

Scientific paper formerly presented

HISTOLOGICAL ANALYSIS OF COMPACT BONE TISSUE IN ADULT LABORATORY RATS

M. MARTINIAKOVÁ^{1*}, R. OMEKKA¹, B. GROSSKOPF², Z. MOKOŠOVÁ¹, R. TOMAN³

¹Constantine the Philosopher University, Nitra, Slovak Republic;

³Institute of Zoology and Anthropology, Georg-August University, Göttingen, Germany;

⁴Slovak University of Agriculture, Nitra, Slovak Republic

ABSTRACT

The aim of our work was to describe in details compact bone tissue microstructure in adult laboratory rats. Five clinically healthy 4 month-old rats (males) were included in the study. The animals were euthanized and the right femur bone was sampled for analyses. 70-80 µm thick bone sections of the femoral diaphysis were prepared using standard histological equipment. Qualitative histological characteristics were determined according to internationally accepted classification systems, whilst quantitative parameters were assessed using the Motic Images Plus 2.0 ML software. Areas, perimeters, minimum and maximum diameters of primary osteon' vascular canals, Haversian canals and secondary osteons were measured. Additionally, an orientation of collagen fibers has also been analyzed using polarized-light microscopy. According to our results, 4 month-old laboratory rats dispose with non-vascular and/or primary vascular radial and/or irregular Haversian bone tissues. Vascular canals of primary osteons and Haversian canals are very short. The collagen fibres run exactly parallel to the axis of the bone and/or run only slightly oblique and/or run, in polarized light, parallel to the direction of the additive position and/or run at right angles to the direction of the position.

Key words: compact bone tissue, laboratory rats, histology

INTRODUCTION

Animal models are commonly used in the study of skeletal biology. Rats are the animals most often used in experiments studying the response of the musculoskeletal system to exercise (Boyde, 2003), because they are simple, relatively inexpensive, and have good animal compliance (Shimano and Volpon, 2009). In the past, rats have been very predictive of the skeletal responses of humans to sex steroids and other bone-active drugs; ovariectomized rats have become a widely accepted model for human postmenopausal osteoporosis (Erben et al., 2004). In all studies mentioned above, mainly cancellous bone tissue has been analyzed in details by various techniques due

to its increased modeling and remodeling activity in comparison to the compact bone tissue.

Compact bone tissue forms the shafts of long bones, the surfaces of their extremities, short bones, and the outer and inner layer (*lamina externa et interna*) of the skull vault in rats. Basic constituents of its structural organization are primary osteons and secondary osteons (Haversian systems). While primary osteons are not surrounded by a reversal (cement) line, and lamellae around them merge smoothly with the surrounding bone, the secondary osteons consist of a central (Haversian) canal, which is surrounded by concentric rings (lamellae) of the matrix (Currey, 2002; Martiniaková et al., 2007). Histological research of the compact bone tissue can

*Correspondence: E-mail: mmartiniakova@ukf.sk

doc. RNDr. Monika Martiniaková, PhD., Department of Zoology and Anthropology,
Constantine the Philosopher University, Nábřežie mládeže 91, 949 74 Nitra, Slovak Republic;
Tel.: +421376408718, Fax: +421376408556

be carried out using two approaches: qualitative and quantitative. With the qualitative approach, a structural pattern of the bone tissue is identified. For the quantitative approach, parameters of the osteons and/or Haversian canals are to be measured.

Compact bone of rats is mainly composed of the non-vascular bone tissue. Primary vascular radial and/or primary vascular reticular bone tissues can also be identified in the compact bone of adult individuals (Enlow and Brown, 1958; Martiniaková et al., 2005). Also, some secondary osteons can be observed in rat bones (Enlow and Brown, 1958). However, no data related to collagen fibers orientation in the bone tissue of rats are available yet.

The aim of our study was to analyze in details a microscopic structure of the compact bone tissue in adult (4 month-old) laboratory rats. The microstructure of the *femur* was evaluated from the point of view of qualitative and quantitative histological characteristics.

MATERIAL AND METHODS

In our experiment, five clinically healthy 4 month-old rats (males) were analyzed. The animals were obtained from an experimental farm of the Slovak University of Agriculture in Nitra (Slovakia). Five right *femurs* were studied using histological analysis. Each of the bones was sectioned at the midshaft of its diaphysis. Obtained segments were macerated and degreased (Martiniaková et al., 2007). Later the samples were embedded in epoxy resin Biodur (Günter von Hagens, Heidelberg, Germany). Transverse thin sections (70 - 80 μm) were prepared with a sawing microtome (Leitz 1600, Wetzlar, Germany) and affixed to glass slides with Eukitt (Merck, Darmstadt, Germany). The qualitative histological characteristics of the bone tissue were determined according to the classification systems of Enlow and Brown (1956) and Ricqles et al. (1991); the quantitative ones were assessed using Motic Images Plus 2.0 ML computer software (Motic China Group Co., Ltd.) in anterior, posterior, medial and lateral views of thin section. Since osteon size can vary in above mentioned views (Skedros et al., 2007), we measured the area, perimeter and the minimum and maximum diameter of primary osteon vascular canals, Haversian canals and secondary osteons in all views of thin section in order to minimize statistical differences in the individual. Additionally, the orientation of collagen fibers has also been analyzed using polarized-light microscopy (NP-400T, Optika microscope, Italy). Using the first order (quartz) retardation plate as compensator, the course of collagen fibers can be colored in purple-red and/or dim-violet and/or blue and/or yellow. Collagen fibers that run exactly parallel to the axis of the microscope show the same purple-red color as the background,

whereas fibers which run only slightly oblique had a dim-violet color. Fibers which run in polarized light parallel to the direction of the additive position are blue and those at right angles are yellow (Schultz, 2001).

RESULTS AND DISCUSSION

The femoral compact bone tissue of analyzed animals had following microstructure in common. The arrangement and distribution of different bone tissue types is given from the medullary cavity towards the periosteal surface:

- The inner layer surrounding the medullary cavity was in general formed by a zone of non-vascular bone tissue in all views (anterior, posterior, medial and lateral). The bone tissue contained cellular lamellae and osteocytes. Vascular canals, either primary or secondary, were absent. There were also some areas of primary vascular radial bone tissue identified in lateral and posterior sides. The tissue was composed of branching or unbranching vascular canals which radiated from the marrow cavity. Using polarized-light microscopy with the first order retardation plate we have found that collagen fibers run exactly parallel to the axis of the microscope (purple-red color) and/or run only slightly oblique (dim-violet color) and/or run in polarized light parallel to the direction of the additive position (blue color).
- Some primary and secondary osteons have been observed in middle parts of *substantia compacta* (Fig. 1). However, dense Haversian bone tissue which is characterized by dense concentration of secondary osteons has not been identified in 4 month-old rats. In polarized light, collagen fibers between primary and

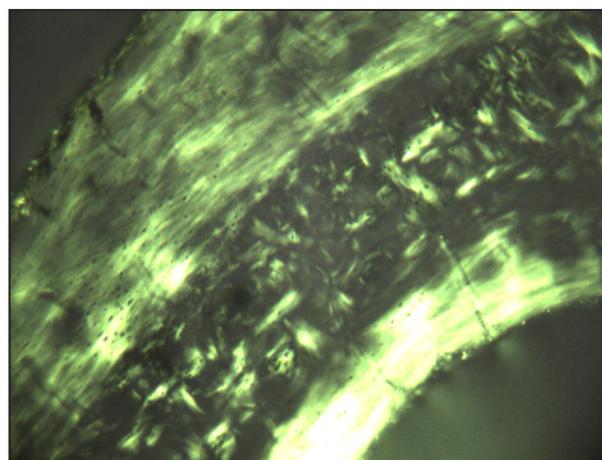


Fig. 1: Localization of primary and secondary osteons in middle part of compact bone tissue in 4 month-old rats [225X]

secondary osteons run parallel to the direction of the additive position (blue color) or only slightly oblique to the axis of the microscope (dim-violet color). In some parts, the course of collagen fibers was colored in yellow; these fibers run in polarized light at right angles to the direction of the additive position.

- Periosteal border was again composed of non-vascular bone tissue. Collagen fibers run only slightly oblique to the axis of the microscope (dim-violet color) or parallel to the direction of the additive position (blue color).

Our results from qualitative histological analysis correspond to those reported by other researchers in rats (Enlow and Brown, 1958; Martiniaková et al., 2005; Reim et al., 2008). The basic structural pattern of the compact bone tissue was non-vascular. In addition, primary vascular radial and/or irregular Haversian bone tissues were observed. However, we did not identify primary vascular reticular bone tissue which had been observed in adult individuals (6 month-old rats) in the studies of Enlow and Brown (1958) and Martiniaková et al. (2005). We have found some secondary osteons in rat's compact bone tissue which lead to the formation of irregular Haversian bone tissue. However, there was no evidence of true Haversian intracortical remodeling in our rats. The newly formed remodeling units within the compact bone originated from endocortical surface

and extended deeply into the underlying compact bone. The same results have also been observed in the study of Reim et al. (2008) in 13 month-old male rats.

Using quantitative histological analysis 84 vascular canals of primary osteons, 42 Haversian canals and 42 secondary osteons were measured. The results are summarized in Table 1. Mean diameter of primary osteon's vascular canals, mean diameter of Haversian canals and mean diameter of secondary osteons were $8.44 \pm 1.11 \mu\text{m}$, $9.46 \pm 1.38 \mu\text{m}$, and $40.21 \pm 6.62 \mu\text{m}$, respectively. Using classifications of Rämisch and Zerndt (1963) and Gladuhsew (1964) we noted that vascular canals of primary osteons and Haversian canals are very short in 4 month-old laboratory rats. Similar results have also been observed in 6 month-old individuals (Martiniaková et al., 2005).

In conclusion, compact bone tissue of 4 month-old laboratory rats has been analyzed by qualitative and quantitative histological methods. We have found that these animals dispose with non-vascular and/or primary vascular radial and/or irregular Haversian bone tissues. Vascular canals of primary osteons and Haversian canals were very short. The orientation of collagen fibres in adult laboratory rats has also been described for the first time. The results can be applied in experimental studies focusing on comparison of the skeletal biology between various age groups of rats.

Table 1: The results of quantitative histological analysis of compact bone tissue from 4 month-old laboratory rats

Measured structures	Vascular canals of primary osteons	Haversian canals	Secondary osteons
Area (μm^2)	225.03 ± 51.16	282.52 ± 66.56	5060.51 ± 1581.84
Perimeter (μm)	53.36 ± 6.13	60.01 ± 7.50	255.93 ± 37.39
Max. diameter (μm)	9.08 ± 1.23	10.34 ± 1.56	46.05 ± 6.28
Min. diameter (μm)	7.79 ± 0.98	8.58 ± 1.19	34.36 ± 6.96

ACKNOWLEDGEMENT

This study was supported by the grant KEGA 3/7338/09.

REFERENCES

- BOYDE, A. 2003. The real response of bone to exercise. *J. Anat.*, vol. 203, 2003, p. 173-189.
- CURREY, J. D. 2002. *Bones: Structure and Mechanics*, 1st edition. New Jersey : Princeton University Press, 2002. ISBN 0-691-09096-3
- ENLOW, D. H. – BROWN, S. O. 1956. A comparative histological study of fossil and recent bone tissues. Part I. *Texas J. Sci.*, vol. 8, 1956, p. 405-412.
- ENLOW, D. H. – BROWN, S. O. 1958. A comparative histological study of fossil and recent bone tissues. Part III. *Texas J. Sci.*, vol. 10, 1958, p. 187-230.
- ERBEN, R. G. – BRUNNER, K. S. – BREIG, B. 2004. Long-term sensitivity of uterus and hypothalamus/pituitary axis to 17β -estradiol is higher than that of bone in rats. *J. Bone Miner. Res.*, vol. 19, 2004, p. 1827-1832.
- GLADUHSEW, J. M. 1964. Problems of the histological investigation of the bone in forensic medicine. *Sudebno Med. Exp.*, vol. 7, 1964, 23-26.
- MARTINIAKOVÁ, M. – GROSSKOPF, B. – VONDRÁKOVÁ, M. – OMEĽKA, R. – FABIŠ, M. 2005. Observation of the microstructure of rat cortical bone tissue. *Scripta medica*, vol. 78, 2005, p. 45-50.

-
- MARTINIAKOVÁ, M. – GROSSKOPF, B. – OMELKA, R. – DAMMERS, K. – VONDRÁKOVÁ, M. – BAUEROVÁ, M. 2007. Histological study of compact bone tissue in some mammals: a method for species determination. *Int. J. Osteoarch.*, vol. 17, 2007, p. 82-90.
- RÄMSCH, R. – ZERNDT, B. 1963. Vergleichende Untersuchungen der Haversschen Kanäle zwischen Menschen und Haustieren. *Archiv für Kriminologie*, vol. 131, 1963, p. 74-87.
- REIM, N. S. – BREIG, B. – STAHR, K. – EBERLE, J. – HOEFELICH, A. – WOLF, E. – ERBEN, R. G. 2008. Cortical bone loss in androgen-deficient aged male rats is mainly caused by increased endocortical bone remodeling. *J. Bone Miner. Res.*, vol. 23, 2008, p. 694-704.
- RICQLES, A. J. – MEUNIER, F. J. – CASTANET, J. – FRANCILLON-VIEILLOT, H. 1991. Comparative microstructure of bone. In: Hall, B. K.: Bone 3, Bone Matrix and Bone Specific Products. Boca Raton : CRC Press, 1991, p. 1-78.
- SCHULTZ, M. 2001. Paleohistopathology of bone: a new approach to the study of ancient diseases. *Yearbook of physical anthropology*, vol. 44, 2001, p. 106-147.
- SHIMANO, M. M. – VOLPON, J. B. 2009. Biomechanics and structural adaptations of the rat femur after hindlimb suspension and treadmill running. *Brazilian Journal of Medical and Biological Research*, vol. 42, 2009, p. 330-338.
- SKEDROS, J. G. – SORENSON, S. M. – JENSON, N. H. 2007. Are distributions of secondary osteon variants useful for interpreting load history in mammalian bones? *Cells Tissues Organs*, vol. 185, 2007, p. 285-307.