



The influence of severe prolonged exercise restriction on the mechanical and structural properties of bone in an avian model

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ABSTRACT

Many studies have described the effects of exercise restriction on the mammalian skeleton. In particular, human and animal models have shown that reduction in weight bearing leads to generalised bone loss and deterioration of its mechanical properties. The aim of this study was to assess the effect of prolonged exercise restriction coupled with heavy calcium demands on the micro-structural, compositional and mechanical properties of the avian skeleton. The tibiae and humeri of 2-year-old laying hens housed in conventional caging (CC) and free-range (FR) housing systems were compared by mechanical testing and micro-computed tomography (μ CT) scanning. Analyses of cortical, cancellous and medullary bone were performed.

Mechanical testing revealed that the tibiae and humeri of birds from the FR group had superior mechanical properties relative to those of the CC group, and μ CT scanning indicated larger cortical and lower medullary regions in FR group bones. Cancellous bone analysis revealed higher trabecular thickness and a higher bone volume fraction in the FR group, but no difference in mineral density. The biomechanical superiority of bones from the FR group was primarily due to structural rather than compositional differences, and this was reflected in both the cortical and cancellous components of the bones. The study demonstrated that prolonged exercise restriction in laying hens resulted in major structural and mechanical effects on the bird skeleton.

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Introduction

The morphological, structural and material features of the vertebrate skeleton are genetically programmed. However these features are modified throughout life by epigenetic factors, the most important of which are locally acting stresses and strains created by intrinsic muscle forces and by the effects of external loads. Many studies have investigated the effects of increased or decreased load on the structure of bones, both *in vivo* and *in vitro* (Woo et al., 1981; Rubin and Lanyon, 1984; Unthoff et al., 1985; Biewener and Bertram, 1994; Hillam and Skerry, 1995; Carter et al., 1996; Giangregorio and Blimkie, 2002; Lentle and Kruger, 2005). Increased load causes an increase in bone mass while decreased load (due to disuse following enforced rest, space flight or other causes of reduced loading) leads to loss of bone mass. These effects occur through bone modelling and remodelling and are mediated by osteoblasts and osteoclasts.

The ability of bone to adapt its mass, shape and internal architecture to the prevailing mechanical loading environment is a fundamental concept of bone biology. Indeed, the concept that bone

structure is controlled locally to best fulfil its mechanical function is a central tenet of orthopaedics and forms the basis of much paleontological and bioarchaeological study of skeletal material (Ruff et al., 2006).

Walking is the most common activity resulting in mechanical loading of bones of the appendicular skeleton. This repetitive and consistent activity results in typical levels of strain produced by muscle contraction and ground reaction forces. Strain is a key regulator of the modelling and remodelling process of the skeleton and is critical to the homeostasis of bone tissue (Huiskes, 2000). Laying hens housed in conventional caging (CC) provide an extreme example of animals with prolonged, severe limitations on their capacity to walk. This form of housing results in decreased weight bearing activity, and also reduces strains on the non-weight bearing bones of the forelimbs due to restricted activity such as wing flapping (Knowles and Broom, 1990; Norgaard-Nielsen, 1990). Furthermore, due to the high calcium requirements of daily egg shell production (an average egg shell contains 2.3 g of calcium; Etches, 1987), laying hens are subjected to prolonged and intensive demands in terms of their calcium metabolism.

The skeletal effects of prolonged movement restrictions associated with CC can be examined by comparing the properties of the bones of these birds with those of laying birds kept under free-

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range (FR) conditions. Such comparisons are particularly useful if birds in both groups belong to the same strain, are fed the same commercial food and are at the same stage of their laying cycle. Previous studies have evaluated the effect of housing on laying hens on bone properties and have shown that the bones of chickens housed in CC have lower breaking strength (Rowland et al., 1968; Meyer and Sunde, 1974; Knowles and Broom, 1990; Fleming et al., 1994; Newman and Leeson, 1998; Leyendecker et al., 2005) and reduced trabecular bone volume (Hughes et al., 1993; Wilson et al., 1993) compared to FR chickens. From a clinical perspective, and although pathological fractures and lameness as well as fractures discovered at slaughter have been reported in flocks held in CC (Meyer and Sunde, 1974; Gregory and Wilkins, 1989; Webster, 2004), the detailed micro-structural properties of bones of CC and FR layers have not been reported.

The aims of this study were to compare the micro-structural, compositional and mechanical properties of weight bearing and non-weight bearing bones in laying birds housed in CC and FR systems. Insights gained from this work are expected to contribute to a better understanding of the physiology of bone modelling and remodelling in laying hens, the pathological effects of prolonged movement restriction, and the welfare needs of caged laying hens.

Materials and methods

Sample selection

Long bones were obtained from 2-year-old layers housed under FR ($n = 12$) and CC conditions ($n = 13$). All birds were of the 'Hy-line W-99' breed, were hatched in the same hatchery (Schreiber Hatchery, Hod Ha-Sharon, Israel) and fed the same commercial standard balanced layer diet. Layers belonging to the FR group were raised in cages with free access to a yard with a density of 4 birds/m², whereas hens belonging to the CC group were raised in a CC with a density of 12.5 birds/m² (the cage size was 40 cm wide × 40 cm deep × 42 cm high, with 2 birds/cage). In both FR and CC systems, all birds are routinely slaughtered at the end of the 2 year laying period. Carcasses were weighed immediately after slaughter, the tibiae and humeri were removed and stripped of all soft tissue, and these bones were wrapped and stored at -20°C within 6 h.

Mechanical testing

On the day of testing the bones (24 humeri and 24 tibiae from the FR group and 26 humeri and 26 tibiae from the CC group) were slowly thawed to room temperature over a period of 1–2 h. Biomechanical testing was performed using an Instron materials testing machine (Model 3345) fitted with a custom-built saline-containing testing chamber. All bones were tested by the three-point bending method while fully immersed in saline. Each bone was placed on two supports having rounded profiles (1 mm in diameter) such that the supports were equidistant from the ends of the bone, and both contacted the posterior aspect of the diaphysis. The distance between the supports was 38 mm. Each bone was loaded on its anterior aspect, at the mid-point between the bottom supports, at the precise mid-point along its length. Loading proceeded at a constant rate (2 mm/min) up to fracture point, as identified by a sudden decrease in load, or to a maximum load of 400 N.

Force–displacement data were collected by Instron software (BlueHill) at 10 Hz. The resulting load–displacement curves were used to calculate whole bone stiffness (slope of the linear portion of the load–displacement curve), yield load, load-to-fracture, ultimate load and area – under-the-curve (AUC) (Lanyon et al., 1982). The yield point was defined as the load at which the load–deformation relationship ceased to be linear, and was determined by adapting the 0.03% offset criterion (Turner, 1989).

Structural analysis

Following mechanical testing, 12 tibiae and 12 humeri from each group were scanned using high-resolution micro-computed tomography (μCT) (eXplore locus SP, General Electric) with customised software (version 5.2.2, MicroView). Medium resolution scans (28 μm voxel size) were acquired. The X-ray source was set at 80 kV and 80 μA . A total of 720 projections were acquired over an angular range of 360°. The image slices were reconstructed using customised software (MicroView reconstruction software, version 2.2). As part of the analysis the scanned images were rotated so as to align their anatomical and scan axes.

Analysis of cortical bone was performed on a 1 mm long transverse section of the diaphysis of the tibiae and humeri (approximately 70 slices), adjacent and distal to the fracture site (all of the bones tested fractured at the same location, at the

point of load application). The following cortical properties were measured: mineral density, cross-sectional moment of inertia (CSMI), mean cortical thickness, marrow area, and cortical cross-sectional area. Analysis of cancellous bone was only performed on tibiae.

Micro-CT scans of a 1 mm long transverse section of the distal epiphyseal area of the tibia (approximately 70 slices) were analysed to determine mineral density, bone volume fraction (BVf), mean trabecular thickness and mean trabecular spacing. All tibiae in both groups had prominent medullary bone, and μCT scans were also used to evaluate BVf and mineral density in 10 tibiae from birds in the FR group and in eight tibiae from CC group hens. Mineral density refers to the mineral content of the bone in g/mm³. When this density is calculated in a volume of interest (VOI) of cortical bone, the mineral quantity is divided by the VOI. When the VOI contains cancellous bone, the mineral content of the VOI is divided by the volume of the bony struts without considering the inter-trabecular voids.

Statistical analysis

Data were analysed using NCSS statistical software (NCSS software, V. 6.0.22). The distribution of the various variables was tested for normality using the Shapiro–Wilk test. Comparison between normally distributed variables from the FR and CC groups was carried out using Student's *t* test. When variables were not normally distributed comparisons were made using the Mann–Whitney *U* test. Correlation between variables was examined using the Spearman's Rank correlation test. For all tests $P < 0.05$ was considered statistically significant.

Results

Significant differences were found in many of the variables, both between the CC and FR groups, as well as between weight bearing (tibiae) and non-weight bearing bones (humeri). These differences primarily related to structural and mechanical features. The results are presented as their median value and range.

Bodyweight and bone length

The bodyweight of the birds in both groups were found to be similar, both within and between groups (1.84 [1.68–2.10] kg and 1.76 [1.64–2.20], respectively, $P = 0.7$). The length of the bones of both groups was similar (the tibiae and humeri varied by a maximum of 2% and 1%, respectively). The external morphology of both bone types was also similar with no differences noted within or between groups.

Mechanical properties

The results of mechanical testing of the tibiae and humeri from both groups are presented in Fig. 1A–E. Of the 98 bones (24 FR and CC tibiae, 24 FR and 26 CC humeri) mechanically tested, only three did not break at or below the maximal load (400 N). Significant differences were found in the mechanical properties of tibiae between the two groups (Fig. 1A–E), despite the within group variation.

The following results are presented by their median values and ranges. The bones of the FR group had higher ultimate loads (250.4 [183.4–401.5] N relative to 193.1 [143.9–405.2] N, $P = 0.002$), higher load-to-fracture (188.0 [113.1–373.4] N relative to 129.7 [54.7–269.3] N, $P = 0.001$) and higher yield load (144.6 [94.8–238.7] N relative to 100.7 [71.5–221.2] N, $P = 0.001$) when compared to the CC group. Moreover, the stiffness (slope of the linear portion of the load–displacement curve) of the tibiae from the FR group was higher than that of the CC group (873.6 [549.1–1200.0] N/mm relative to 486.6 [323.1–889.9] N/mm, $P = 0.001$). However, the AUC of the FR group bones was smaller than that of the CC group (192.4 [127.3–878.9] N mm relative to 256.3 [164.6–689.3] N mm, $P = 0.02$). These findings suggest that bones from the FR group are stiffer, but require less energy to fracture.

Significant differences were also noted in all measured variables (with the exception of the fracture load) in the humeri of both groups (Fig. 1A–E) with the FR group having higher ultimate loads (163.1 [140.0–385.0] N relative to 108.9 [73.7–393.9] N, $P < 0.001$),

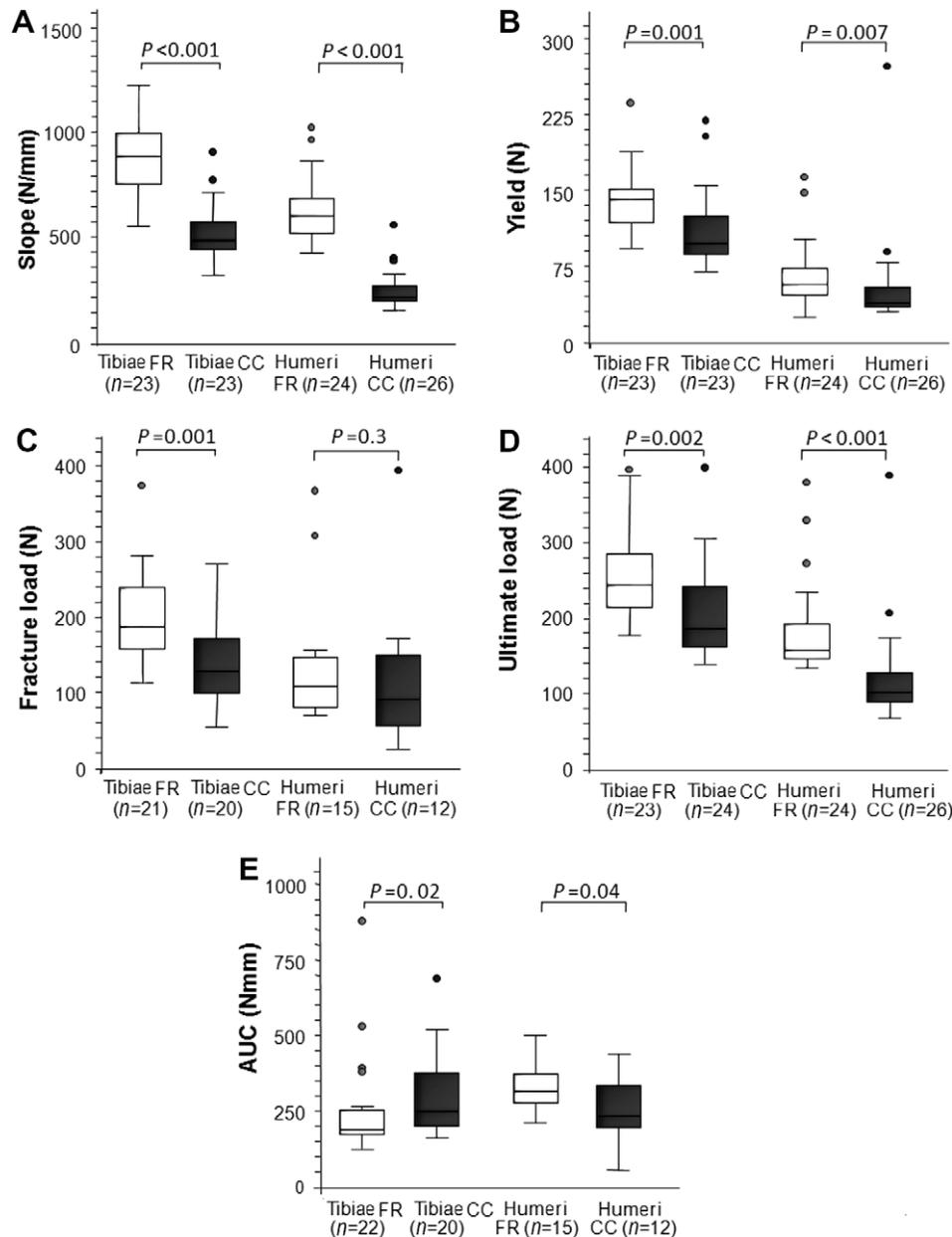


Fig. 1. Box and whisker diagrams illustrating the results of three-point bending tests on tibiae and humeri of birds from the FR and CC groups. Boxes are bounded by the 25th and 75th percentiles with the median shown by the line bisecting the box. Whiskers extend to the full range of the data. Outliers are represented by dots. *P* values shown above the boxes relate to a Mann–Whitney test for difference in medians. (A) Slope of the linear portion of the load–displacement curve (N/mm); (B) yield point (N); (C) fracture load (N); (D) ultimate load (N); (E) area under the load–displacement curve (work to failure, N/mm).

higher yield load (60.0 [26.8–165.0] N relative to 41.6 [33.0–274.8] N, $P = 0.007$) and higher stiffness (601.4 [420.8–1001.9] N/mm relative to 219.6 [161.4–553.6] N/mm, $P < 0.001$) when compared to the CC group. In the humeri the AUC in the FR group was higher than in the CC group.

Morphological characteristics

The results of assessments of the structural characteristics of the cortical bone at the mid diaphysis of the tibiae in both groups are presented in Fig. 2A–G. Significant differences were found in all variables tested except the CSMI. Tibial mineral density was found to be slightly (but significantly) higher in the CC group (965.6 [913.5–1013.6] mg/cm³ relative to 929.5 [894.6–993.0] mg/cm³, $P = 0.019$). Bones from the FR group had significantly larger mean cortical area and thickness compared to those of the CC group

(14.33 [11.19–17.73] mm² relative to 12.49 [10.91–14.22] mm², $P < 0.001$, and 0.85 [0.69–1.07] mm relative to 0.72 [0.63–0.85] mm, $P < 0.001$, respectively). The medullary area was higher in the CC group (17.3 [15.03–24.23] mm² relative to 15.53 [13.02–17.49] mm², $P = 0.007$), while the total bone area was not statistically different between the groups ($P = 0.64$). These findings suggest that the larger cortical area in the CC group is due to greater bone resorption at the endosteal surface rather than greater bone formation at the periosteal surface.

The geometric properties and mineral composition of the mid-diaphysal cortical bone of the humeri of both groups are presented in Fig. 2A–F. As noted for the tibiae, a larger cortical area and cortical thickness were found in humeri from the FR group (13.48 ± 1.25 mm² relative to 10.77 ± 1.38 mm², $P < 0.001$ and 0.73 [0.64–0.84] mm relative to 0.55 [0.48–0.69] mm, $P < 0.001$, respectively), whereas the medullary area was larger in the CC

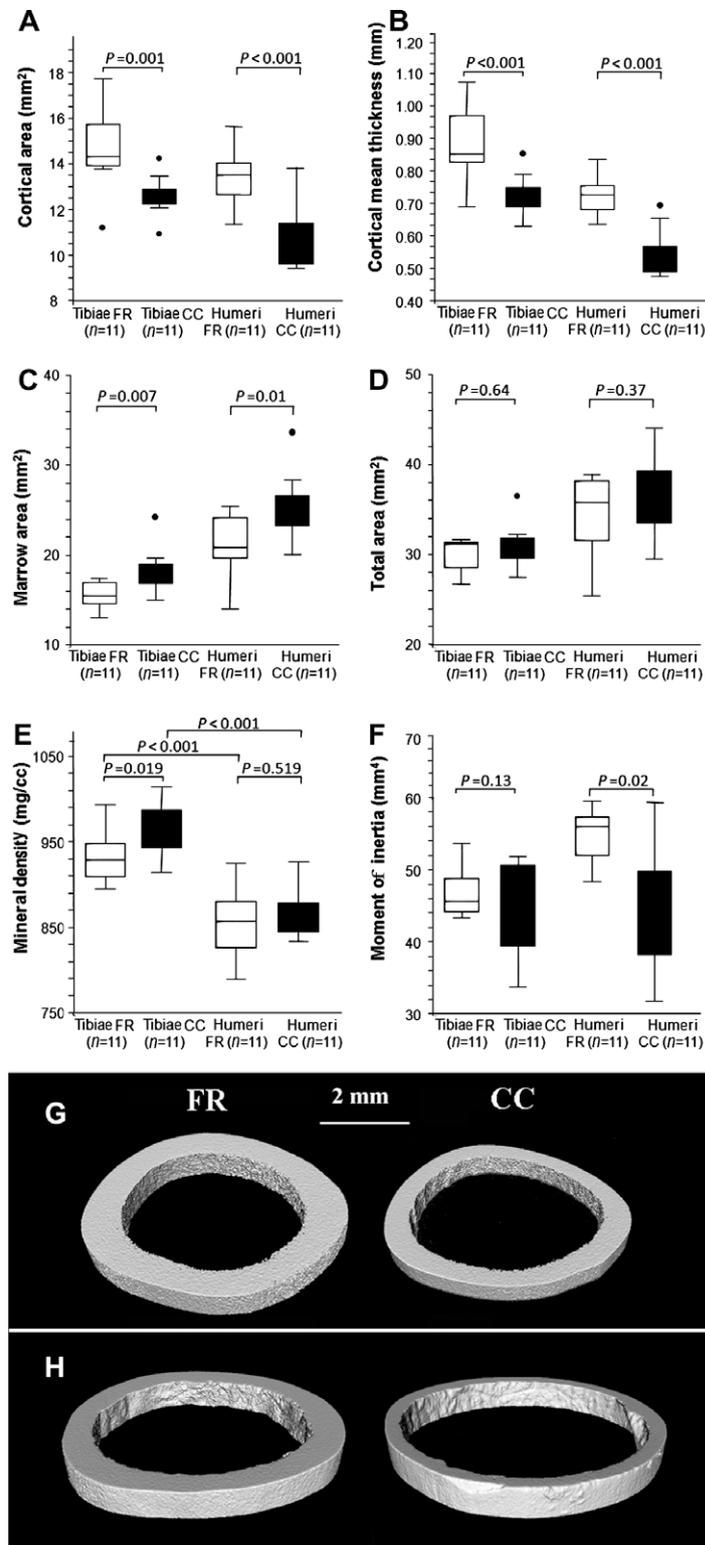


Fig. 2. Box and whisker diagram illustrating micro-architectural characteristics of the cortical bone at the mid-shaft of tibiae and humeri of birds from the FR and CC groups: (A) cortical area (mm²); (B) cortical mean thickness (mm); (C) marrow area (mm²); (D) total bone area (mm²); (E) mineral density (mg/cm³); (F) moment of inertia (mm⁴); (G) micro-computed tomographic image of cortical bone from the tibia of birds from the FR and CC groups illustrating greater cortical thickness and medullary area in the FR and CC hens, respectively; (H) micro-computed tomographic image of cortical bone from the humerus of birds from the FR and CC groups illustrating greater cortical thickness and medullary area in the FR and CC hens, respectively.

group (25.4 [20.1–33.6] mm² relative to 20.9 [14.0–25.5] mm², $P = 0.01$). The CSMI was significantly higher in the FR group (55.9 [48.2–59.5] mm⁴ relative to 46.3 [31.7–59.1] mm⁴, $P = 0.02$). No significant difference in mineral density was found between the

two groups. These findings suggest biomechanical superiority in three-point bending tests of the bones in the FR group, most likely due to architectural rather than compositional features such as mineral density.

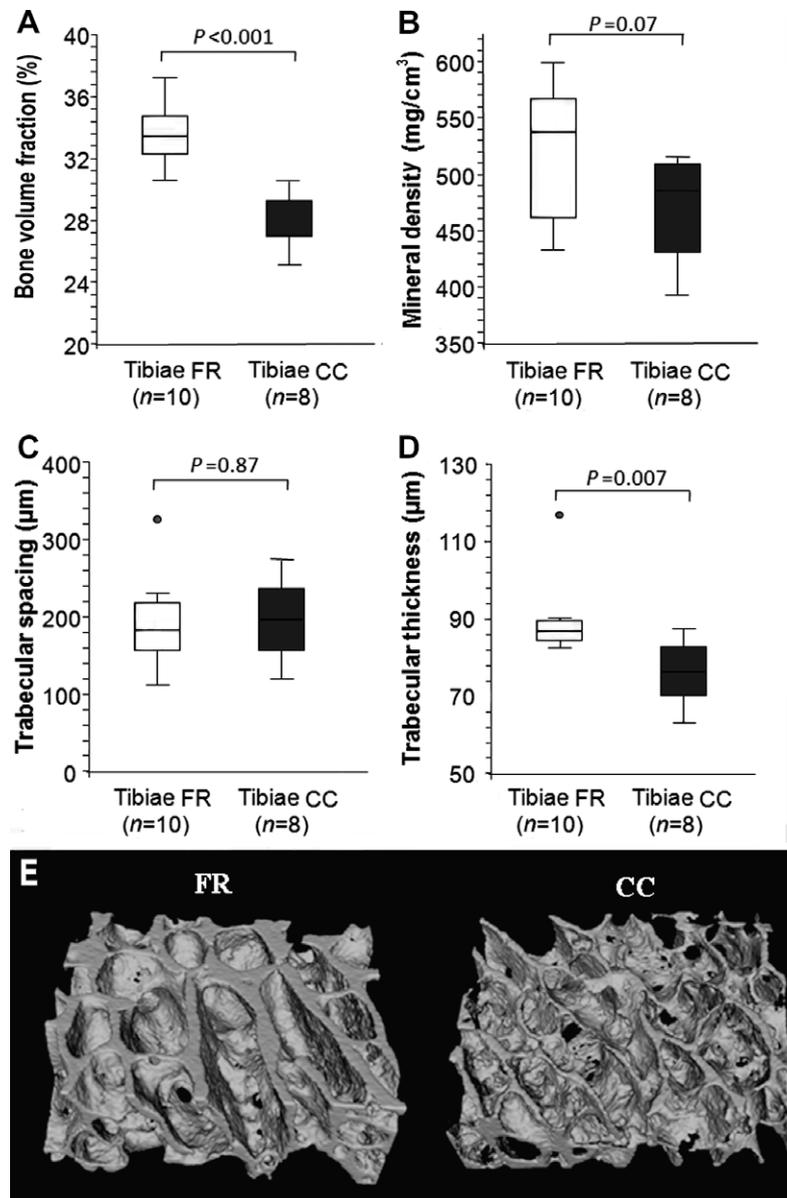


Fig. 3. Box and whisker diagram illustrating micro-architectural characteristics of the cancellous bone at the distal epiphysis of tibiae from birds in the CC and FR groups: (A) bone volume fraction (BVF%); (B) mineral density (mg/cm³); (C) trabecular spacing (μm); (D) trabecular thickness (μm); (E) micro-computed tomographic image of cancellous bone from the CC and FR groups, illustrating greater trabecular thickness in the FR group.

The structural characteristics of the cancellous bone of the two groups at the distal epiphysis of the tibiae are presented in Fig. 3A–E. The bone volume fraction was significantly higher in the FR compared to the CC group (0.33 [0.30–0.37] relative to 0.27 [0.25–0.30]%, respectively, $P < 0.001$). Similarly, the mean trabecular thickness was higher in the FR group (86.9 [58.9–116.8] μm relative to 76.6 [63.2–87.3] μm, $P = 0.007$). This suggests that the cancellous bone of the FR group is denser (i.e. has a higher material content per volume of tissue), and is therefore likely to be more effective in resisting the loads acting on the tibiae.

All tibiae in both groups contained high amounts of medullary bone, which entirely filled the medullary area. Medullary bone refers to woven bone within the medullary canal of the long bones of birds and crocodylians that is formed under the influence of oestrogen (Whitehead, 2004). This medullary bone enables these species to withstand the high calcium demands of eggshell production, facilitating the rapid mobilisation of large quantities of calcium. Analysis of μCT scans of medullary bone of the tibial mid-shaft

yielded similar BVF (0.50 [0.36–0.76] in the FR and 0.49 [0.36–0.66] in the CC group, $P = 0.4$) and mineral density (371.8 [180.0–1013.0] mg/cm³ in the FR relative to 412.4 [205.8–1006.0] mg/cm³ in the CC group, $P = 0.5$) values (Fig. 4B). This finding suggests that the metabolic control of egg-laying, rather than exercise and weight bearing, regulate the production of medullary bone.

Three bones (two tibiae, one from each group, and one humerus from the CC group) consistently yielded results suggestive of extreme biomechanical superiority in the three-point bending tests in all measured variables. These bones were also found to have a high content of medullary bone of high mineral density, as demonstrated by μCT analysis. These bones were excluded from the analysis as it was not possible to differentiate this very dense medullary bone from cortical bone (Fig. 4A).

Spearman correlation tests were performed between the mineral density and BVF of the medullary bone, and the results of mechanical testing of the respective bones, in order to evaluate

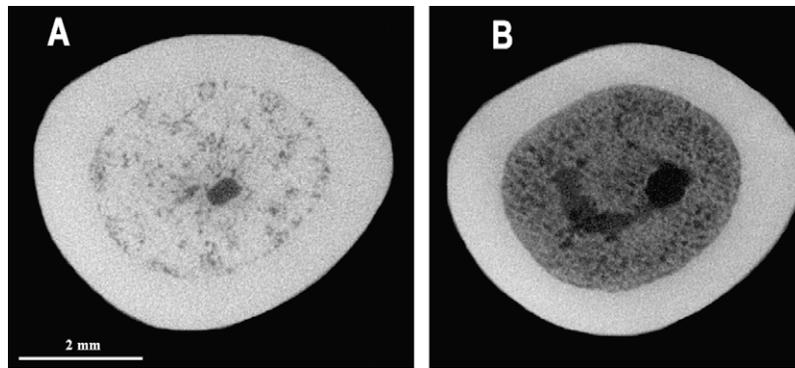


Fig. 4. Micro-computed tomographic image illustrating the density of medullary bone in the tibial mid-shaft of a: (A) free-range and (B) conventionally caged birds.

the potential contribution of medullary bone to the mechanical properties of the whole bone. A weak correlation was found between the mineral density of the medullary bone and maximal load ($r = 0.472$, $P = 0.024$), and correlations were not found with the other mechanical variables evaluated. These findings suggest that medullary bone makes only a marginal contribution to the mechanical performance of the bone as a whole.

Discussion

Bone resorption and formation are closely associated in the process of bone remodelling. This process is essential for maintaining the mechanical properties of bone, for regulating calcium homeostasis and in the repair of skeletal micro-damage caused by daily loading. In mammals, bone remodelling provides a means of mobilising calcium during physiological states such as lactation (Rosen, 2008). Such calcium demands are met by temporarily shifting the balance of bone resorption–production towards resorption. On cessation of lactation, bone mass is rapidly recovered by shifting this balance back towards formation (Rosen, 2008). It is thus intriguing to study the skeletons of laying hens housed in conventional cages, as these birds are subjected to prolonged and continuous exercise restriction coupled with heavy calcium demands. The results of this study demonstrated very significant effects of severe and prolonged exercise restriction on the morphological, compositional and mechanical properties of bones.

The results of whole bone mechanical testing of humeri and tibiae from the CC and FR groups, demonstrate that the bones of free-range layers were mechanically superior in most variables tested. In particular the bones of the FR hens exhibited higher whole bone stiffness, yield load, maximal load and load-to-fracture. Such differences can be attributed to geometrical features of the bones, to superior material composition, or to a combination of these factors (Sharir et al., 2008). These differences can be primarily attributed to the severe exercise restriction imposed on the caged birds, although secondary effects resulting from stress associated with high-density housing may also contribute. These results are supported by previous findings of higher values of several mechanical variables in FR compared to CC birds (Rowland and Harms, 1972; Norgaard-Nielsen, 1990; Leyendecker et al., 2005).

It should be noted that not only the weight bearing tibiae but also the humeri were affected. In the CC system, birds are not only restricted in their movement but also in their ability to flap their wings or perform short flights (Knowles and Broom, 1990; Norgaard-Nielsen, 1990). These activities produce some degree of load and resulting strain on the bones of the wing. Elimination or a significant reduction in these strains can affect bone in a manner akin to reduced weight bearing.

Analysis of μ CT scans of the cortical region indicated that bones from the FR group had a significantly larger mean cortical area and

thickness compared to those of the CC group. The diaphyseal region of long bones can be likened to a thick-walled tube. This geometric design results in a stiff (especially on bending) yet light structure (Turner, 2006). The stiffness of a cylindrical tube is proportional to the CSMI. In a perfectly round tube, the CSMI is proportional to the difference between the 4th power of its outer and inner diameters. Thus a small increase in the outer (or a small decrease in the inner) diameter will result in a large increase in the CSMI and a corresponding increase in whole bone stiffness. In the current study, the cross-sectional areas of the humeri and tibiae (the sum of the marrow and cortical areas) were not significantly different between the two groups, while the marrow area was larger in the CC group. These results imply that the greater cortical area in the FR group was due to increased endosteal resorption by osteoclasts in the CC hens and not to increased periosteal bone deposition by osteoblasts in the FR birds. A report by Rubin and Lanyon (1984) that decreased load-bearing resulted in substantial bone loss endosteally (and to a lesser extent periosteally) in a functionally isolated avian bone supports our findings.

In humans, exercise during growth causes deposition of periosteal bone, post-pubertal exercise results in endosteal bone deposition, and age-related bone loss occurs primarily on the endosteal surface (Ahlborg et al., 2003). Alternatively, exercise restriction in various mammalian models results largely in bone loss by endosteal resorption (O'Doherty et al., 1995; Eser et al., 2004). It should be noted that in layers at the onset of sexual maturity osteoblasts exclusively form medullary bone and do not build periosteal bone, while osteoclasts resorb both medullary and cortical bone (Whitehead, 2004). This unique feature of avian bone biology further supports our conclusion that the higher cortical area of the FR group bones resulted from higher endosteal bone resorption in the CC group rather than from additional bone formation in the FR group.

Bone strength and the consequent risk of fracture are dependent on the interplay between the material and structural properties of the bone. Bones cannot be both very tough and very stiff, and an inverse relationship exists between these properties (Curry, 2002a). In our study, the AUC of tibiae from the CC group was greater than that of tibiae from the FR group. AUC is a measure of the amount of energy required to cause fracture and ductile bones require a larger amount of energy to failure than brittle bones. Our results demonstrated that whereas the bones of the FR group were stiffer and stronger than those of the CC group, they had a smaller post-yield displacement at fracture and were therefore more brittle. Similar results were reported in a study investigating the influence of axial compression on bone in rats (Warden et al., 2007) where loaded bones had higher breaking strength but lower AUC due to lower post-yield deflection.

The results of the present study did not indicate that bones from hens in the FR group had higher mineral densities than those of the

CC group. The cortices of humeri from the CC and FR groups had similar mineral densities, while a marginally (although statistically significant) higher mineral density was found in the tibiae from the CC group. Moreover, the mineral density of cancellous bone in both groups was similar. Comparable findings were reported in broiler chickens raised in CC with statistically significant differences in the breaking strength of tibiae, but not in their content of ash (Tolon and Yalcin, 1997) or calcium (Newman and Leeson, 1998). However, studies in humans and in mammalian models indicate that a reduction in load and exercise decreases the mineral content of both cortical and cancellous bone (O'Doherty et al., 1995; Frey-Rindova et al., 2000; Vico et al., 2000). The reasons for this difference in the avian and mammalian response to bone unloading is not clear but may be associated with the unique physiology of the avian skeletal system, and its highly efficient mechanism of calcium mobilisation through medullary bone resorption. Thus layers may be able to preserve the mineral density in their bones, and the effect of exercise restriction is decreased cortical area and thickness rather than mineral resorption.

The effect of exercise restriction was also quite pronounced in cancellous bone. Bone volume fraction and trabecular thickness were higher in cancellous bone from FR compared to CC birds. Histomorphometry has previously indicated that mean trabecular bone volume in the proximal tarso-metatarsus of birds with access to perches was significantly greater than in controls (Hughes et al., 1993; Wilson et al., 1993). Thus our findings have confirmed the importance of exercise to both the cortical as well as the cancellous component of avian bone.

Medullary bone is a phenomenon unique to egg-laying birds, thought to act as a reservoir of calcium for eggshell production, and to have only minimal structural effects, except in the prevention of buckling of the thin cortical walls (Currey, 2002b). Analysis of medullary bone in the tibiae of both the CC and FR groups did not detect statistically significant differences in their mineral density and BVF. This finding is in agreement with those of previous studies which found no difference in medullary bone volume in the proximal tarso-metatarsus of birds permitted to exercise (Hughes et al., 1993; Wilson et al., 1993). Furthermore, no correlation was found between most of the whole bone mechanical properties determined by three-point bending and the BVF and mineral density of medullary bone. The only exception was a weak correlation between medullary bone mineral density and whole bone maximal load in three-point bending.

Severe exercise restriction probably also results in physiological and mental stress with deleterious welfare implications. Given that in a rat model, mild stress induced bone loss (Yirmiya et al., 2006), it is possible that some of the effects demonstrated in this study are due to elevated stress in birds in the CC group.

The study examined structural, compositional and mechanical effects of exercise restriction on the bones of laying birds at one time-point, at the end of the prolonged restriction period. It would be interesting to chart how these variables changed with time, as previous studies have indicated that cortical bone formation ceases at the onset of laying but cortical resorption continues (Hudson et al., 1993; Whitehead, 2004). Thus, differences between the two groups in the current study were expected to peak at this end time-point due to the prolonged movement restriction, combined with the equally long period of heavy calcium demand of daily egg production.

Conclusions

This study has demonstrated that severe, prolonged exercise restriction in laying hens has major structural and mechanical implications for the skeletal long bones. Our results suggest that,

in layers, the deterioration in the mechanical properties of the bones of caged birds is due largely to structural modelling rather than any decrease in mineral density.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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