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Bone Density and Breaking Strength in UK Raptors Exposed to Second Generation Anticoagulant Rodenticides

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Second generation anticoagulant rodenticides (SGARs) such as difenacoum, brodifacoum, and bromadiolone are more persistent and acutely toxic than their first generation counterparts (such as warfarin), and are designed to deliver a lethal dose during a single feeding (US EPA 2004). These properties make SGARs effective primary rodenticides and they have become extremely important for rodent control worldwide. However, their high acute toxicity and relatively long tissue half-lives present the potential for secondary exposure in predatory birds and mammals that feed upon exposed rodents. Mortality incidents have been documented amongst non-target predators but, perhaps more striking yet is the wide-scale (large proportions of each population, multiple species) exposure (Stone et al. 1999; Howald et al. 1999; Eason et al. 2002; Shore et al. 2006).

There is considerable concern that this widespread and large-scale 'sub-lethal' exposure (or at least, exposure not visibly associated with hemorrhagic symptoms) may potentially be associated with other adverse effects. SGARs elicit their acute toxicity by inhibiting the synthesis of vitamin K, which leads to increased coagulation times followed by lethal internal hemorrhage (US EPA, 2004). However, vitamin K also plays a role in bone metabolism as it is required for the formation of γ-carboxyglutamyl, a component of bone proteins such as osteocalcin (Weber 2001). Studies on humans have shown that warfarin therapy and low dietary vitamin K intake are related to reduced bone density, increased frequency of bone fractures and osteoporosis (Barnes et al. 2005). Given this, we tested the hypothesis that wild birds exposed to periodic sub-lethal concentrations of SGARs may also exhibit decreased bone density and bone strength, possibly placing them at risk for bone fractures.

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Materials and Methods

Twenty-eight barn owls, *Tyto alba* (n = 28; 13 males, 9 females, 6 undetermined; 6 adults, 16 juveniles, 6 undetermined) and 20 kestrels, *Falco tinnunculus* (n = 20; 9 males, 8 females, 3 undetermined; 8 adults, 9 juveniles, 3 undetermined) were analyzed for this study. Birds were found dead in the UK between February and December 2003 and submitted to the Centre for Ecology & Hydrology's predatory bird monitoring scheme (http://www.pbms.ceh.ac.uk). None of the birds were diagnosed to have died from rodenticide poisoning; most were road traffic victims or had died from starvation. The right humerus, femur and liver were excised from each bird and stored frozen. Livers were later analysed for brodifacoum, difenacoum, bromadialone, and flocoumafen residues as described in Shore et al. (2003). It has been reported that freezing bones can lead to a reduction in overall breaking strength and may not be representative of the true breaking strength of individuals' bones; however, for studies where comparative and not absolute breaking data is necessary, freezing is considered an acceptable storage technique (Merkley and Wabeck 1975).

Prior to making radiographs (Minxray; 52kVp, 15mA, 0.04 sec exposure, 24 inch focal plate-film distance with Kodak MG-1 green sensitive, high-speed film), bones were denuded with dermestid beetles, dried for 24 hours at 105°C and placed in a desiccator. Each radiograph was digitized (Epson Perfection 3200 Pro scanner with the transparency unit adapter), and density determined by obtaining an average opacity of four 5 x 5 pixel sites along the length of the bone (Adobe Photoshop 6.0). Repeatability of density measures was verified by blindly re-measuring a subset of the bones. To ensure measurement consistency, a reference opaque object (a penny) was scanned with each radiograph and the images adjusted so the density values for the penny and for air were always 100 and 0, respectively (i.e., denser bones have greater values than less dense bones).

Before testing breaking strength (Instron Universal Testing Machine), the minimum and maximum diameter of each bone was measured. Only barn owl humeri were assessed for breaking strength; the small size of owl femora and both kestrel bones prohibited their use with the available testing apparatus. Proximal and distal epiphyses were placed on specially made supports and a load applied to the centre of the bone (i.e., 3-point breaking test). The load (Newtons) required to break the shaft was divided by the product of the minimum and maximum diameter to correct for differences in bone sizes. Published reports assessing bone strength in domestic chickens have used 10 and 50 mm/min as the downward speed of the head used in the 3-point breaking test. We tested the variation in breaking strength of chicken humeri using both speeds and found the coefficient of variation to be smaller at 10 than 50 mm/min (0.16 versus 0.24); thus, 10 mm/min was used for this study.

Results and Discussion

Of the 48 birds, 30 had measurable concentrations of brodifacoum (8 of 30), difenacoum (25 of 30) and bromadialone (15 of 30). No birds had detectable levels of flocoumafen. Brodifacoum, difenacoum and bromadialone residues ranged from 0.012 to 0.238 μg/g, 0.003 to 0.336 μg/g, 0.013 to 0.581 μg/g (wet weight), respectively. These were within the typical range previously observed in predatory birds in the UK (Shore et al. 2006). The majority of birds (16 of 30) had one rodenticide residue present; 10 had two different residue types present; and 4 birds had all three residues present.

Humerus and femur density, and humerus breaking strength were normally distributed, and total rodenticide values (summed residues assuming that SGARs have similar toxicities and modes of action (US EPA 2004)) were normal after $\log_{10}$ transformation (Lilliefores > 0.05). Humerus density and humerus breaking strength from barn owls were strongly related ($r^2 = 0.49$, $p < 0.0001$), with denser bones having a higher breaking strength than less dense bones (Fig. 1A). Average density, breaking strength, and rodenticide residues were not significantly related to species (ANOVA; $p > 0.016$ (Bonferroni adjusted p)). Thus, species data were pooled and a three-way mixed model ANOVA conducted, accounting for the potential influence of sex and age (when known) to determine the relationship between bone density and breaking strength and total SGARs liver residue. Femur density was not significantly related to any of the main effects or to the interactions ($p = 0.16$–0.72).

Humerus density was likewise not significantly related to any of the main effects ($p = 0.46$–0.68), but was related to the sex × age and sex × age × total rodenticide interactions ($p = 0.02$ and 0.03, respectively). Post-hoc assessment of the data revealed that adult females had significantly greater humerus density than adult males, while juvenile males and females had similar humerus density (Fig. 1B). Humerus breaking strength was not significantly related to any of the main effects or to the interactions ($p = 0.44$–0.82).

Results of this study suggest that bone breaking strength and density in wild barn owls and kestrels were not related to liver SGARs residues. Only the age of birds and sex

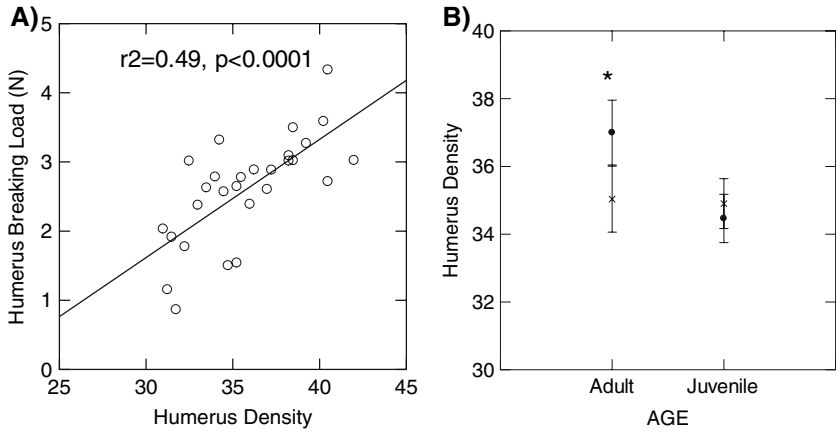

Fig. 1 **A)** Relationship between barn owl humerus density and breaking strength; **B)** Relationship between age, sex (female •; male x) and humerus density.* indicates significant difference

significantly explained some of the variation in these bone measurements. The lack of association between liver SGARs residues and bone measures may be because the exposure was insufficient in magnitude, timing and/or duration to have a significant long-term effect on bone measures. It is also possible that any depletion of calcium in bones that may have been caused by SGARs exposure is reversible once normal vitamin K levels are restored. There is no standard procedure for measuring breaking strength and density in birds, and other researchers have utilized different tests than we have in this study (e.g., breaking strength: tension, torsion, 4-point bending; density: bone mineral assessment, dual energy x-ray absorptiometry). It is possible that other strength and density measures may be more sensitive to the effects of SGARs in wild birds. In conclusion, there appears to be no evidence to date that sub-lethal exposure to SGARs affects long-term bone integrity in wild predatory birds.

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