Thickness of the substantia compacta of porcine long bones

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ABSTRACT: Mechanical load on the bone influences bone tissue and its inner tension, subsequently affecting bone formation and its histological structure. A precise understanding of this load and the development of strategies to influence it would contribute to principles of fracture management and to solving other bone pathologies of both humans and animals. The long bones of the thoracic and pelvic limb of a pig were here used as possible models to test new devices and implant materials. The purpose of this study was to investigate the thickness of compact bone of the diaphysis of porcine long bones and to establish ideal insertion points for tensometer probes, where 2.3 mm is the minimal required thickness, and to evaluate the histological structure of the compact porcine bone. A total number of 96 long bones from 12 pigs was investigated. The investigations consisted of morphometric assessment of the diaphysis of the bones by measuring thickness of the compact bone in different segments of the diaphysis and of microscopical evaluation of the compact bone. Macroscopical assessment of the bones revealed that the minimal required thickness of 2.3 mm of compact bone was found only in the middle and distal segment of the humerus, middle segment of the femur, proximally only on cranial aspect. The radius showed suitable thickness on the medial aspect of its proximal segment and on the lateral and caudal aspect of the middle segment. Tibial compact bone is suitable across the whole middle segment and on its lateral and medial aspect of the distal segment. Microscopical structure of the compact bone revealed characteristics of growing/immature bone characterised by both lamellar and osteonic bone. This study confirms the suitability of porcine radius and tibia for tensometer testing. However, one needs to take into account the different thickness of different parts of the bone planning experiments using tensometers.

Keywords: porcine bones; compact bone; bone measurement; swine

Mechanical load on the bone influences bone tissue and its inner tension. Bone adapts to mechanical loads largely by changing its size and shape, which are major determinants of its resistance to injury (Turner 2006). Much has been published on the mechanical properties of compact bone, the factors which affect its properties, and variation between species (Currey 1979, 1984; Zioupos and Currey 1998; Tommasini et al. 2005). Studies report little variation in the mechanical properties of compact bone from the limbs of adult mammalian quadrupeds, but considerable adaptive variation in the mechanical properties of different bone types. Quadrupedal animal models are extensively used as animal models for human research. Porcine bone has been used for the development of different surgical techniques, the use of fixation devices (Seil et al. 1998; Kousa et al. 2005, Stembirek et al. 2012) and in forensic studies investigating trauma (Southern et al. 1990; Humphrey and Hutchinson 2001). Despite the widespread use of the porcine model in orthopaedic research, few studies have investigated the mechanical properties and composition of compact porcine bone when considering an appropriate animal model for bone research (Szilagyi et al. 1980; Aerssens et al. 1998). Mechanical properties of femoral bone are likely to correspond to the loading and strain patterns of the bone during normal locomotion (Skuban et al. 2009). These patterns will differ considerably between bipedal and quadrupedal animals.

A precise understanding of this load and the development of strategies to influence it would contribute to fracture management and to the solving of other bone pathologies of both humans and animals (Macione et al. 2011; Verbruggen et al. 2012). Many studies have employed mechanical testing to evaluate how the structural properties of the bone are affected (Currey 1979; Turner and Burr 1993; Turner 2006). Of these, the fundamental structural properties of greatest importance are generally the stiffness, strength and toughness, together with more complicated properties such as the fatigue resistance. Biomechanical testing to evaluate the stiffness of the bone, which is defined in terms of the elastic modulus (shear force), and the strength (tension forces and compression forces) are documented; however, the reproducibility of results is problematic in such studies (Currey 1979; Turner and Burr 1993).

Recently, a detection device with a tensometer was developed at the Faculty of Engineering (Czech Technical University, Prague, Czech Republic) which is capable of detecting the actual tension forces within the bone. However, ideal bone specimens for testing have not yet been established (Volf et al. 2002). The device requires full insertion within the compact bone of minimal thickness of 2.3 mm. The purpose of this study was to investigate the thickness and histological structure of compact bone of the diaphysis of porcine long bones and to establish ideal insertion points for tensometer probes.

MATERIAL AND METHODS

Animals. Bones of the thoracic and pelvic limbs of twelve domestic pigs (*Sus scrofa domestica*) of six to seven months of age and average weight of 110 kg were used. Only humerus, radius, femur and tibia were used from each limb. A total number of 96 bones was investigated.

Sample preparation. Selected bones (humerus, radius, femur and tibia) were harvested from the limbs of animals immediately after slaughter. Harvested bones were stripped of all soft tissues and macerated using a biological maceration technique which consisted of maceration in water of temperature between 25–30 °C for a period of two to three months. The maceration process was considered finished once all the remaining soft tissues on the bone (e.g., muscles, ligaments and periosteum) were detached. Bones were mechanically cleaned and fat was removed from the bone using soap solution (Jar, Procter & Gamble, Rakona s.r.o., Czech Republic). Final cleaning and whitening of the bones was performed using 6% hydrogen peroxide at 25 °C for 48 h.

Osteometric measurements. Clean bones were investigated using an osteometric method described by Von den Driesch (1976). A digital calliper was used to gather metric values. Bone greatest length (GL) was measured from the most proximal to the most distal extent and was cut transversally into four equal segments using three cutting planes (proximal plane A, middle plane B, distal plane C). The tibia was cut into five equal segments using four cutting planes (proximal plane A, middle proximal plane B, middle distal plane C, distal plane D). Plane B corresponded to the half of GL, plane A 2 cm above plane B and plane C 2 cm below plane B. In the case of tibia plane D was measured 4 cm below plane B. The following measurements were taken on the bones:

- GL – (greatest length) the longest distance of the long bone (distance between most proximal and most distal points on the bone)
- AW = width – lateromedial distance in plane A
- BW = width – lateromedial distance in plane B
- CW = width – lateromedial distance in plane C
- DW = width – lateromedial distance in plane D
- AH = height – craniocaudal distance in plane A
- BH = height – craniocaudal distance in plane B
- CH = height – craniocaudal distance in plane C
- DH = height – craniocaudal distance in plane D
- AK = thickness of the compact bone on the cranial aspect of plane A
- AL = thickness of the compact bone on the lateral aspect of plane A
- AM = thickness of the compact bone on the medial aspect of plane A
- AC = thickness of the compact bone on the caudal aspect of plane A
- BK = thickness of the compact bone on the cranial aspect of plane B
- BL = thickness of the compact bone on the lateral aspect of plane B
- BM = thickness of the compact bone on the medial aspect of plane B
- BC = thickness of the compact bone on the caudal aspect of plane B
- CK = thickness of the compact bone on the cranial aspect of plane C
- CL = thickness of the compact bone on the lateral aspect of plane C
- CM = thickness of the compact bone on the medial aspect of plane C

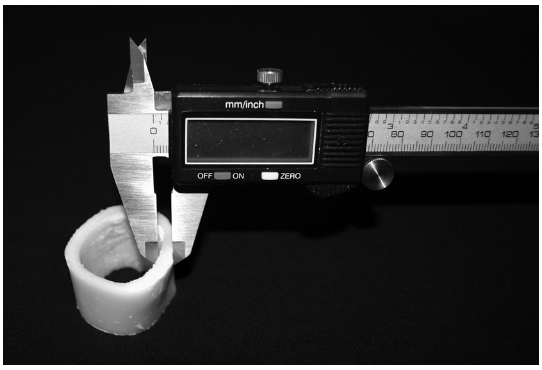

Figure 1. Calliper used to measure compact bone thickness

– CC = thickness of the compact bone on the caudal aspect of plane C
– DK = thickness of the compact bone on the cranial aspect of plane D
– DL = thickness of the compact bone on the lateral aspect of plane D
– DM = thickness of the compact bone on the medial aspect of plane D
– DC = thickness of the compact bone on the caudal aspect of plane D

Measurements of the thickness of the substantia compacta were always taken on each cutting plane using a digital calliper (Figure 1). The surface of the substantia compacta was determined as the outer smooth surface and the inside as the smooth inside surface excluding any macroscopically visible thin bony prominences. All values were evaluated using descriptive statistics in statistical software (Unistat 5.0, Czech Republic). No further statistical comparison of bone morphometry was used in this study.

Ground section preparation. Samples of the substantia compacta were harvested from cutting plane B. Slices of approximate thickness of 1–2 mm were harvested using a hand saw blade parallel with the cutting plane. A total number of three ground sections was evaluated from each aspect of the corresponding plane and were collected as full thickness compact bone on medial, lateral, cranial and caudal aspects of a bone at the level of the cutting plane B. Ground sections were prepared from each sample according to Maat et al. (2001). Harvested samples were initially manually grinded on one side using grinding papers of increasing calibration (P 800, P 1500, P 2000 and P 2500). This prepared site (smooth and shiny) was attached to a microscopic slide using resin. The remaining tissue of the sample was gradually grinded to a final size of 20–30 μm using incremental calibrations of grinding papers (P 320, P 450, P 600, P 800, P 1500, P 2000 and P 2500). The thickness and quality of the sample was continually checked under a light microscope using 40× and 100× magnification. The finalised samples were removed from microscopic slides using immersion in xylene for a period of 60–180 min. Removed samples were then mounted on clean microscopic slides using Solacryl (Solakryl BMX 900 ml, Dr. Kulich Pharma, s.r.o., Hradec Kralove, Czech Republic). Microscopic structure and orientation of osteons was assessed qualitatively. The long axis of the diaphysis was used as a reference point for the description of osteon orientation.

RESULTS

Morphometric assessment revealed differing thicknesses of cortical bones within individual bones

The osteometric analysis of selected bones is summarised in Tables 1–4.

The minimal required thickness of 2.3 mm which is required for insertion of the tensometer probe was met in the following areas of the bone planes: on the humerus in plane B and C across the whole section (medial, lateral, cranial and caudal); on the radius in plane A medially and in plane B laterally and caudally; on the femur in plane A cranially and plane B across the whole circumference and on the tibia in plane B across the whole circumference and in plane C medially and laterally.

Microscopic structure revealed differing spatial organisations of the compact bone

Differing organisations of spatial orientation of osteons and lamellae were observed between different bones and within the bone and its plane.

There were differences in the spatial orientation of the compact bone of the osteon in different types of bones and their diaphysis (i.e., humerus, radius, femur and tibia). Furthermore, the histological structure of bone ground sections revealed both lamellar bone and osteonic bone characterised by presence of newly formed osteons and large vascular canals forming within the lamellar bone.

Humerus. *Cranial aspect* (Figure 2C) – the layer of outer circumferential lamellae consisted of three

Table 1. Statistical results of the measurements of the humerus (n = 24)

Measured values	Minimum (mm)	Maximum (mm)	Average (mm)	Median (mm)	SD	$S_{\bar{x}}$
GL	138.0	156.0	147.13	147.00	5.788	1.182
AW	20.6	22.9	21.93	22.20	0.767	0.157
BW	16.0	19.1	18.06	18.50	0.950	0.194
CW	17.4	19.6	18.63	18.70	0.699	0.143
AH	28.3	30.5	29.51	29.50	0.814	0.166
BH	21.1	23.6	22.64	23.00	0.829	0.169
CH	23.3	26.0	24.83	25.00	0.879	0.179
AK	1.3	2.2	1.78	1.85	0.280	0.057
AL	1.0	1.6	1.38	1.40	0.187	0.038
AM	1.0	2.5	1.84	1.85	0.458	0.093
AC	1.4	1.9	1.64	1.60	0.147	0.030
BK	2.5	3.9	3.09	3.10	0.360	0.073
BL	2.5	2.9	2.69	2.70	0.128	0.026
BM	2.0	3.1	2.71	2.80	0.343	0.070
BC	2.9	3.6	3.25	3.25	0.219	0.045
CK	2.1	4.0	3.32	3.55	0.643	0.131
CL	1.8	2.8	2.35	2.50	0.335	0.068
CM	1.5	2.9	2.27	2.40	0.463	0.095
CC	2.0	4.9	3.33	3.15	0.886	0.181

SD = standard deviation,$S_{\bar{x}}$ = sample standard deviation, GL = the longest distance of the long bone (distance between most proximal and most distal points on the bone); AW-width = lateromedial distance in plane A, BW-width = lateromedial distance in plane B, CW-width = lateromedial distance in plane C, AH-height = craniocaudal distance in plane A, BH-height = craniocaudal distance in plane B, CH-height = craniocaudal distance in plane C, AK = thickness of the compact bone on the ranial aspect of plane A, AL – thickness of the compact bone on the lateral aspect of plane A, AM = thickness of the compact bone on the medial aspect of plane A, AC = thickness of the compact bone on the caudal aspect of plane A, BK = thickness of the compact bone on the cranial aspect of plane B, BL = thickness of the compact bone on the lateral aspect of plane B, BM = thickness of the compact bone on the medial aspect of plane B, BC = thickness of the compact bone on the caudal aspect of plane B, CK = thickness of the compact bone on the cranial aspect of plane C, CL = thickness of the compact bone on the lateral aspect of plane C, CM = thickness of the compact bone on the medial aspect of plane C, CC = thickness of the compact bone on the caudal aspect of plane C

to four lamellae overlaying circularly oriented osteons with scant distribution of longitudinal osteons. The layer of inner circumferential lamellae was composed of two to three lamellae.

Lateral aspect – three to four lamellae formed the outer circumferential lamellae layer that was overlaying a lower layer which was composed of predominantly circularly oriented osteons with scant longitudinally oriented osteons. Inner circumferential lamellae layer had three lamellae.

Medial aspect – the layer of outer circumferential lamellae was represented by four lamellae. Osteons were evenly oriented circularly and longitudinally with high numbers of osteons in the irregular orientation. The layer of inner circumferential lamellae consisted of four lamellae.

Caudal aspect – three to four lamellae formed the outer circumferential lamellae layer. Circular and irregular osteons were present in the compact bone. Longitudinal osteons were scarce. The layer of inner circumferential lamellae consisted of four lamellae.

Radius. *Cranial aspect* – diaphysis was smooth with small irregularities and composed of two to four outer circumferential lamellae. The osteons closer to the periosteum had circular orientation and those closer to the endosteum showed “plexiform” or irregular orientation. Two to three inner

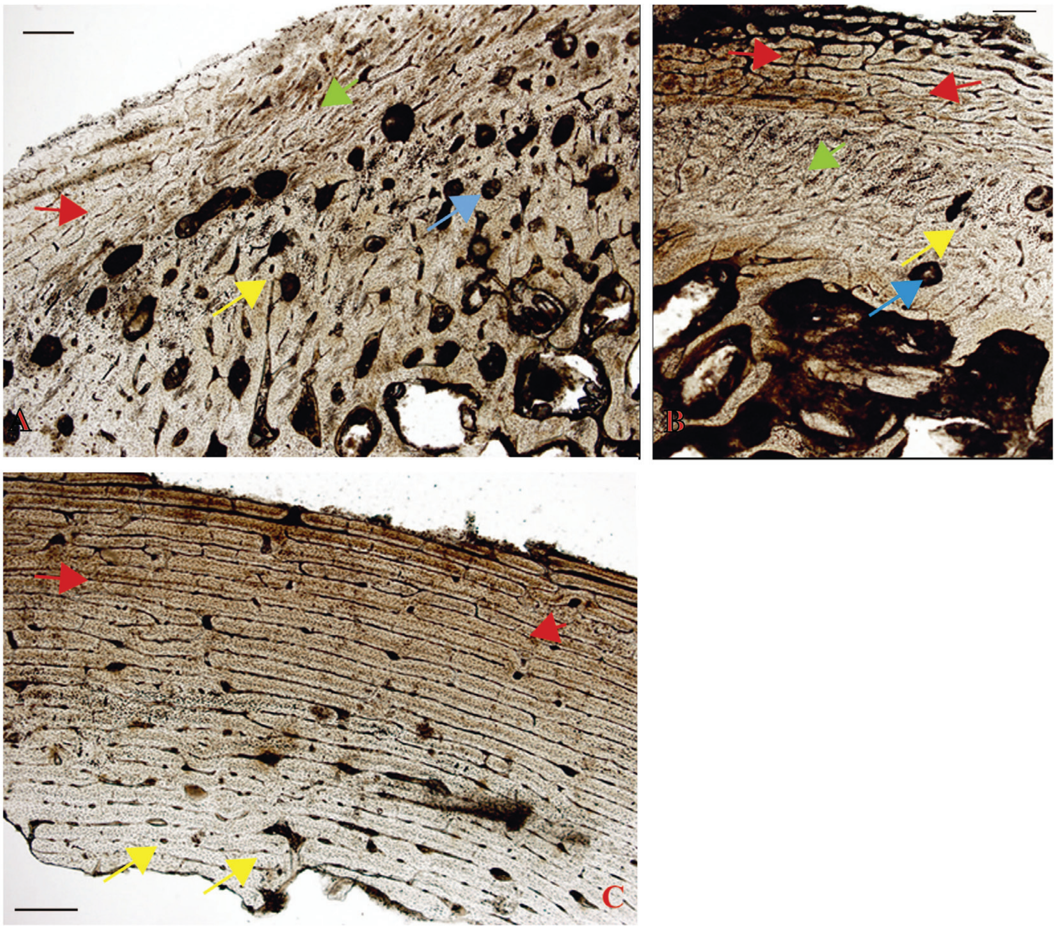

Figure 2. **A**. Ground section of radius; plane B, lateral aspect. Circular and irregular osteons present closer to the periosteum. Longitudinal osteons present closer to the endosteum. The inner layer of the compact bone follows with small and short prominences of spongious bone. **B**. Ground section of radius; plane B, medial aspect. The outer layer closer to the periosteum consists of circular osteons overlaying a layer consisting of predominantly irregular osteons with scant longitudinal osteons. **C**. Ground section of humerus; plane B, lateral aspect. Circular osteons are predominantly present in this ground section. Scarce longitudinal osteons are present close to the endosteal surface. Red arrow = circular osteons, yellow arrow = longitudinal osteons, blue arrow = vascular compartments/canals, green arrow = irregular osteons, bar = 100 μm. Plane B corresponds to the cut in the centre of the diaphysis

circumferential lamellae formed a thin layer below the endosteum.

Lateral aspect (Figure 2A) – the layer of outer circumferential lamellae consisted of three to four lamellae. The osteons closer to the periosteum showed a circular orientation, whereas those closer to the endosteum were predominantly irregular. Longitudinal osteons were scarce. The inner layer of circumferential lamellae consisted of three lamellae and the endosteal surface showed numerous projections of thin spongious bone.

Medial aspect (Figure 2B) – the outer circumferential lamellae consisted of four lamellae. Osteons had a predominantly circular orientation with some presence of longitudinal and irregular osteon orientation. The inner circumferential lamellae consisted of three lamellae with numerous projections of spongious bone.

Caudal aspect – three to four lamellae formed the outer circumferential lamellae layer which overlayed a layer of predominantly circularly oriented osteons with scant longitudinally oriented osteons. Three inner circumferential lamellae were present.

Femur. *Cranial aspect* – the outer circumferential lamellae consisted of three to four lamellae. Osteons located closer to the periosteum were

Table 2. Statistical results of the measurements of the radius (n = 24

Measured values	Minimum (mm)	Maximum (mm)	Average (mm)	Median (mm)	SD	$S_{\bar{x}}$
GL	102.0	120.0	111.96	110.50	5.433	1.109
AW	19.4	21.4	20.41	20.50	0.659	0.134
BW	19.2	22.1	20.61	20.80	1.030	0.210
CW	21.9	24.2	23.15	23.35	0.825	0.168
AH	11.3	13.7	12.43	12.40	0.891	0.182
BH	12.8	15.1	14.03	14.30	0.849	0.173
CH	15.7	19.3	17.68	18.15	1.273	0.260
AK	1.2	1.4	1.31	1.30	0.078	0.016
AL	1.2	2.6	1.83	1.90	0.470	0.096
AM	2.2	2.5	2.36	2.40	0.097	0.020
AC	1.0	1.5	1.28	1.30	0.137	0.028
BK	1.0	1.3	1.21	1.25	0.106	0.022
BL	2.0	2.6	2.32	2.40	0.195	0.040
BM	1.7	2.2	1.98	1.95	0.171	0.035
BC	1.9	2.8	2.36	2.40	0.269	0.055
CK	1.0	1.5	1.33	1.40	0.182	0.037
CL	1.4	2.0	1.76	1.75	0.179	0.037
CM	1.9	2.1	1.98	2.00	0.070	0.014
CC	1.3	1.8	1.58	1.60	0.145	0.030

For explanation see Table 1

predominantly circular and those closer to the endosteum were predominantly irregular and longitudinal. The inner circumferential lamellae were three in number.

Lateral aspect – the layer of outer circumferential lamellae consisted of three to four lamellae. Osteons were evenly distributed and represented by both irregular and circular osteons. The layer of inner circumferential lamellae consisted of three to four lamellae.

Medial aspect – the layer of outer circumferential lamellae was represented by four lamellae. Observed osteons were evenly represented by circular, longitudinal and irregular osteons. The layer of inner circumferential lamellae was composed of four lamellae.

Caudal aspect – the outer circumferential lamellae consisted of four lamellae overlaying a layer of circular and irregular osteons which were evenly represented. The inner circumferential lamellae consisted of four lamellae.

Tibia. *Cranial aspect* – the layer of outer circumferential lamellae was formed by four lamellae. The underlying layer of osteons consisted of predominantly circular osteons and scarce irregular and longitudinal osteons. The inner layer of circumferential lamellae was composed of four lamellae.

Lateral aspect – the layer of outer circumferential lamellae was formed by four lamellae. Osteons were predominantly circular with irregular and longitudinal osteons located closer to the endosteum. The layer of inner circumferential lamellae consisted of three to four lamellae.

Medial aspect – four lamellae formed the outer circumferential lamellae. Osteons were predominantly irregular and longitudinal with a low number of circular osteons. The layer of inner circumferential lamellae consisted of three lamellae.

Caudal aspect – four lamellae formed the outer circumferential lamellae. Osteons were predominantly circular and the layer of inner circumferential lamellae was composed of four lamellae.

DISCUSSION

Assessment of compact bone has revealed differences within and between bones from both the macroscopic and microscopic point of view. Knowledge

Table 3. Statistical results of the measurements of the femur (n = 24

Measured values	Minimum (mm)	Maximum (mm)	Average (mm)	Median (mm)	SD	$S_{\bar{x}}$
GL	156.0	172.0	164.83	166.50	5.231	1.068
AW	20.7	22.5	21.61	21.65	0.574	0.117
BW	19.9	22.5	21.20	21.35	0.846	0.173
CW	26.3	29.4	28.05	28.15	1.067	0.218
AH	20.2	23.3	21.61	21.55	1.018	0.208
BH	19.3	23.3	21.30	21.50	1.353	0.276
CH	23.3	24.8	24.06	24.05	0.442	0.090
AK	2.3	2.9	2.57	2.55	0.193	0.039
AL	1.5	2.1	1.84	1.85	0.206	0.042
AM	1.9	2.5	2.22	2.30	0.186	0.038
AC	2.0	2.5	2.23	2.20	0.151	0.031
BK	2.2	2.8	2.49	2.50	0.153	0.031
BL	1.9	2.7	2.34	2.35	0.272	0.055
BM	2.0	2.6	2.32	2.40	0.195	0.040
BC	2.5	3.2	2.86	2.85	0.245	0.050
CK	1.4	1.6	1.50	1.50	0.083	0.017
CL	1.7	2.0	1.86	1.90	0.078	0.016
CM	1.6	1.8	1.70	1.70	0.083	0.017
CC	1.7	1.9	1.80	1.80	0.072	0.015

For explanation see Table 1

of bone thickness is imperative in the selection process for tensometer testing. We have used porcine bones due to their anatomical similarities with those of humans. Six to seven month old pigs were selected because of their size, age and due to the availability of bone material from the slaughterhouse (most pigs are slaughtered at this age). The demand for a large quantity of bone material for tensometer testing can be therefore easily fulfilled. The tensometer is composed of an oval-shaped detection probe connected to a recording device. The actual detecting device within the tensometer probe is 2.3 mm in length and has to be fully inserted within the bone tissue to record accurate measurements (Volf et al. 2002). The minimal required thickness of the bone tissue is therefore 2.3 mm.

Macroscopic assessment of the bones revealed that the minimal required thickness of 2.3 mm of compact bone was found only in the middle and distal segment of the humerus (planes B and C), middle segment of the femur (plane B), proximally only on the cranial aspect. The radius showed suitable thickness on the medial aspect of its proximal segment (plane A) and on the lateral and caudal aspects of the middle segment (plane B). Tibial compact bone is suitable across the whole middle segment (plane B) and on its lateral and medial aspects of the distal segment (plane C).

The method of Von den Driesch (1976) was used and modified to take measurements from the bones. We selected four segments on the radius and five segments on the tibia due to its relative length compared to the radius. The division into segments was made based on the predictive suitability of the bones for tensometer insertion. However, bone thickness is not the only criterion in specimen selection. The tensometer device is designed to be used *in vivo*. Therefore, the tensometer probe needs to be surgically implanted within the bone. A suitable surgical approach has to be chosen and vital soft tissues overlapping the bone must be considered during the surgical approach. The humerus and femur are less suitable for tensometer testing because of the large mass of soft tissues overlapping these bones (Kolda 1950; Seiferle and Frewein 1986). Further studies inves-

Table 4. Statistical results of the measurements of the tibia (n = 24)

Measured values	Minimum (mm)	Maximum (mm)	Average (mm)	Median (mm)	SD	$S_{\bar{x}}$
GL	154.0	167.0	160.46	160.50	3.912	0.799
AW	24.2	26.0	24.98	24.95	0.594	0.121
BW	22.4	23.8	22.99	22.90	0.416	0.085
CW	23.1	24.7	23.86	23.85	0.518	0.106
DW	26.2	28.1	27.28	27.45	0.594	0.121
AH	25.0	26.1	25.65	25.65	0.362	0.074
BH	16.0	16.6	16.35	16.40	0.174	0.036
CH	14.5	15.7	15.04	14.95	0.390	0.080
DH	17.0	18.4	17.76	17.80	0.430	0.088
AK	1.6	1.8	1.70	1.70	0.072	0.015
AL	1.6	2.4	1.98	1.95	0.246	0.050
AM	1.6	1.8	1.68	1.70	0.074	0.015
AC	1.4	2.0	1.72	1.75	0.208	0.042
BK	2.6	3.3	2.93	2.90	0.214	0.044
BL	2.3	3.6	3.01	3.00	0.463	0.095
BM	2.2	2.7	2.45	2.40	0.135	0.028
BC	2.2	2.6	2.39	2.40	0.135	0.028
CK	2.1	2.5	2.23	2.20	0.103	0.021
CL	2.2	2.9	2.57	2.60	0.206	0.042
CM	2.8	3.3	3.04	3.05	0.147	0.030
CC	2.1	2.3	2.21	2.20	0.078	0.016
DK	1.5	1.7	1.59	1.60	0.078	0.016
DL	1.6	2.0	1.83	1.80	0.113	0.023
DM	2.0	2.4	2.22	2.20	0.109	0.022
DC	1.5	1.9	1.74	1.75	0.128	0.026

For explanation SD to CC see Table 1
DK = thickness of the compact bone on the cranial aspect of plane D, DL = thickness of the compact bone on the lateral aspect of plane D, DM = thickness of the compact bone on the medial aspect of plane D, DC = thickness of the compact bone on the caudal aspect of plane D

tigating/describing safe corridors for tensometer insertion are needed.

Bones of the zeugopodium, mainly the radius, are positioned almost perpendicular to the ground during weight bearing and are therefore exposed to a large mechanical load as described previously (Hert et al. 1994; Frost 2004). Due to this large mechanical stress, compact bone of the radius and tibia thickens significantly and becomes suitable for tensometer testing. Differences were observed between the investigated bones mainly when comparing the radius and tibia. The compact bone of the radius is thinner along the whole cranial and caudal aspect compared to its medial and lateral aspects. The thickness of this bone is probably determined by the weight-bearing function of this bone as force vectors act along the long axis of the radius (Barone 1966; Nickel et al. 1986; Frost 2004).

The ulna and fibula were not used in our study due to their small size, shape and large muscle mass overlapping these bones which makes them unsuitable for tensometer insertion (Kolda 1936; Nickel et al. 1986; Konig and Liebich 2003). Measurement of the bone thickness of macerated bones is affected by the removal of the periosteum during the maceration process, which reduces the overall thickness of the bone. The thickness of the diaphyseal periosteum is reported to vary from 0.5–1 mm in thickness (Augustin et al. 2007). This inaccuracy in the measurement could have been overcome by using CT transverse images of the bone segments in this study. However, this does not affect the decision on tensometer insertion as it has to be fully inserted within the cortical bone.

We studied the microscopic structure of the bone to complement the macroscopic study. All investigated samples showed a structure of immature bone compared to the known structure of bones from adult pigs (Tichy et al. 2000). This immature bone can be characterised by circumferentially oriented lamellae and the forming of osteons. Newly formed osteons can be further characterised by concentric laminae around large vascular canals forming within the lamellar bone. This description of immature or maturing bone is in agreement with other studies (Enlow and Brown 1956; Locke 2004; Martiniakova et al. 2008). However, even mature porcine bone possesses characteristics of both lamellar and osteonic bone unlike that of humans, which is composed mostly of osteons (Locke 2004). All samples were selected and harvested from the middle part of the diaphysis (plane B) because the perichondral ossification originates in this area of the bone. Therefore, the maturity of the bone in this area is predictably most advanced (Tichy et al. 2000).

Ground section samples were used for investigation because this method is superior for our purposes when compared to decalcification methods (Maat et al. 2001; Thorat et al. 2011). The advantages of the use of ground sections include permanent control of specimen orientation, the possibility for immediate correction during the preparation process and availability of the whole cross section of the compact bone. Another important factor is the low cost of sample preparation.

The microscopic study revealed developing and immature compact bone. Differing microscopic bone structures were observed between and within different bones. Compact bone often consisted of two layers - an outer layer which consisted of osteons with circular orientation and an inner layer with plexiform orientation, and occasional longitudinal osteons. This differed in different segments. This structure was not observed in the humerus, femur and medial tibia and was present in the radius and most commonly in the middle segment of the tibia. It can therefore be concluded that the areas of thick bone with high bone turnover are areas of bone with the highest mechanical stress (Parfitt 1984; Volf et al. 2002). The higher bone turnover can be explained by the different positions of the bones. The humerus, which is not perpendicular to the ground, is not capable of transforming the weight forces longitudinally and its bone turnover is therefore slower compared to the femur (Tichy et al. 2000; Frost 2004).

This study confirms the suitability of porcine radius and tibia for tensometer testing. The circumference of the central part of the radius and tibia possesses an ideal thickness for tensometer insertion. However, in *in vivo* studies/experiments one must keep in mind vital structures which might be injured during surgical approach to the bone.

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