The relative density of bone types in laying hens

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Bone breakages caused by osteoporosis in laying hens remain commonplace. Studies of this disease are complicated by the presence of medullary bone (MB), a bone type deposited during lay to provide calcium for eggshell formation. In vivo technologies such as Quantitative Computed Tomography (QCT), Dual energy X-ray Absorptiometry (DXA) and Quantitative Ultrasound (QS) could be used to assess laying hen bone but it may be difficult to separate MB and structural bone. In 12 end-of-lay hens, we measured structural (cortical) bone density (CBD) and medullary bone density (MBD) in 250micron x-ray slices prepared post-mortem on a diamond saw with a hydroxyapatite (HA) step-wedge as a reference standard. These measurements were made alongside bone breaking strength (BStr), whole bone radiographic density (RD) and histological measures of the volume fractions of bone types. Measurements of plasma total calcium (Ca) were also made. Results revealed significant correlations between tibia BStr and MB volume fraction (r =0.83, P =0.004), tibia BStr and whole tibia RD (r =0.81, P =0.006), tibia BStr and plasma Ca (r =-0.71, P =0.03), humerus BStr and whole humerus RD (r =0.91, P <0.001), MB volume fraction and whole tibia RD (r =0.79, P<0.01) and whole tibia and humerus RD values (r =0.69, P =0.04). Mean values for CBD and MBD densities (when expressed per unit total tissue) were significantly different (CBD = 1.09±0.01 mg HA/mm^3 total tissue, MBD = 0.38±0.06 mg HA/mm^3 total tissue, paired t-test, P <0.001). When corrections were made for volume fractions of bone types, MBD approached CBD levels in some hens (especially when volume fraction of MB exceeded 50%) but mean MBD remained significantly lower than CBD (CBD = 1.27±0.02 mg HA/mm^3 bone, MBD = 1.12±0.07 mg HA/mm^3 bone, paired t, P = 0.02). These results suggest that it should be possible to separate densities derived from in vivo measurements made in QCT slices into structural and MB contributions respectively. Difficulties may be encountered only when MB volume fractions are very large. However, the above correlations suggest that this may only increase overall bone strength in any case; as long as there is adequate MB surface area to allow mobilisation of calcium for eggshell formation, larger amounts of MB on endocortical surfaces may protect structural bone and may actually be beneficial for bone strength in ageing hens.

Keywords: Hens; Osteoporosis; Bone density; Bone strength; Medullary bone.

Introduction

Recent studies suggest that bone fractures caused by osteoporosis (OP) in laying hens remain a serious welfare issue (Budgell and Silversides, 2004, Sandilands *et al.,* 2005). Studies of this disease *in vivo* are often complicated by the presence of medullary bone (MB), a bone type deposited during lay to provide calcium for eggshell formation that is considered of limited structural value. In vivo technologies such as Quantitative Computed Tomography (QCT), Dual energy X-ray Absorptiometry (DXA) and Quantitative Ultrasound (QS) could be used to assess partitioning of bone types in laying hen bone but there are difficulties in separation of MB and structural bone using these technologies (Hester et al, 2004, Korver et al, 2004, Fleming et al 2004). If MB could be adequately separated from structural bone by density value then it should be possible to set an appropriate density threshold for research use. However there is little or no empirical data available for the density value of MB in relation to structural bone types in the laying hen. In 12 end-of-lay hens, we set out to

accurately measure the relative contributions of structural (cortical) bone density (CBD) and medullary bone density (MBD) post-mortem, and also the relationships between these and other post-mortem bone measurements.

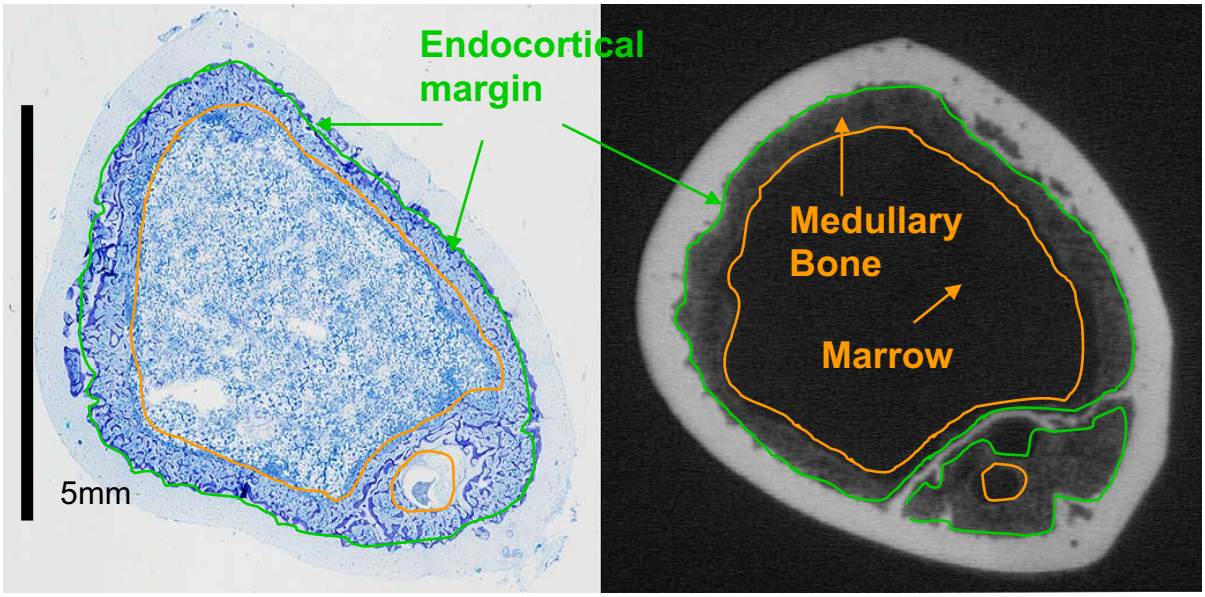
Materials and Methods

Measurements of plasma total calcium (plasma Ca) were made prior to culling in 12 individually caged end-of-lay hens (68 weeks) fed on standard layer rations. Following culling and dissection of whole tibiotarsi and humeri, 3x 250µm x-ray slices from the mid–diaphysis of each left tibiotarsus were prepared on a Microslice diamond saw. Slices were radiographed alongside a hydroxyapatite (HA) step-wedge (as a bone mineral reference standard) on Kodak MRE x-ray film in a Faxitron x-ray cabinet. Films were digitised in a Kodak LS-75 x-ray line scanner and mean densities were calculated within each bone type (in mg HA/mm^3 of total tissue) using the public domain image analysis program *ImageJ* (http://rsb.info.nih.gov/ij/). Linear radiographic densities (RD) of whole dissected bones were also determined. Measurements were also made of bone breaking strength (BStr) on right bones using a 3–point bending jig on a JJ Lloyd LRX materials tester. Spatial measurements from slice x-rays were utilised to calculate the 2nd moment of area (I) using the formula:

$$I = \pi/4(\text{External radius}^4 - \text{Internal radius}^4).$$

This was then used to calculate tibia bending stress (in N/mm^2) in accordance with ASAE standard methodology (1997). Histological measurements were made of the volume fractions of bone types in tibial mid-diaphysis using toluidine blue staining to differentiate structural and MB bone types and confirm their location in x-ray slices (see Figure 1). Volume fractions of bone types calculated from histological slices were also used to correct the mean x-ray slice densities to mg HA/mm^3 of bone tissue.

Figure 1. A toluidine blue stained histological section of mid-tibiotarsus, with adjacent slice x-ray to show distribution of cortical (light blue), medullary bone (dark blue) and bone marrow, and radiographic appearance of each bone type. Volume fraction of MB and mean radiographic density was measured for all tissue between the green and orange boundaries. Cortical bone density and volume fraction were measured outside the green boundary.



Results and Discussion

All data from individual birds are presented in Table 1.

Mean values for CBD and MBD densities (when expressed per unit total tissue, not shown in table) were significantly different (CBD = 1.09+/-0.01 mg HA/mm^3 total tissue, MBD = 0.38+/-0.06 mg HA/mm^3 total tissue, paired t-test, P <0.001). When corrections were made for volume fractions of bone types, MBD approached CBD levels and exceeded them in some hens (especially when volume fraction of MB exceeded 50%) but as a group, means remained significantly lower (CBD = 1.272+/-0.02 mg HA/ mm^3 bone, MBD = 1.122+/-0.07 mg HA/mm^3 bone, paired t, P = 0.02).

Table 1. All measurements from individual birds.

Bird ID	Tibia BStr (N)	Humerus BStr (N)	Tibia Bending Stress (N/mm^2)	Plasma Ca (mmol/l)	MB volume fraction (%)	Tibia linear RD (mm HA equiv)	Humerus linear RD (mm HA equiv)	CBD (mg HA/mm^3 bone)	MBD (mg HA/mm^3 bone)
O-2	188.3	338.8	59.3	5.5	29.1	1.618	1.028	1.286	1.367
O-1	128.4	117.8	55.2	6.0	24.3	1.724	1.390	1.314	1.070
P-1	185.8	184.3	69.9	5.5	26.2	1.627	1.133	1.165	1.177
P-2	209.4	88.7	94.6	4.7	20.8	1.706	1.159	1.312	.965
P-3	200.5	105.8	80.1	5.5	30.6	1.860	1.477	1.316	1.110
O-4	306.4	251.3	110.4	4.8	50.7	1.926	1.670	1.425	1.469
O-5	261.1	472.2	108.5	5.0	39.0	1.872	1.093	1.244	.809
O-6	249.3	113.0	96.8	5.5	31.5	1.777	1.302	1.234	.877
P-4	215.5	237.4	93.6	.	20.2	1.726	1.116	1.214	1.005
P-5	180.0	89.8	80.4	.	30.2	1.677	1.153	1.221	1.118
P-6	197.3	120.8	80.6	5.4	24.2	1.906	1.361	1.214	.994
O-3	412.2	192.5	123.6	.	63.9	1.760	1.398	1.315	1.510

Results also revealed significant correlations between the following measurements in Table 2:

Table 2. Significant correlations between measurements.

	r	P
Tibia BStr v. MB volume fraction	0.83	0.004
Tibia BStr v. whole tibia linear RD	0.81	0.006
Tibia BStr v. Tibia Bending Stress	0.92	0.001
Tibia BStr v. plasma Ca	-0.71	0.03
MB volume fraction v. whole tibia linear RD	0.79	<0.001
Whole tibia linear RD v. humerus linear RD	0.69	0.04
Humerus BStr v. whole humerus linear RD	0.91	<0.001
Humerus BStr v. whole tibia linear RD	0.68	0.04
Tibia Bending Stress v. plasma Ca	-0.80	0.008
MBD v. MB volume fraction	0.64	0.02

There were no significant correlations between CBD and other bone characteristics, suggesting that overall bone strength is perhaps related more to bone mass rather than material quality in this group of hens. There is a clear indication that as MB volume increases, MBD also increases, suggesting that the larger amounts of MB are better mineralised and less transient in nature. In this way MB may contribute to overall bone structure. The negative correlations with plasma calcium and parameters of strength are of interest and appear to be unrelated to MB volume fraction. However they may suggest more vigorous osteoclastic activity in those birds with poorer bones. This is in agreement with previous findings on osteoclast numbers in OP susceptible hens (Fleming et al, 2005).

These results indicate that it should be possible to separate densities derived from *in vivo* measurements made in QCT slices into structural and MB contributions respectively if careful thresholding adjustments are made. Difficulties may be encountered when MB volume fractions are

very large (> 50%). However, the above correlations suggest that this may only increase overall bone strength in any case as strength and MB volume fraction are well correlated (r =0.83, P =0.004); as long as there is adequate MB surface area to allow mobilisation of calcium for eggshell formation, larger amounts of better mineralised MB on endocortical surfaces may protect structural bone and may actually be beneficial for bone strength in ageing hens.

References

ASAE STANDARDS (1997) Shear and Three-Point Bending Test of Animal Bone. *ANSI/ASAE S459 Feb93, Approved FEB 1993 by American National Standards Institute*, 572-574.

BUDGELL, K. L. and SILVERSIDES, F. G. (2004) Bone breakage in three strains of end-of-lay hens. *Can. J. Anim. Sci*. **84:**745-747.

FLEMING, R.H., KORVER, D.R., McCORMACK, H.A. and WHITEHEAD, C.C. (2004) Assessing Bone Mineral Density in vivo: Digitised Fluoroscopy and Ultrasound. *Poultry Science* **83:** 207-214.

FLEMING, R.H., McCORMACK, H.A. McTEIR, L. and WHITEHEAD, C.C (2005) Environmental and Genetic Effects on Osteoclast Recruitment in Osteoporotic Avian Bone. *Bone and Tooth Society Annual Meeting, Birmingham, 4-5 July 2005. Journal of Bone and Mineral Research* **20**: 1307.

HESTER, P.Y., SCHREIWEIS, M.A., ORBAN, J.I., MAZZUCO, H., KOPKA, M.N., LEDUR, M.C. and MOODY, D.E. (2004) Assessing Bone Mineral Density in vivo: Dual Energy X-Ray Absorptiometry. *Poultry Science* **83:** 215-221.

KORVER, D.R., SAUNDERS-BLADES, J.L. and NADEAU, K.L. (2004) Assessing Bone Mineral Density in vivo: Quantitative Computed Tomography. *Poultry Science* **83**: 222-229.

SANDILANDS, V., SPARKS, N., WILSON, S., and NEVISON, I. (2005) Laying hens at depopulation: the impact of the production system on bird welfare. *British Poultry Abstracts,* **1(1):** 23-24.

Acknowledgements

We wish to acknowledge Defra for funding these studies and Laura Durie for her excellent technical assistance.