LONGITUDINAL VARIATION IN MECHANICAL COMPETENCE OF BONE ALONG THE AVIAN HUMERUS

RICHARD H. C. BONSER

University of Bristol, School of Biological Sciences, Woodland Road, Bristol, BS8 1UG, UK

Summary

Vickers microhardness tests were used to gauge the mechanical 'competence' (ability to resist bending and failure) of cortical and trabecular bone along the humeri of three bird species. Hardness was greatest at the mid-length portion of the shaft. The mean hardness of trabeculae, where present, was between 78.7 and 90.9 % of that of the adjacent cortical bone. The possible causes of this are briefly discussed. Microhardness tests offer the opportunity to gauge differences in mechanical properties over small distances and might usefully be applied to test the homogeneity of mechanical properties within specimens for tensile or compressive tests.

Key words: Vickers microhardness, cortical bone, trabecular bone, longitudinal variation, mechanical competence, *Larus argentatus*, *Columba livia*, *Lagopus lagopus*.

Introduction

The mechanical properties of avian bones have received little attention in the literature, probably because of difficulties in machining regular test specimens from small bones. In this paper, I will describe how microhardness tests can be used as a means to examine changes in the mechanical competence of compact and trabecular bone along the length of the avian humerus. 'Competence' is defined here as being the ability of a material to resist bending and failure.

Microhardness testing involves the application of an indenter under constant load to the material under test. Although shear failure is responsible for forming the indentation, the physical properties controlling shear failure in bone are also those responsible for stiffness and tensile failure (i.e. mineralisation). As the indentations made during hardness testing may be very small (20–40 μm), the technique is suitable for use on very small bone samples, such as individual trabeculae. Carlström (1954) first used microhardness tests on bones, and many authors have subsequently investigated the relationships between hardness and physical properties, such as Young's modulus, of bone (see Szilágyi *et al.* 1980; Hodgskinson *et al.* 1989; Currey and Brear, 1990; Evans *et al.* 1990; Bonser, 1993). Microhardness has been shown by these studies to have a strong positive relationship with Young's modulus, yield stress and ultimate stress.

Because the indentation is so small, the technique is very suitable for examining the mechanical competence of trabecular bone. Lénárt *et al.* (1968) investigated differences in hardness between the corticalis and spongiosa of mammalian bone. Hodgskinson *et al.* (1989) found that, in mammalian bones, the cortical bone was 10–20 % harder than the adjacent cancellous bone; this difference was related to the calcium content of the two bone types. Longitudinal variation in cortical hardness has also been demonstrated (Weaver, 1966; Szilágyi *et al.* 1980).

In this paper, I will address the following two questions: how does the competence of bone vary along the length of the avian humerus, and are there any differences between the competence of trabeculae and adjacent cortical bone?

Materials and methods

Three humeri were examined from each of three species of bird: herring gull (*Larus argentatus* Pontoppidan), rock pigeon (*Columba livia* Gmelin) and willow ptarmigan [*Lagopus lagopus* (Linnaeus)]. The specimens were all air-dried prior to use. Each humerus was sectioned with a small handsaw at 5 mm intervals along its entire length. Each section was then degreased in a 2:1 chloroform:ethanol mixture, and any adherent fat or cutting debris blown away using compressed air. The clean, dry sections were then embedded in Acrulite casting resin (Rubert and Co., Cheadle, UK) to which had been added a small quantity of carbon black to increase the visual contrast between the bone and resin. After curing, the sections were polished flat using 500 grit emery paper and finally polished with an abrasive alumina paste.

Microhardness tests were performed using a Leitz 'Wetzlar' Miniload test machine (Ernst Leitz, Wetzlar, Germany), with an indenter for the Vickers hardness test. Vickers hardness number (VHN) is given by:

$$VHN = 1854P/d^2, \qquad (1)$$

where P is applied mass in g, and d is the length of the indentation diagonal in μm. VHN is expressed in kg mm^{-2}.

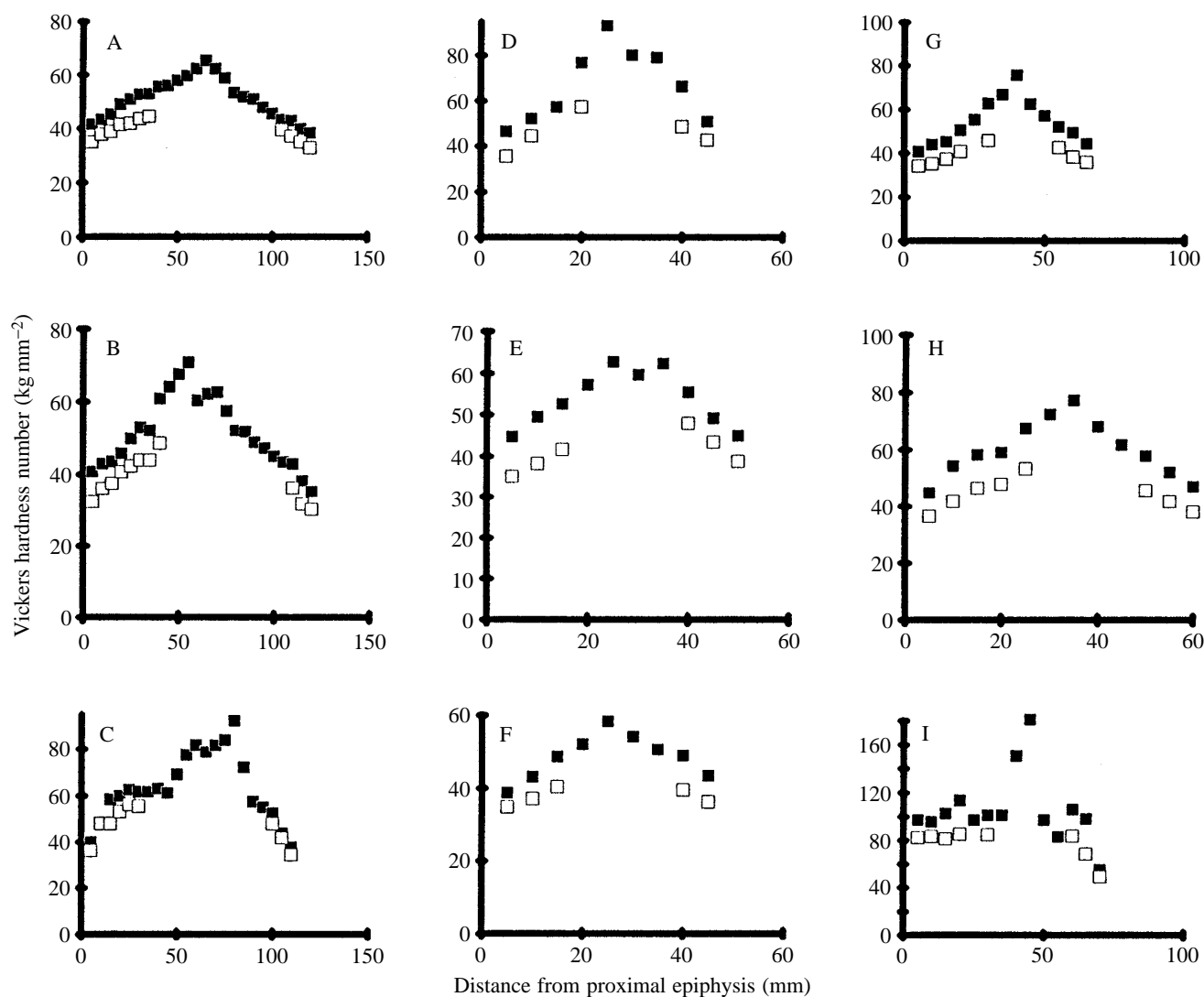Descent time for the indenter was approximately 10 s, after

Fig. 1. Variation in the Vickers hardness number along the length of humeri from three herring gulls (A,B,C), three rock pigeons (D,E,F) and three willow ptarmigan (G,H,I). ■ indicates cortical bone; □ indicates trabecular bone.

which the indenter was allowed to remain in contact with the bone for 15 s. The indenter was elevated and a further 45 s allowed before the indentation was measured. Ten indentations were made at random sites on the cortical and trabecular regions of each section. The hardness of each section was the mean of these ten measurements. A load of 25 g was used throughout.

The herring gulls were individuals from a captive colony and were all aged 43 months on death. The pigeons and willow ptarmigan were all wild birds; in all cases, the birds had attained full adult plumage and the epiphyses of their limb bones had fused. None of the birds was killed specifically for the study. The masses of the birds (kg) were as follows: herring gulls, A, 0.89, B, 0.75, C, 0.8; rock pigeons, D, 0.36, E, 0.34, F, 0.35; willow ptarmigan, G, 0.77, H, 0.75, I, 0.76.

Results

The variation in Vickers hardness along the length of the humeri from the three species are presented graphically in Fig. 1. It is immediately apparent that the bone mineral was hardest at the mid-portion of the humerus and decreases in hardness towards the epiphyses. For all nine humeri, the hardness of trabeculae (where present) was significantly less than that of the adjacent cortical bone (Table 1). The mean hardness of the trabeculae was, at most, 90 % or less than that of the adjacent cortical bone (Table 1).

Discussion

These results represent the first attempt to investigate the regional differences in mechanical properties along avian bones. Clearly, there is considerable variation in the mechanical properties of bone mineral along the length of the humerus, with the hardest bone being found in the mid-portion of the shaft. Additionally, there is a consistent difference between the hardness of cortical and trabecular bone. It is worth speculating why such differences between cortical and trabecular bone occur along humeri. The pattern of these differences may be

Table 1. *Significance of, and percentage difference between, cortical and cancellous bone hardness*

Species	Bird	t	d.f.	P	Trabecular hardness (% of cortical hardness)	S.E.M.
Herring gull	A	13.111	10	<0.001	85.5	0.7
	B	12.148	10	<0.001	83.8	0.8
	C	4.732	8	<0.01	90.9	1.6
Rock pigeon	D	5.194	4	<0.01	78.7	2.5
	E	8.748	5	<0.001	82.5	2.0
	F	7.388	5	<0.001	85.6	1.4
Willow ptarmigan	G	8.874	7	<0.001	79.8	1.2
	H	16.239	8	<0.001	79.7	0.5
	I	6.683	7	<0.001	81.0	2.2

Comparisons were made using two-tailed paired t-tests.

due to the manner in which bones mature. It is known that the mineralisation of cartilage in immature bones commences at the mid-length, and a 'front' of mineralisation sweeps towards the epiphyses (see Simkiss, 1967; Sissons, 1971), resulting in bone at the mid-diaphyseal region being 'older' than that at the epiphyses. There is some confusion in the literature concerning the development and maintenance of trabecular bone. In birds, trabecular bone develops after cortical mineralisation has commenced (see Taylor *et al.* 1971), so trabeculae are 'less mature' than cortical bone. Many birds have additional granular calcium stores present in the medullary region of their long bones. Investigators have often failed to distinguish clearly whether the mobility of these deposits is similar to or distinct from that of trabecular bone. There are technical problems associated with differentiating between trabecular bone and medullary calcium deposits (Lynch and Maxwell, 1991), so it is possible that workers have failed to distinguish between the two bone types and, hence, have attributed high mobility to mineral in trabeculae. Hurwitz (1965) found that avian medullary and epiphyseal bone were both considerably more 'mobile' than diaphyseal cortical bone. Of particular interest to this study is the observation that, in poultry, cortical bone from the humerus was the least mobile type; bone from the epiphyses was more mobile. In any case, there appear to be differences between the turnover rates of mineral from different regions of limb bones. Certainly, if time elapsed since the initiation of ossification was the principal factor affecting bone mineralisation, then one would expect that the mid-shaft of bones should be harder than both the epiphyses and trabeculae.

The differences observed may result from 'adaptive remodelling' (for a review, see Lanyon, 1981) corresponding to the *in vivo* pattern of strain distribution along the humerus. If strain is important in determining the maintenance of mineral, then one would expect that areas of bone subject to low strains should show decreased mineralisation, resulting in a lowering of mechanical competence. Lanyon (1974) found that the magnitude and direction of cortical strain, measured *in vivo*,

was a good predictor of the orientation and distribution of trabeculae. Hayes and Snyder (1981) produced a finite-element model of a patella and found that trabeculae were orientated mainly along the principal stress trajectories within the bone. Goldstein (1987) concluded that variability in cancellous bone properties could be attributed to functional adaptation. Indeed, examination of the gross morphology and distribution of cancellous bone within long bones suggests that the trabeculae are orientated along the axes of stress trajectories. Given that trabeculae appear to be orientated along strain axes, the lower mechanical competence of trabeculae compared with that of cortical bone may indicate that lower stresses are customarily present in trabeculae. When we have a thorough understanding of the direction and magnitude of the forces acting on the avian humerus it will be possible to determine whether the patterns of bone competence reported here are due to adaptation to the magnitude and direction of functional strains.

Rice *et al.* (1988) reviewed values of Young's modulus of cancellous bone and found that it was generally lower and far more variable than that reported for cortical bone. Most of the studies from which this review drew data had tested samples of cancellous bone rather than individual trabeculae. It is quite possible that the accuracy of the results was dependent, to a large degree, on the method by which the 'fabric' of the cancellous bone was quantified. Using microhardness tests removes this problem, as the mechanical competence of individual trabeculae can be determined directly and may be compared with that of the adjacent cortical bone. It may prove feasible to investigate how mechanical properties change in the interface between trabeculae and the cortex. Indeed, Blackburn *et al.* (1992) have demonstrated that it is possible to measure the difference in properties of microcallus tissue on trabeculae and normal, adjacent sections of trabeculae.

The results of the present study have important implications for future work on avian bone mechanics. Clearly, if profound longitudinal differences in bone properties are present, then methodological problems may occur if one wishes to machine tension-test specimens from whole bones. The apparent Young's modulus of the specimen will be the result of serial interactions of regions of bone having subtly different properties; this may result in erroneously low estimates of Young's modulus. A better approach may be to use miniature test specimens to minimise the problem of longitudinal variability. Microhardness testing may offer a simple test for homogeneity of properties within test specimens.

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