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Osteonic organization of limb bones in mammals, including humans, and birds: a preliminary study

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Summary

As it is well known, bone tissue is characterized by a calcified extracellular matrix which makes this tissue suitable to support the body and protect the inner organs. Lamellar bone tissue is organized in lamellae, 3-7 μm in thickness, and arranged concentrically around vascular channels: the basic structure in this type of organization is called *Haversian system* or *osteon* and the diameter of osteons depends on the number of lamellae. Shape and regional density of osteons are related to the bone segment and the specific functional requirements to meet. Aim of this study is to correlate the compact bone tissue microstructure in various classes of mammals, including humans, and birds in order to find an adequate identification key. The results of our study show that in bone tissue samples from various classes of mammals, including humans, and birds the osteonic structure shows peculiar features, often depending on the rate of bone remodelling, different in different animal species. We conclude that a careful microscopic analysis of bone tissue and the characterization of distinctive osteonic features could give a major contribution to forensic medicine to obtain a more reliable recognition of bone findings.

Key words

Osteon, diaphysis, mammals.

Key to abbreviations:

HB = human bone
RB = rodent bone
BB = bovine bone
PB = pig bone
SB = sheep bone
GB = Gallus gallus bone

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Introduction

As it is well known, bone tissue is characterized by a calcified extracellular matrix which makes this tissue suitable to support the body and protect the inner organs. It also represents an important calcium store. Macroscopically it is possible to distinguish *compact* bone and *spongy* bone. Histologically, adult lamellar bone tissue is organized in lamellae, 3-7 μm in thickness, arranged concentrically around vascular Havers channels: such a group of lamellae with its central channel is called *Haversian system* or *osteon* and the diameter of osteons depends on the number of lamellae.

In the bone matrix there are lacunae in which osteocytes reside: branched canaliculi depart from lacunae and anastomose with similar ones from adjacent lacunae, thereby forming a complex network. Besides within osteons, lamellar bone tissue is also present among osteons (interstitial tissue) and lines the internal and external aspects of the diaphysis (circumferential tissue). From the existing literature (Pacini et al., 1999), it is known that shape and regional density of osteons are related to the bone segment and the specific functional/mechanical requirements that shall be met. Aim of this study is to correlate the compact bone tissue microstructure in various classes of mammals, including humans, and birds and to find an adequate identification key (Martiniakovà et al., 2006a) even for fragments of bone of unknown origin (Cattaneo et al., 2009). This work is the first part of an extensive investigation on different classes of animals, using specific histological staining for bone tissue and quantitative histological techniques, such as immunohistochemistry and image analysis.

Materials and methods

In our study we used samples from femur diaphysis obtained after surgical amputation in a diabetic patient and from rodent, bovine, pig, sheep and Galliformes upper limbs. Bone fragments (thickness ~2 cm) were collected from each sample; they were fixed in 4% formalin and then decalcified in EDTA (ethylene-diamin-tetra-acetate) for 36 hours. After decalcification, samples have been processed for routine paraffin inclusion. Transverse sections, 6 μm thick, were cut and stained with haematoxylin-eosin staining (Cattaneo et al. 1999; Hillier and Bell, 2007).

Samples have been called: HB = human bone; RB = rodent bone; BB = bovine bone; PB = pig bone; SB = sheep bone; GB = Gallus gallus bone.

Results

Human Femoral Diaphysis

Transverse section of human femoral diaphysis (Fig. 1) showed: round osteocytes with similar size to each other; osteons with a diameter of ~280 μm; a thin layer of connective tissue lining the Havers channels; interstitial tissue located among osteons. The lamellae were 16-20 per osteon; bone lacunae, containing the osteocyte bodies, and bone canaliculi were easily recognizable.

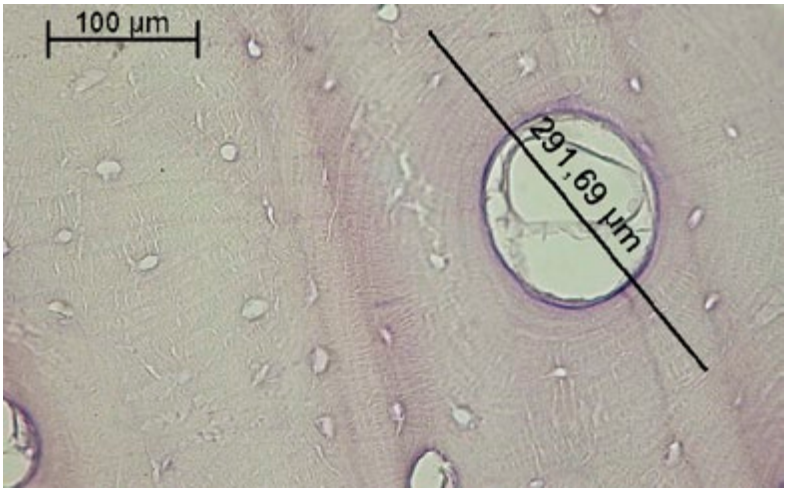

Figure 1 – Transverse section of human femoral diaphysis. Bone lacunae with osteocytes and canaliculi, a thin layer of connective tissue lining Havers canals, and interstitial tissue among osteons are evident. Light microscopy; haematoxylin and eosin; calibrating bar = 100 μm.

Bovine Femoral Diaphysis

In transverse section of bovine femoral diaphysis (Fig. 2), osteons appeared with a lower number of lamellae (12-15) and osteocytes than in human femur; the diameter of osteons was ~230 μm. Bone tissue, as a whole, showed a more homogeneous appearance: in fact, the borders between adjacent lamellae were barely evident.

Pig Femoral Diaphysis

Sections from pig femur showed osteons characterized by smaller size (diameter ~200 μm) than those observed in human samples, so they also appeared with a lower number of ill-defined lamellae (10-12) and osteocytes than in human femur. Interstitial tissue was relatively scarce (Fig. 3).

Sheep Femoral Diaphysis

Well-defined osteons were made up by a moderate number of lamellae (14-16), with a diameter of ~230 μm. In this mammal species, it was possible to appreciate the peculiar structure of bone tissue, in which osteons show an irregular profile and interstitial lamellae are numerous, possibly as a consequence of intense osteoclastic resorption. It was also possible to observe a greater number of osteocytes with intensely stained nuclei (Fig. 4) than in human femur.

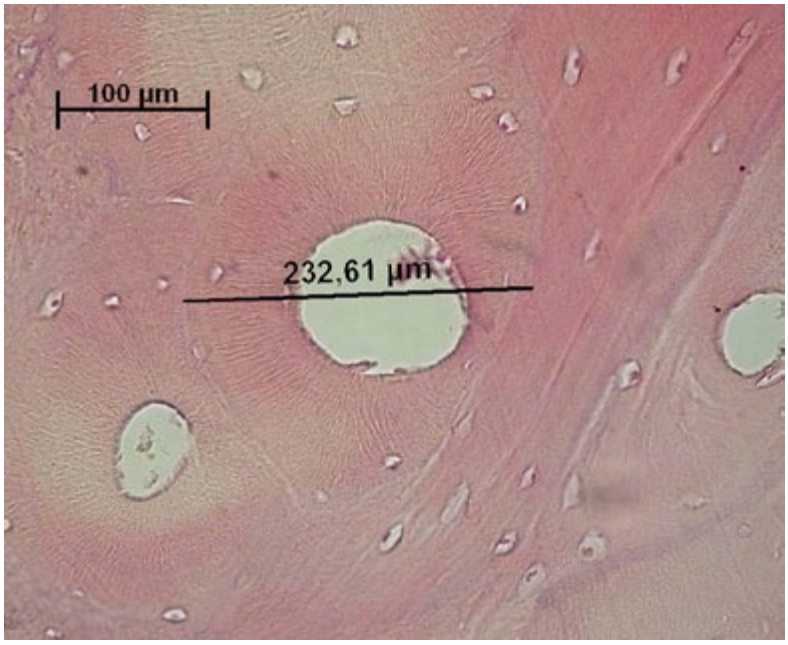

Figure 2 – Transverse section of bovine femoral diaphysis. Osteons appear with a lower number of lamellae and osteocytes and bone tissue appears more homogenous than in human femur. Light microscopy; haematoxylin and eosin; calibrating bar = 100 μm.

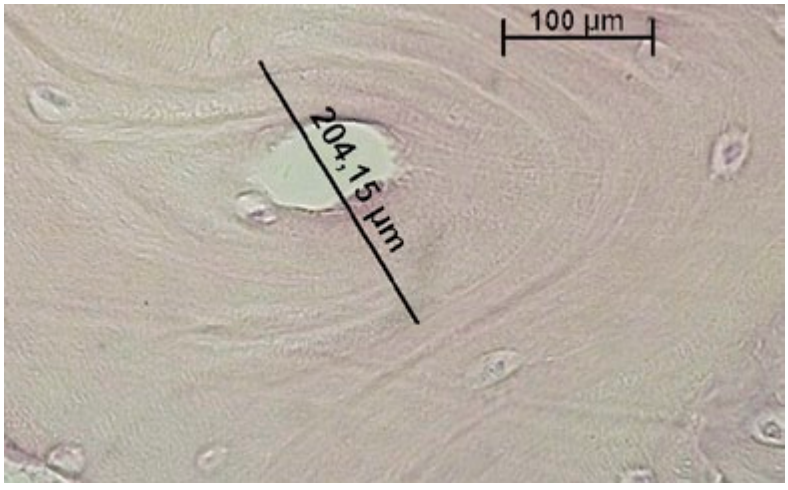

Figure 3 – Transverse section of pig femoral diaphysis. Osteons are small with a low number of lamellae and osteocytes; interstitial tissue is moderately represented. Light microscopy; haematoxylin and eosin; calibrating bar = 100 μm.

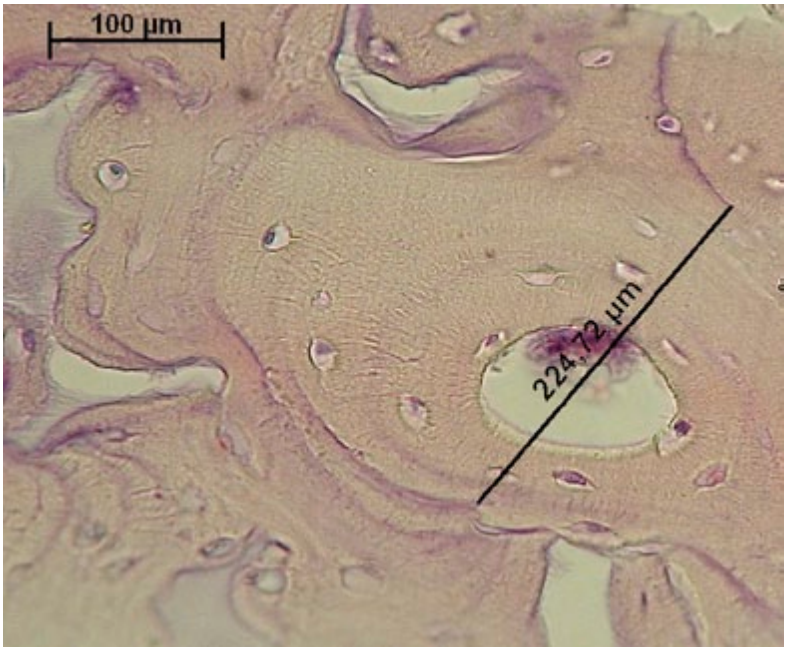

Figure 4 – Transverse section of sheep femoral diaphysis. Osteons are well defined and with a moderate number of lamellae. Their profile is irregular, and interstitial lamellae are numerous. Light microscopy; haematoxylin and eosin;calibrating bar = 100 μm.

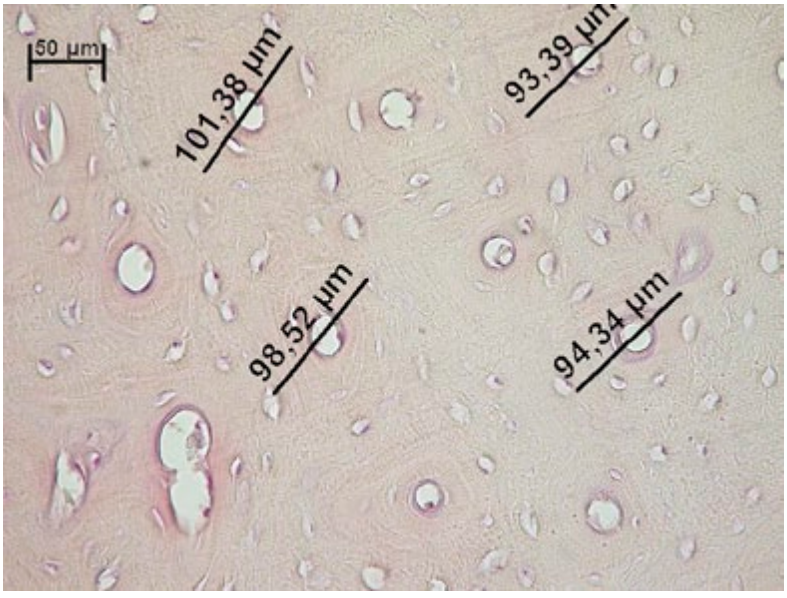

Figure 5 – Transverse section of rodent femoral diaphysis. Osteons are small and tightly packed. Lacunae with osteocytes characterized by intensely stained nuclei, and canaliculi are evident. Light microscopy; haematoxylin and eosin; calibrating bar = 50 μm.

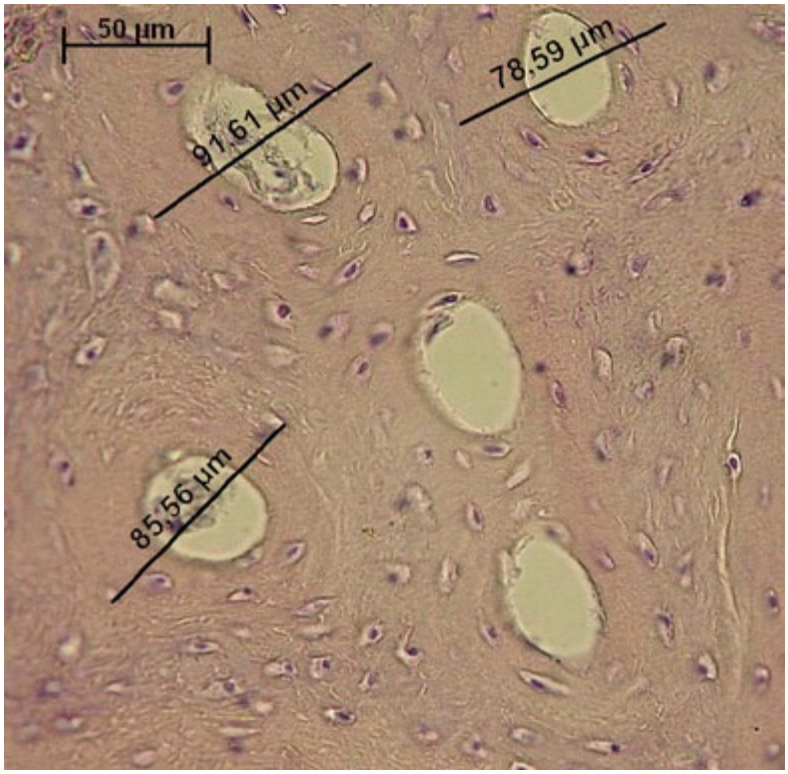

Figure 6 – Transverse section of galliforme femoral diaphysis. A great number of small osteons are evident; the nuclei of the numerous osteocytes are well stained. Light microscopy; haematoxylin and eosin; calibrating bar = 50 μm.

Rodent Femoral Diaphysis

In this sample, osteons were very small, with a diameter of ~100 μm and a low number of lamellae (5-7), and were densely packed (Fig. 5). Osteocytes were characterized by intensely stained nuclei and were interconnected by canaliculi.

Galliforme Femoral Diaphysis

This sample was also characterized by many small osteons (diameter ~80 μm) with a low number of lamellae (4-6); osteonic lamellae had ill-discernible limits between each other; the nuclei of the numerous osteocytes were markedly stained. The number of osteocytes was significantly greater than in the other species (Fig. 6).

Discussion

Based on the current histological analysis made on bone samples from mammals, including humans, and a bird, a Galliforme, it is possible to come to the following conclusions.

The typical osteonic structure is not always well recognizable, especially in birds and sheep (Martiniakovà et al., 2007): in fact, in the former species, single osteons are ill-defined because of lack of cementing lines; in the latter species, bone tissue has a similar structure as that of flat bones of the human skull, where lamellae are arranged around blood vessels and organized to form a three-dimensional network; osteons show an irregular profile with incomplete external lamellae.

It is possible to suppose that the regularity of osteon shape and dimensional parameters may depend on both species-related characteristics and rate of bone remodelling: in fact, in animals such as bovine (Martiniakovà et al., 2006b), in which bone remodelling is slower than in humans, osteons have a more regular shape and fewer osteocytes; in sheep, as a consequence of intense osteoclastic resorption, osteons show an irregular profile and interstitial lamellae are numerous and incomplete.

Compared with other animal species, the process of bone remodelling in humans is much more intense for a long life period; therefore, osteons can attain a more regular shape and a greater numbers of cells, with larger osteocytes at the osteon periphery and gradually smaller osteocytes in proximity of the Havers channel (Szulc et al., 2005). Obviously, these features are less evident in relation to ageing, as the rate of bone remodelling decreases gradually.

The presence of clear-cut interstitial bone indicates continuous bone remodelling; the semicircular shape of interstitial lamellae reveals their origin from previous osteons which have undergone partial resorption.

Human, bovine and pig samples are characterized by much less intensely stained nuclei of osteocytes than the other species examined; this is probably due to artefacts related to formalin fixation, perhaps because of the different fixative penetration among samples related to the greater or lesser abundance of canaliculi and the frequency of osteocyte lacunae.

As a future direction of this study, we aim at investigating additional bone tissue samples from different animal species, in which the osteonic structure may show peculiar features (Mori et al., 2005). Such investigation could give a major contribution to forensic medicine, expanding the knowledge required for a proper identification of fragmented and damaged bone findings (Grevin et al., 1998). In fact, a careful microscopic analysis of bone tissue and the characterization of distinctive osteonic features (Hoc et al., 2006) might help to obtain a more reliable recognition of bone findings.

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