identified based on several criteria, such as the amount of tissue porosity, osteocyte lacunar shape, and the distribution and organization of the collagen fiber matrix. The bone type classification used in the present study is similar to that used by McFarlin et al. (2016).

We distinguished the following bone types: (a) periosteal and endosteal lamellar-zonal bone (PLM, ELM), (b) periosteal and endosteal fibrolamellar bone (complex) (PFLC, EFLC), (c) intermediate fibrolamellar-zonal bone (IFL), and (d) Haversian bone containing secondary osteons. Characteristic examples of the distinguished bone types are presented in Figures 3–5.

Figure 3 shows examples of lamellar-zonal bone found in the pericortex (PLM) and the endocortex (ELM) of the Soay sheep humeri. Lamellar-zonal bone has been defined as a slow-forming non-vascular tissue type characterized by a highly ordered arrangement of individual lamellae. The variation in collagen orientation between successive lamellae can be demonstrated by polarized light microscopy (Francillon-Vieillot et al., 1990; de Margerie et al., 2002; Kolb et al., 2015a; 2015b). The osteocyte lacunae in this bone type are spindle-shaped to oval.

Figure 4 shows examples of fibrolamellar bone in the pericortex (PFLC) and endocortex (EFLC) of the Soay sheep humeri. Fibrolamellar bone is a fast forming, highly vascularized tissue. It is characterized by the presence of a framework (scaffold) of woven bone. Infilling of the primary vascular spaces lined by these scaffolds occurs initially by parallel-fibered or lamellar bone and subsequently by concentric lamellar systems (primary osteons) with central vascular spaces (Huttenlocker et al., 2013; Marin-Moratalla et al., 2013). The osteocyte lacunae in the lamellar bone are small and roundish. Whereas Currey (2002) treats the terms fibrolamellar, plexiform, and laminar bone as broadly synonymous, other authors distinguish between different sub-types of fibrolamellar bone based on blood vessel orientation in the vascular compartment, or use terms such as 'laminar' only for the orientation of the vascular system (Huttenlocker et al., 2013). In this study, we use the terms 'laminar' and 'plexiform' to describe vascular orientation in PFLC/EFLC, but not to characterize sub-types of fibrolamellar bone.

Figure 5(a,b) depicts a bone type that exhibits characteristics of both PFLC/EFLC and PLM/ELM. This bone type was designated as intermediate fibrolamellar-zonal bone (IFL). It is characterized by a scaffold formed predominantly of parallel-fibered bone with only occasional occurrence of woven bone. The primary vascular spaces...
have been filled in with primary osteons. Osteocyte lacunae in the parallel-fibered bone are elongated. The primary osteons are elliptical in shape and consist of a few concentric lamellae around the primary vascular canal(s). This tissue type, with a growth rate intermediate between that of PFLC/EFLC and PLM/ELM, was only found in the pericortex.

Finally, secondary (remodeled, Haversian) bone was recorded (Figure 5c,d). The secondary osteons formed in the course of bone remodeling are delimited by reversal lines.

With respect to cement lines, we followed the terminology of Francillon-Vieillot et al. (1990) and distinguished two types of lines under this heading. Rest lines mark a temporary cessation of growth and are formed during slow resumption of bone growth. They occur on un-resorbed bone surfaces and show a smooth outline. Reversal lines mark the restart of bone formation at bone surfaces following resorption and show a scalloped outline, reflecting the former position of resorption (Howship’s) lacunae. Both types of cement lines are typically hypermineralized compared with the surrounding bone (Francillon-Vieillot et al., 1990; Skedros et al., 2005; Huttenlocker et al., 2013).

In the sections, we first determined the relative proportions of the total cross-sectional area covered by, respectively, the medullar cavity, mineralized bone tissue, and cortical porosities (unfilled or partly filled resorption cavities [infilling secondary osteons] and large blood vessel spaces). The Haversian canals of fully formed secondary osteons were excluded. To quantify the percentages of the cortical cross-sectional area covered by the different bone types, we used the spatial analysis software ArcGIS 10.4 (ESRI), following the method of Cambra-Moo et al. (2012). Pre-processed images were added to an ArcMap document and georeferenced. Subsequently, all areas covered by a specific bone type were digitized as polygons in a feature-class, and area size was calculated in the attribute table. Large resorption cavities and larger blood vessel spaces of the bone were mapped separately and erased from the measured area prior to the calculation of the respective area percentages for the bone types and the medullary cavity.
The proportions of cortical bone and medullary cavity were determined along the maximum antero-posterior (cranio-caudal) and the medio-lateral axes.

3 RESULTS

Basic morphometric data of the analyzed humeri are given in Table 1. Values of left and right humeri from individual sheep varied only slightly, thus demonstrating a high degree of symmetry between antagonists. The results show a general trend for an increase with age of the recorded morphological parameters; however, some size differences between individuals of the same age were also present.

Data for tissue compartmentalization in the different age classes, i.e. the respective percentages of the total cross-sectional areas of the humeri covered by the medullary cavity, mineralized bone, and cortical porosities are presented in Table 2. There was a high degree of concordance for these parameters in the paired humeri from individual sheep. The values for the cross-sectional area covered by mineralized bone (overall means: 34.6%–37.0%) varied only slightly between the age classes, thereby demonstrating retention of a relative constant ratio of mineralized to non-mineralized portions in the humeral midshaft, despite the marked increase in overall size of the humeri with age. By contrast, mean cortical porosity showed a declining trend with age, with highest values (mean: 1.9%) occurring in the age class 13 months and much lower mean values (0.3% and 0.4%) in, respectively, the 25- and 33-month-old animals. The main factor influencing cortical porosity was the high number of resorption cavities in the humeral cortex of the youngest age class. The much lower values in the older age classes point to a reduced remodeling activity.

The distribution of the different bone types in the cross-sections is depicted for all analyzed humeri in Figure 6. The percentages of the cross-sectional areas covered by the respective bone types are listed in Table 3. Generally, bone type distribution was relatively similar in the paired humeri of an individual. Larger differences occurred, however, between individuals of the same age class. The most pronounced inter-individual difference was observed for the percentage of secondary osteons in the 13-month-old sheep.
In the 13-month-old individuals, PFLC was the dominating bone type with an age class mean area coverage of 72.8% (Figure 6, Table 3). In the outermost pericortex, large primary vascular spaces were present that had not yet been filled in with parallel-fibered or lamellar bone (Figure 7a,b), indicating rapid bone growth at the time of death of these individuals (end of May). The primary vascular canals in the outer and central cortex showed a predominantly circular orientation (Figure 7a,b), whereas in the inner cortex, a more irregular, plexiform orientation of the vascular canals was present (Figure 7b). In the un-remodeled portion of the central cortex, presence of a rest line indicated a seasonal growth arrest during the animal’s first winter (Figure 7b).

Remodeled (secondary) bone was the second most common bone type present in the humeri of 13-month-old sheep. However, the proportion of the cortical area covered by this bone type differed markedly between the two sheep, with 7.1% in individual no. 17 and 22.2% in individual no. 13 (Figure 6, Table 3). This indicates an overall higher bone remodeling activity in the latter. In both animals, presence of fully formed secondary osteons was limited to the antero-medial (cranio-medial) cortex, and here mainly confined to the inner and central cortical portions, although resorption cavities and infilling secondary osteons were also present in the outer cortex anteriorly (cranially) (Figures 7c,d and 8a,b). The remodeling process had caused resorption of the primary cortex and formation of mostly non-overlapping (first-generation) secondary osteons with interspersed remnants of primary bone (Figure 8a,b). However, in places, the presence of overlapping secondary osteons and larger resorption cavities indicated that some remodeling had already occurred within the secondary bone (Figure 8a).

**FIGURE 4** Micrographs of ground sections through the midshaft of the humerus showing fibrolamellar bone tissue in the pericortex (periosteal fibrolamellar bone, PFLC; a,b) and in the endocortex (endosteal fibrolamellar bone, EFLC; c,d) viewed in normal transmitted (a,c) and in linearly polarized light with 1λ compensator (b,d). (a,b) In the PFLC, primary vascular spaces lined by scaffolds of woven bone (black arrows) have initially been filled in with parallel-fibered bone followed by the deposition of lamellar bone resulting in the formation of primary osteons (PO). Asterisks: vascular canals; arrowheads: rest lines running parallel to the bone surface. Periosteal surface to the bottom right corner of the images. (c,d) In the EFLC, a primary vascular space has been filled in with a primary osteon (PO). Asterisks: vascular canals of the primary osteon; black arrows: woven bone of the scaffold; black arrowhead: reversal line; white arrow: Volkmann’s canal. Endosteal surface to the bottom of the images.
A seam of endosteally deposited bone (ELM and EFLC) surrounded nearly the entire medullary cavity (Figure 6). The endosteal lamellar bone that had been deposited in this process was thickest in the anterior (cranial) quadrant, forming an endosteal lamellar pocket (ELP), and tapered in posterior (caudal) direction (Figure 7b,d). The endosteally deposited bone was delimited from the original endocortex by a reversal line (Figures 7d and 8c,d) that indicated resorption of primary and secondary bone of the inner cortex prior to ELP formation (Figure 8c,d). Bony scaffolds whose intertrabecular compartments had not yet been filled in with lamellar or parallel-fibered bone were present at the boundary of the ELP with the medullary cavity in both individuals, indicating rapid ELP formation at the time of death (Figure 8c). The presence of IFL and PLM, indicating periods of reduced bone growth rate, was confined to relatively small cortical areas in the 13-month-old age individuals, with age class means of 2.8% (PLM) and 4.6% (IFL), respectively (Figure 6, Table 3).

Compared with the 13-month-old sheep, in the 25-month-old individuals a reduction of the area coverage by PFLC and an increase in the area coverage by secondary bone was recorded (Table 3). In individual no. 18, in which both humeri could be analyzed, bone type distribution in the antimeres was highly symmetrical (Figure 6). Remodeled bone was largely confined to the inner and central portions of the antero-medial (cranio-medial) cortex and only a thin seam of secondary bone extended over a short distance into the inner portion of the lateral cortex.

The outermost humeral cortex of both 25-month-old individuals consisted almost entirely of the IFL or PLM bone types (Figures 6 and 9) and did not show the unfilled vascular spaces lined by woven bone scaffolds that characterized the rapidly growing outer cortex in the 13-month-old individuals. This indicates a much reduced radial bone growth rate in the 25-month-old than the 13-month-old individuals.
**TABLE 2** Area percentages of mineralized bone (MA), medullary cavity (MC), and cortical porosity (PO) in the sheep humeri (measured on midshaft cross-sections). MA + MC + PO = total cross-sectional area.

| Age at death (months) | Animal no | Side  | Single humerus | Mean of paired humeri | Mean of age class | Single humerus | Mean of paired humeri | Mean of age class | Single humerus | Mean of paired humeri | Mean of age class |
|-----------------------|-----------|-------|----------------|-----------------------|------------------|----------------|-----------------------|------------------|----------------|-----------------------|------------------|
| 13                    | 13        | Right | 64.8           | 64.7                  | 63.7             | 31.8          | 33.2                  | 34.6             | 3.4            | 2.2                    | 1.9              |
|                       |           | Left  | 64.6           |                       |                  |                |                       |                  |                |                        |                  |
| 17                    | 15        | Right | 61.9           | 62.7                  |                | 36.5          | 35.9                  |                | 1.7            | 1.5                    |                  |
|                       |           | Left  | 63.5           |                       |                  |                |                       |                  |                |                        |                  |
| 25                    | 15        | Right | 62.0           | 62.0                  | 62.8            | 37.6          | 37.6                  | 37.0             | 0.4            | 0.4                    | 0.3              |
|                       |           | Left  | 63.3           |                       |                  | 36.1          | 36.4                  |                | 0.1            | 0.1                    |                  |
| 33                    | 14        | Right | 59.8           | 61.1                  | 64.4            | 39.5          | 38.3                  | 35.4             | 0.7            | 0.7                    | 0.4              |
|                       |           | Left  | 62.4           |                       |                  | 37.0          |                       |                  |                |                        |                  |
| 16                    | 15        | Right | 68.6           | 67.7                  |                | 31.4          | 32.4                  |                | 0.6            | 0                     |                  |
|                       |           | Left  | 66.7           |                       |                  | 33.3          |                       |                  |                |                        |                  |

*a* Compacta excluding resorption spaces.

*b* Area occupied by resorption spaces.

*c* Calculated from means of paired humeri (only one humerus measured in animal 15).
In the 25-month-old sheep, two rest lines were present in the humeri, located, respectively, in the central and the outer cortex. Both rest lines could be followed along most of the cortical circumference (Figure 9a,b) but were missing in the remodeled bone areas. Bands of PLM or IFL were associated with these rest lines, indicating periods of reduced bone growth rate. In places, a third (discontinuous) rest line was present between these two rest lines. The two rest lines traceable through most of the cortex are regarded to reflect periods of seasonal growth arrests in, respectively, the first and second winter of the individuals, while the intermediate rest line is considered to reflect a temporary non-seasonal growth arrest affecting only certain midshaft regions. The distance between the two seasonal rest lines was highest in the postero-lateral (caudo-lateral) quadrant of the cortical bone, thereby indicating the preferential cortical growth direction. While vascular canals in the outer cortex showed an almost entirely circular orientation, the canals in the inner cortex exhibited a more plexiform orientation (Figure 9b).

Resorption of primary bone along the posterior (caudal) rim of the medullary cavity and endosteal new bone apposition on the opposite side were evident in both individuals of this age group. While the ELP occupied an extended area in the right humerus of individual no. 15, it was much smaller in both humeri of individual
| Age at death (months) | Animal no | Side | % PFLC | Mean of paired humeri | Mean of age classa | % PLM | Mean of paired humeri | Mean of age classa | % IFL | Mean of paired humeri | Mean of age classa | % EFLC | Mean of paired humeri | Mean of age classa | % ELM | Mean of paired humeri | Mean of age classa | % SO | Mean of paired humeri | Mean of age classa |
|----------------------|-----------|------|--------|----------------------|-------------------|------|----------------------|-------------------|------|----------------------|-------------------|------|----------------------|-------------------|------|----------------------|-------------------|------|----------------------|-------------------|
|                      |           |      |        |                      |                   |      |                      |                   |      |                      |                   |      |                      |                   |      |                      |                   |      |                      |                   |
| 13                   | 13        | Right | 65.8   | 66.8                 | 72.8              | 1.7  | 1.8                  | 2.8               | 3.8  | 4.2                  | 4.6               | 1.3  | 1.0                  | 1.6               | 4.0  | 4.2                  | 3.8               | 23.4 | 22.2                 | 14.7              |
|                      |           | Left  | 67.8   | 3.3                  |                   | 1.8  | 4.5                  |                   | 0.6  | 4.4                  |                   | 2.2  | 2.2                  |                   | 3.9  | 3.3                  |                   | 4.9  | 7.1                  |                   |
| 17                   | 77.8      | Right | 77.8   | 8.1                 | 4.5               | 0.4  | 7.1                  | 1.9               | 3.9  | 3.3                  |                   | 2.2  | 2.2                  |                   | 3.9  | 3.3                  |                   | 4.9  | 7.1                  |                   |
|                      |           | Left  | 79.5   | 2.4                 |                   | 2.2  | 2.2                  |                   | 3.9  | 3.3                  |                   | 2.2  | 2.2                  |                   | 3.9  | 3.3                  |                   | 4.9  | 7.1                  |                   |
| 25                   | 15        | Right | 49.3   | 49.3                 | 57.0              | 10.6 | 10.6                 | 8.0               | 6.6  | 6.6                  | 6.4               | 3.4  | 3.4                  | 2.0               | 5.5  | 5.5                  | 3.8               | 24.7 | 24.7                 | 23.1              |
|                      |           | Left  | 63.5   | 64.7                 |                   | 5.2  | 5.3                  | 11.5              | 6.1  | 6.1                  |                   | 0.2  | 0.6                  |                   | 1.7  | 2.0                  |                   | 17.8 | 21.4                 |                   |
| 33                   | 14        | Right | 34.8   | 37.1                 | 38.2              | 11.8 | 11.8                 | 11.6              | 9.5  | 8.9                  | 9.7               | 4.2  | 3.4                  | 5.7               | 6.8  | 6.4                  | 4.7               | 32.9 | 32.6                 | 30.5              |
|                      |           | Left  | 39.3   | 3.3                  |                   | 11.8 | 8.2                  |                   | 2.6  | 2.6                  |                   | 10.6 | 7.9                  |                   | 2.5  | 2.9                  |                   | 24.3 | 28.4                 |                   |
| 16                   | 43.8      | Right | 43.8   | 39.2                 |                   | 10.6 | 11.3                 | 8.3               | 10.5 | 10.6                 | 2.9               | 5.2  | 3.3                  |                   | 3.3  | 3.3                  |                   | 32.4 | 32.4                 |                   |
|                      |           | Left  | 34.6   | 11.9                 |                   | 12.6 | 5.2                  |                   | 5.2  | 5.2                  |                   | 5.2  | 5.2                  |                   | 5.2  | 5.2                  |                   | 32.4 | 32.4                 |                   |

Abbreviations: EFLC, endosteal fibrolamellar complex; ELM, endosteal lamellar-zonal bone; IFL, intermediate fibrolamellar-zonal bone; PFLC, periosteal fibrolamellar complex; PLM, periosteal lamellar-zonal bone; SO, secondary osteons, Haversian (secondary) bone.

aCalculated from means of paired humeri (only one humerus measured in animal 15).
no. 18 (Figure 6). The ELP was largely composed of lamellar bone (ELM) that was mainly confined to the anterior (cranial) half of the humeri (Figures 9c,d and 10a,b). As in the 13-month-old individuals, some areas within the lamellar bone of the ELP contained primary osteons (SO) are present medially. (b) Peripherally, the outer cortex consists of primary bone scaffolds (black arrow) whose intertrabecular spaces are yet unfilled. In the central and the outer cortex (double-headed arrow), the primary vascular spaces show a circumferential (laminar) orientation, but in the inner cortex (double-headed arrow with asterisk) they show a more plexiform arrangement. Arrowheads: rest line in the central cortex. (c) PFLC and SO are the dominant bone types in the anterior (cranial) cortex. The ELP is characterized by the presence of ELM and EFLC. (d) At the outer and inner rims of the humerus, the primary vascular spaces (arrowheads) are still unfilled. Larger, partially infilled resorption spaces (asterisks) are present throughout most of the cortex. The ELP is separated from the remodeled bone by a reversal line (white arrows).

The most obvious changes recorded in the 33-month-old compared with the 25-month-old individuals were a further increase in the area covered by secondary bone (age class means of 23.1% in the 25-month-old and of 30.5% in the 33-month-old individuals) and a marked increase of ELP extension (Table 3, Figures 6 and 11). The humeri of the latter age class revealed a high concordance in the spatial distribution of secondary bone and the ELP (Figure 6), both structures occurring in the anterior (cranial) and medial cortical quadrant. Medially, nearly the entire cortex was composed of secondary bone. Multiple generations of secondary osteons could be distinguished in the secondary bone, denoting a high remodeling rate (Figures 6 and 11c,d).

In the humeri of the 33-month-old individuals, bone circumference had only a slightly increased compared with the 25-month-old individuals. Limited periosteal new bone apposition had generated layers of either PLM or IFL in the former (Figures 6 and 11), but no
newly formed PFLC was present in the outermost cortex. Several rest lines were visible in the cortical bone, indicating repeated growth arrests. (Figure 11b,d). Within the ELP, presence of several rest lines likewise indicated multiphasic bone apposition (Figure 12a). Whereas in places, the presence of EFLC indicated bone formation activity along the boundary of the ELP with the medullary cavity, in other regions the presence of Howship’s lacunae indicated resorption activity along this boundary (Figure 12a,b). Remodeling activity in the older portions of the endosteally formed primary bone of the ELP had completely obscured the original border with the inner cortex.

The amount of modeling drift that had occurred in the sheep humeri over the study period is demonstrated in Figure 13 for representative humeral cross sections from the three age classes. Bone resorption in the course of modeling drift had caused a shift of the medullary cavity in a posterior (caudal) direction, but endostreal new bone formation had predominantly taken place in the anterior (cranial) and medial quadrants (Figures 6 and 13). Modeling drift had not markedly changed the relative proportion occupied by the medullary cavity along both the maximum antero-posterior (cranio-caudal) and medio-lateral axes (Table 4). However, a slight increase in thickness of the anterior (cranial) cortex relative to that of the posterior (caudal) cortex and of the medial relative to that of the lateral cortex can be deduced from our data. These findings can be ascribed to ELP formation and, in the case of the medial cortex, to a distinct bulging at the tuberositas teres major (attachment site of the teres major muscle) that is especially prominent in the 33-month-old individuals (Figure 6). Our data further demonstrate that the ontogenetic changes in the relative proportions occupied by the respective cortical areas and the medullary cavity occurred in a highly symmetrical way in the paired humeri (Table 4).
DISCUSSION

The present study reports data on the degree of bilateral symmetry in tissue compartmentalization and bone type distribution in humeri of Soay sheep from different ontogenetic stages. The relative proportions of mineralized cortical bone and medullary cavity remained remarkably unchanged in the analyzed age classes, although the humeri were still growing in length and width over the study period. Compared with the data given by Cambra-Moo et al. (2015) for sub-adult and adult sheep of the Merina breed, our results in Soay sheep showed a higher percentage of the cross-sectional area covered by mineralized bone and a lower percentage covered by the medullary cavity. We further observed only minor changes in the relative thicknesses of the cortical areas in the four quadrants over the study period, with a slight increase for the anterior (cranial) and the medial cortex and a slight decrease for the posterior (caudal) and the lateral cortex in the course of cortical drift. Thus, although intense cortical resorption in the posterior (caudal) cortex and prominent new endosteal bone formation in the anterior (cranial) and the medial cortex occurred over the study period, these processes were well balanced, so that the medullary cavity maintained not only its central position but also its relative size in relation to the cortical bone areas.

Analysis of bone type distribution in the humeral cortex revealed a dominance of PFLC in the 13-month-old sheep (age class mean of 72.8%), indicating rapid new bone formation in their humeri. The proportion of PFLC was reduced to 57.0% in the 25-month-old sheep and further to 38.2% in the 33-month-old individuals. The factors responsible for this trend were an intensified remodeling and formation

4 | DISCUSSION

The present study reports data on the degree of bilateral symmetry in tissue compartmentalization and bone type distribution in humeri of Soay sheep from different ontogenetic stages. The relative proportions of mineralized cortical bone and medullary cavity remained remarkably unchanged in the analyzed age classes, although the humeri were still growing in length and width over the study period. Compared with the data given by Cambra-Moo et al. (2015) for sub-adult and adult sheep of the Merina breed, our results in Soay sheep showed a higher percentage of the cross-sectional area covered by mineralized bone and a lower percentage covered by the medullary cavity. We further observed only minor changes in the relative thicknesses of the cortical areas in the four quadrants over the study period, with a slight increase for the anterior (cranial) and the medial cortex and a slight decrease for the posterior (caudal) and the lateral cortex in the course of cortical drift. Thus, although intense cortical resorption in the posterior (caudal) cortex and prominent new endosteal bone formation in the anterior (cranial) and the medial cortex occurred over the study period, these processes were well balanced, so that the medullary cavity maintained not only its central position but also its relative size in relation to the cortical bone areas.

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of secondary bone, and a marked reduction in radial growth already in the 25-month-old sheep. Here, and in the 33-month-old animals, new bone growth at the outer cortical surface occurred exclusively by formation of either PLM or IFL, demonstrating a slow radial bone growth rate.

Cambra-Moo et al. (2015) reported 67.4% and 67.2% of the cross-sectional area of humeri covered by fibrolamellar bone, and 30.4% and 24.8% covered by Haversian bone in, respectively, 18- and 36-month-old sheep (Merina breed). For 9- and 12-year-old sheep, they reported values of 65.4% and 58.9% for fibrolamellar bone, and of 31.6% and 36.1% for Haversian bone. The values for fibrolamellar bone in the older individuals studied by Cambra-Moo et al. (2015) are much higher than those found for PFLC in the 33-month-old Soay sheep of our study. However, in contrast to our study, Cambra-Moo et al. (2015) did not distinguish between PFLC, PLM, and IFL, but combined these three bone types into the category of fibrolamellar bone. If we use the same approach for our 33-month-old individuals, the values for fibrolamellar bone (broad sense) are in the range of those reported by Cambra-Moo et al. (2015).

In the studied Soay sheep humeri, presence of small bands of PLM or IFL in the central cortex reflect periods of reduced bone growth that were associated with the formation of rest lines denoting phases of temporary (seasonal or non-seasonal) bone growth arrests, a situation previously reported for different species of ungulates (Köhler et al., 2012; Kolb et al., 2015a; Jordana et al., 2016; Nacarino-Meneses and Köhler, 2018; Orlandi-Oliveras et al., 2018) and primates (Castanet et al., 2004; McFarlin et al., 2016). Distances between consecutive rest lines were highest in the posterior (caudal) cortex, thereby indicating a preferential apposition of periosteal new bone in this area. In the anterior (cranial) and the medial cortex, secondary bone formation in the course of remodeling activity had caused the disappearance of the rest lines.

Our study revealed a characteristic distribution of Haversian bone in the midshaft of the sheep humeri. It was found in the anterior (cranial) and medial quadrants of the humeri. The posterior (caudal) and lateral quadrants retained their primary bone structure. In paired humeri of single individuals, secondary bone occurred in a highly symmetrical fashion with respect to both its position and extent. The amount of Haversian bone (age class means) present in the humeral midshaft more than doubled, from 14.7% in the youngest (13 months) to 30.5% in the oldest (33 months) individuals. A corresponding finding has previously been reported for humeri of adult and senile sheep of the Merina breed (Cambra-Moo et al., 2015). Even in the oldest sheep studied by these authors, the cortical area covered by Haversian bone was in the same range (31.6% in a 9-year-old and 36.1% in a 12-year-old individual) as in the 33-month-old Soay sheep of our study. This suggests that, in contrast to the midshaft area of the human humerus, where Haversian bone in adults typically covers almost 100% of the cortical area (Cambra-Moo et al., 2014), in the midshaft of sheep humeri a different biomechanical loading causes a restriction of bone remodeling to certain cortical areas. Our data and those of Cambra-Moo et al. (2015) further suggest that the distribution of secondary bone in the humeral midshaft of sheep reaches a plateau level at about 3 years of age. In this respect, it would be interesting to analyze the distribution of secondary bone along the entire shaft of the sheep humerus, as studies in goats and cattle demonstrated marked differences in the amount of remodeling activity along the femoral shaft (Mayya et al., 2013, 2016).

We recorded a high degree of bilateral symmetry in the distribution and amount of Haversian bone in sheep humeri. Previously, Marotti (1963) and Currey (2002) have reported a similar bilateral symmetry in remodeling activity for limb bones of cats and dogs under normal physiological loading. These observations prompted Currey et al. (2017) to postulate that damage-driven repair alone

**FIGURE 10** Higher magnification of the inner cortex of the humerus of individual 15 (age at death: 25 months) viewed in transmitted light with phase contrast (a) and in linearly polarized light (b). The original border between the ELP and the inner cortex is no longer visible due to remodeling of the former. Rest lines (white arrows) indicate intermittent growth of the ELP. Asterisks: Infilling secondary osteons; MC, medullary cavity; PO, primary osteons; SO, secondary osteons.
cannot be the factor causing remodeling, as micro-damage cannot be expected to occur in such a symmetric fashion. They argue that the similar biomechanical loading may be the decisive factor for the symmetrical distribution of remodeling activity in antimeres. In this respect, it is important to consider that some authors (e.g. Skedros, 2012; Gocha and Agnew, 2016) suggest that remodeling can probably be activated by high local strain magnitudes sensed by osteocytes, even in the absence of microdamage. This would make intracortical remodeling a mechanism for adapting a bone to its mechanical environment. Osteocytes have been shown to be key modulators of bone resorption through the production of the receptor activator of nuclear factor kappa B ligand (RANKL) (Nakashima et al., 2011; Wijenayaka et al., 2011). Osteocyte-derived RANKL induces osteoclast differentiation and activity, and thus regulates osteoclastic bone resorption during remodeling (Prideaux et al., 2016).

Currey et al. (2017) argue that the dominant role of remodeling in longer-lived animals is bone maintenance and adaptation. The fact that in the studied sheep humeri secondary bone also shows signs of ongoing remodeling is consistent with this view. Furthermore, it was shown that modeling and remodeling processes occur

**FIGURE 11** Bone type maps (a,c) and light microscopic images (plain transmitted light, b,d) of the postero-medial (caudo-medial) cortex of the right humerus of individual 16 (a,b), and the antero-medial (crania-medial) cortex of the right humerus of individual 14 (c,d). Age at death for both individuals: 33 months. (a) The outer cortex is formed by IFL and PLM, both indicative of relatively slow bone formation. The central cortex is still dominated by PFLC. (b) Three rest lines (arrowheads) are visible in the cortex. The innermost rest line presumably indicates the growth arrest during the individual's first winter of life. In the posterior (caudal) cortex (double-headed arrow), the primary vascular canals show a laminar orientation. Vascular orientation in the central portion of the medial cortex is more plexiform (double-headed arrow with asterisk). (c,d) Except for the outermost zone, the primary periosteal cortex has been replaced by secondary bone (SO). The outermost cortex is formed by either avascular PLM or by IFL. In the inner cortex, at the boundary between the ELP and the remodeled cortex, large blood vessel spaces can be seen. Several rest lines (arrowheads) are visible in the outer cortex. The central primary cortex is largely remodeled, with secondary bone extending almost up to the bone surface in the anterior (cranial) cortex. The ELP is separated from the remodeled cortex by a reversal line (white arrows). In places, small areas of the primary fibrolamellar bone (large asterisk) are still present. The lumina of larger blood vessel spaces (small asterisks) have become incorporated into the inner cortex.
simultaneously during the formation of the sheep humerus, a situation also known to occur during other bone-forming processes, e.g. antler growth (Gomez et al., 2013; Kierdorf et al., 2013b).

Taking into account the role of remodeling in adapting bone to compressive loading (Riggs et al., 1993a; 1993b; Mason et al., 1995; Mayya et al., 2013; 2016; Zedda et al., 2015), our study suggests that in the midshaft of sheep humeri, the anterior (cranial) and the medial cortical portions are especially subjected to high compressive loads. In the stance phase of the gait, the sheep humerus is inclined posteriorly (caudally) at an angle of about 105–110º to a vertical axis connecting the shoulder joint with the toes (Nickel et al., 1992). In this situation, bodyweight will cause a bending of the humerus, with compressive strain occurring predominantly in the anterior (cranial) and tensile strain in the posterior (caudal) cortex. We further assume that the force exerted by the musculus deltoideus that is responsible for abduction and rotation of the front limb causes differences in the mechanical loading of the humerus. This muscle inserts at the tuberositas deltoidea, which is positioned laterally in the proximal third of the humerus. Action of the deltoid muscle will create tensile strain in the lateral and compressive strain in the medial portions of the humeral midshaft during locomotion. Our findings on the distribution of Haversian bone in the midshaft of sheep humeri are in accordance with the prediction made by Mayya et al. (2013) about the distribution of Haversian and non-Haversian bone in sheep humeri.

Microscopic studies comparing morphological parameters of secondary osteons and Haversian canals in the cross-sectioned femoral midshafts of goats (Capra hircus) and sheep (Ovis aries) (Zedda et al., 2017), and between domestic sheep (Ovis aries aries) and free-ranging mouflon (Ovis aries musimon) (Giua et al., 2014) reported intra- and interspecific variation. This variation was attributed to differences in lifestyle (behavioral and locomotion patterns) and the related variation in mechanical stress acting on the femora of the animals. In this respect, we would like to stress that the Soay sheep used in our study lived in an enclosure under human control and were not free-ranging.

Our study revealed a considerable amount of cortical drift in the humeral midshaft of sheep over the studied age-span. This involves bone resorption predominantly in the posterior (caudal) cortex, and endosteal new bone formation predominantly in the anterior (cranial) and the medial cortex. The latter process is reflected by a prominent ELP, whose formation caused a relative thickening of the anterior (cranial) and the medial cortex compared with the posterior (caudal) and the lateral one, a process that can be considered an adaptation to the loading asymmetry of the sheep humerus. Our results are thus in line with the statement (Maggiano, 2012; Maggiano et al., 2016) that the endosteum has a high osteogenic potential and that the resulting formation of the ELP may contribute to the achievement of an adaptive adult bone morphology. However, especially in older sheep, the exact extent of the ELP is difficult to assess, as remodeling activity in the older portions of the ELP progressively obscures the original boundary between the inner cortex and the endosteally formed new bone.

Our study provided evidence that endosteal new bone is formed in an intermittent fashion, with resorptive phases occurring along the border to the medullary cavity prior to apposition. Maggiano (2012) suggested that the term modeling should be applied to all processes occurring at periosteal and endosteal surfaces during bone development and growth, including those where bone deposition follows resorption at a given site. According to this view, all described processes at the endosteal surface of sheep humeri can be characterized as bone modeling.

In a pooled sample of human humeri from Mexico that comprised modern and archaeological material, the cortical area covered by the ELP was found to be quite variable, mostly averaging 10–12% but
sometimes accounting for up to 45% of the total cortical area (Maggiano et al., 2016). Cambra-Moo et al. (2014) reported highly varying percentages of endosteal bone in the cortices of human humeri dating from the 12th to the 18th century. Mean values declined from infants (18.9%) to juveniles (12.8%) to adults (4.5%). The reduction in cortical area covered by endosteal bone was attributed to the increase in the percentage of Haversian bone to almost 100% in the humeri of adults as a result of intense remodeling. In the sheep humeri, we observed an increase in the amount of the cortical area covered by endosteal bone with age. The highest combined age class mean (EFLC + ELM: 10.4%) was recorded in the 33-month-old individuals. For sheep humeri, our data and those of Cambra-Moo et al. (2015), which include data for senile sheep, suggest that the difference in the age trends of the cortical area coverage by endosteal bone between human and sheep humeri can largely be attributed to a much higher remodeling rate in the former.

5 | CONCLUSION

Our study demonstrated a marked change in bone type distribution of sheep humeri with age. In the cortical midshaft area of 13-month-old individuals, PFLC was the dominating bone type. This indicates a rapid bone growth during the first year of life that is only interrupted by a seasonal growth arrest in the animals’ first winter. In older individuals (ages at death of 25 and 33 months, respectively), new bone formation had produced bone types (PLM and IFL at the periosteal, and ELM at the endosteal surface) indicative of a reduced bone growth rate. Distribution and extent of secondary bone showed a marked bilateral symmetry in the sheep humeri. Presence of secondary bone was largely restricted to the anterior (cranial) and the medial cortex, thereby demonstrating a characteristic distribution of remodeling activity compatible with the view that remodeling activity is caused by compressive loading. Our study also demonstrated a considerable amount of cortical drift in the sheep humerus, with endosteal resorption occurring predominantly in the posterior (caudal) quadrants and formation of a prominent ELP in the anterior (cranial) and medial cortical quadrants. A simultaneous occurrence of modeling and remodeling processes was recorded during bone formation of the humerus in all age classes studied.

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The data that support the findings of this study are available from the corresponding author upon reasonable request.
## Table 4

| Age at death (months) | % of AP axis covered by | % of ML axis covered by | % of AP axis covered by | % of ML axis covered by | % of AP axis covered by | % of ML axis covered by | % of AP axis covered by | % of ML axis covered by | % of AP axis covered by | % of ML axis covered by |
|-----------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
|                       | Medullary cavity        | Anterior cortex         | Posterior cortex        | Medullary cavity        | Medial cortex           | Lateral cortex           | Medullary cavity        | Anterior cortex         | Posterior cortex        | Medullary cavity        |
| Right                 | 13                      | 22.2                    | 13.1                    | 17                      | 19.0                    | 18.2                    | 14.4                    | 16.4                    | 18.2                    | 19.0                    |
| Left                  | 17                      | 16.7                    | 18.3                    | 17                      | 17.6                    | 19.1                    | 14.1                    | 15.8                    | 17.6                    | 15.8                    |
| Right                 | 17                      | 16.5                    | 18.3                    | 17                      | 17.5                    | 17.1                    | 14.1                    | 15.8                    | 17.5                    | 15.8                    |
| Left                  | 18                      | 16.5                    | 18.3                    | 17                      | 17.6                    | 17.1                    | 14.1                    | 15.8                    | 17.6                    | 15.8                    |
| Right                 | 20                      | 19.0                    | 18.3                    | 20                      | 19.5                    | 17.9                    | 14.0                    | 15.9                    | 18.0                    | 16.0                    |
| Left                  | 16                      | 19.0                    | 18.3                    | 16                      | 19.5                    | 17.9                    | 15.9                    | 14.0                    | 15.9                    | 16.0                    |

Calculated from means of paired humeri (only one humerus measured in animal 15).

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