Health of Herring Gulls (*Larus argentatus*) in Relation to Breeding Location in the Early 1990s. III. Effects on the Bone Tissue

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Health effects associated with the Great Lakes environment were assessed in adult herring gulls (*Larus argentatus*) in the early 1990s, including the size and quality of their bones. Femurs were excised from 140 individuals from 10 colonies distributed throughout the Great Lakes and 2 reference colonies in Lake Winnipeg (freshwater) and the Bay of Fundy (marine). Femurs of gulls from the Great Lakes differed from the freshwater or marine reference for 9 of 12 variables of size, composition, and strength assessed using peripheral quantitative computed tomography (pQCT) and biomechanical testing. Femurs of Great Lakes gulls were significantly smaller in length (–2.9%), periosteal circumference (–2.4%), and cross-sectional area (–5.4%) than freshwater reference birds. Femurs of the Great Lakes gulls had a lower significant cortical bone mineral content (–8.1%) and density (–2%) than the marine reference. A significant increase in the amount the bone could bend before it broke (+34%) and the energy required to break it (+44%) and a significant decrease (–16.3%) in stiffness during three-point biomechanical bending test were also detected in Great Lakes versus the freshwater gulls. These differences are indicative of impaired mineralization. When divided into high and low 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity equivalent (TCDD-TEQ) colonies, the amount the bone could bend before it broke and the energy required to break it were significantly higher in the high TEQ colonies, but not high polychlorinated biphenyl (PCB) colonies. Breeding location and dietary choices of Great Lakes herring gulls in the early 1990s resulted in modulations of physiological processes

that affected the size, mineralization, and biomechanical properties of bone.

Bone tissue provides the body with a rigid, hard framework that protects internal organs, maintains body posture, and provides leverage for muscles, thus enabling locomotion and specific motor functions. All bones consist of living cells embedded in a mineralized collagenous matrix. The mineralized component is composed of hydroxyapatite [$\text{Ca}_5(\text{PO}_4)_3(\text{OH})$], creating a metabolic reservoir of calcium and phosphate salts whose homeostasis is regulated by mechanisms coordinated by bone together with the kidneys and the parathyroid glands. Bone is formed and destroyed (remodeled) continuously in response to physical stresses, estrogens, and other hormones (Seibel et al., 1999). Bone tissue homeostasis is also influenced by a variety of dietary factors including calcium, magnesium, potassium, copper, zinc, silicon, and sodium; vitamins D₃, C, A, K, and B₁₂; and macronutrients such as protein and fatty acids (Lanham-New & Bonjour, 2003).

Bone tissue homeostasis is also influenced by environmental pollutants. The metals cadmium (Bhattacharyya et al., 1988), aluminum (Hahn, 1989; Firling et al., 1999), and lead (Potula et al., 2006) are known to induce harmful effects on bone tissue. Experimental studies suggest that bone tissue could be an important target for a number of endocrine-disrupting persistent organohalogen pollutants, including hexachlorobenzene (Andrews et al., 1988), polychlorinated biphenyls (PCBs) (Andrews, 1989; Hoffman et al., 1996; Lind et al., 1999, 2000; Lundberg et al., 2006), and polychlorinated dibenzo-*p*-dioxins (PCDD) (Jämsä et al., 2001; Miettinen et al., 2005).

Alterations in the composition and amount of bone and its geometry affect different aspects of its mechanical properties, including the amount of energy it can absorb before breaking

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and its stiffness. The stiffness of bones is largely dependent on the degree of mineralization. Unlike the widely used dual-energy x-ray absorptiometry (DEXA) technique, bone mineral density (BMD) measurements by peripheral quantitative computed tomography (pQCT) imaging are not confounded by bone size. The pQCT technique allows selective measurement of cortical density, bone area, cortical area, cortical thickness, and periosteal and endosteal circumference. The biomechanical determinants of bone strength can be quantified by measuring the load necessary to break the bone and the amount of bending at the time of failure using the three-point bending method. This information is used to calculate the energy required and the bone's stiffness. These are the four most important basic variables when describing the mechanical properties of a structure such as a long bone.

Studies of free-ranging wildlife including East Greenland polar bears (*Ursus maritimus*) (Sonne et al., 2004), Baltic gray seals (*Halichoerus grypus*) (Lind et al., 2003), mink (*Mustela vison*) (Beckett et al., 2005), American alligators (*Alligator mississippiensis*) (Lind et al., 2004), clapper rails (*Rallus longirostris*) (Rodriguez-Navarro et al., 2006), and great blue (*Ardea herodias*) (Hart et al., 1991) and gray heron (*A. cinerea*) (Thompson et al., 2006) chicks exposed to ambient mixtures of pollutants have revealed negative effects on bone tissue.

Herring gulls are long-lived and breed in both the freshwater and marine environments. As adults, herring gulls in the Great Lakes basin form what is essentially an isolated, closed population (Weseloh, 1984; Yauk & Quinn, 1999) of year-round residents (Gilman et al., 1977; Weseloh, 1984). The choice of breeding location constrains the variety, quantity, and quality of food available, and individual preferences/specializations (i.e., lifestyle) greatly influence what is consumed by this opportunistic species (Fox et al., 1990; Ewins et al., 1994; Hebert et al., 1999b). Because much of the chemical pollution of the Great Lakes results from point sources, it differs in quantity and nature among lakes, and between sites within lakes (Hebert et al., 1999a). Gulls that breed in colonies north of 44° 30', where the lakes ice over for extended periods in winter, must migrate to more southern lakes where their diet differs and pollutant exposure is greater (Hebert, 1998). Diet quality varies among lakes and is correlated with female body condition, egg size, and productivity (Hebert et al., 2002).

The health of adult herring gulls resident within the five Great Lakes was assessed relative to individuals breeding in colonies located on freshwater and marine habitats outside the Great Lakes basin, using an intensive cross-sectional survey, sampling colonies during incubation in the early 1990s (Fox et al., 2002). By sampling adults at the same point in the breeding cycle using a single method of capture, the influence of season, reproductive condition, method of capture, and to a lesser extent age was effectively controlled, thereby allowing us to detect the influences of sex, local environment (including the bioavailability of pollutants), and availability and nutritional quality of food.

Tissue pollutant concentrations (Fox et al., 2002), hematological effects (Grasman et al., 2000), decreased DNA strand length (Fox et al., 2005), and biochemical, cellular, and histopathological variables of health (Fox et al., 2007a, 2007b) have been reported previously for these individuals. Gulls from one or more Great Lakes differed from one or both reference colonies for 17 of 19 biochemical variables, whereas the freshwater and marine reference sites differed for only 3 (Fox et al., 2007b). Herring gulls from some of the Great Lakes had lower plasma calcium, inorganic phosphorus, and magnesium concentrations than those from the reference colonies. Since bone represents the major reservoir of the minerals in the body, their circulating concentrations are indicators of bone and mineral dynamics. These minerals are leaked from the body by damaged kidneys. Fox et al. (2007a) found that chronic granulomatous interstitial nephritis was more severe in gulls from Great Lakes colonies and associated with pollutants. These findings prompted our investigation of bone composition, size, and strength in these gulls using pQCT and three-point bending which is reported here. Our aim was to determine whether properties of bones of these adult gulls differ spatially in a manner consistent with pollutant exposure.

MATERIALS AND METHODS

Study Sites

Collections were made from 10 colonies, representing all 5 Great Lakes and the Detroit River, and included colonies for which there was or was not historical evidence of point source pollution or adverse reproductive effects. For comparison, a colony from Lake Winnipeg was chosen as a reference freshwater lake with comparable surface area, but without obvious point source pollution. Kent Island, in the Bay of Fundy, was chosen as a marine, and therefore iodine-sufficient, reference colony with low levels of pollution (Table 1).

Sampling

Adult herring gulls were collected under federal permit using protocols and procedures approved by the National Wildlife Research Centre Animal Care Committee for the Canadian Council on Animal Care as described previously (Fox et al., 2002, 2007b). Briefly, collections were timed to coincide with mid-incubation (d 7 to 21 of the 28-d incubation period). The incubating gulls were trapped on their nests using self-triggered drop traps. Gulls were weighed to the nearest 10 mg; blood was collected and various organs and tissues were collected and preserved appropriately for biochemical assays, histology, or contaminant analysis (Fox et al., 2002, 2007a, 2007b). Sex was determined by gonadal inspection. The left femur was excised, partially defleshed, and placed in an airtight polyethylene "Whirl-Pac" bag, placed on ice, and frozen at -40°C upon return to the National Wildlife Research Centre. In May 2005, the bags containing femurs were shipped on dry

TABLE 1

Details of Locations of Collection Sites of Adult Herring Gulls, Year, and Geographic and Exposure Codes

Region/lake	Location	Latitude, longitude	Year	Group ^a
L. Ontario	Scotch Bonnet Island, eastern basin	43° 50' N, 77° 30' W	1991	GLs, HTEQ, HPCB
L. Ontario	Hamilton Harbour, western shore	43° 17' N, 79° 50' W	1991	GLs, LTEQ, HPCB
L. Erie	Middle Sister Island, central western basin	41° 51' N, 83° 00' W	1993	GLs, LTEQ, HPCB
L. Erie	Middle Island, off Pelee Island, western basin	41° 41' N, 82° 41' W	1991	GLs, HTEQ, HPCB
Detroit River	Fighting Island	42° 12' N, 83° 07' W	1991	GLs, LTEQ, HPCB
L. Huron	Chantry Island near Southhampton, ON	44° 30' N, 81° 27' W	1991	GLs, LTEQ, LPCB
L. Michigan	Big Gull Island, west of Beaver Island, northeast end	45° 31' N, 86° 43' W	1991	GLs, HTEQ, HPCB
L. Michigan	Kidney Island, mouth of Fox River, in lower Green Bay	44° 34' n, 88° 00' W	1991	GLs, HTEQ, LPCB
L. Superior	Island east of mouth of Aquasabon River, near Terrace Bay, ON	48° 46' N, 87° 05' W	1991	GLs, HTEQ, LPCB
L. Superior	Granite Island in Black Bay, north shore	48° 43' N, 88° 28' W	1991	GLs, LTEQ, LPCB
L. Winnipeg	Unnamed island in north basin, near Grand Rapids, MB	53° 17' N, 99° 05' W	1991	Freshwater, LTEQ, LPCB
Bay of Fundy	Kent Island, off Grand Manan, NB	44° 43' N, 66° 46' W	1991	Marine, L TEQ, LPCB

^aGrouping: GLs = Great Lakes colony, LTEQ = low TCDD-TEQs (<500 pg/g), HTEQ = high TCDD-TEQs (>500 pg/g), LPCB = low ΣPCBs (<9500 ng/g), and HPCB = high ΣPCBs (> 9500 ng/g).

ice to Karolinska Institute, Stockholm, Sweden. They were stored at -20°C until August 2005, and thereafter completely defleshed and their size, composition, and biomechanical properties were determined.

Peripheral Quantitative Computed Tomography (pQCT)

The bone composition and dimensions were evaluated by peripheral quantitative computed tomography (pQCT; Stratec XCT Research SA+ with software version 5.50R; Norland Stratec Medizintechnik GmbH, Birkenfeld, Germany). The pQCT method has been used, with modifications, in previous studies (Lind et al., 2000, 2004; Lundberg et al., 2006). Before analysis, the bones were placed in plastic test tubes filled with Ringer solution (1 L contains 0.3 g Tris; 0.24 g CaCl₂(H₂O); 0.4 g KCl; and 2.05 ml 1 M HCl; pH 7.4). The bones were scanned, in the test tubes with the Ringer solution, at 40% of the total bone length from the distal tip. The bones were scanned only at the diaphysis because in birds the medullary (trabecular) bone formation alternates with periods of bone depletion due to the ovulation-oviposition cycle. A voxel size of 0.07 mm was used and the scan line was adjusted using the scout view of the pQCT system. A threshold of 710 mg/cm³ was used to define cortical bone. For the analyses, measurements of total cross-sectional area (CSA in mm²), cortical bone mineral content (BMC in mg/mm), cortical bone mineral density (BMD in mg/cm³), cortical area (mm²), cortical thickness (mm), periosteal circumference (mm), and endosteal circumference (mm) were used.

To evaluate the reproducibility of the pQCT measurements, the coefficients of variation (CVs) for the different variables

were calculated from 10 repeated measurements with a single sample being repositioned before each measurement. The coefficients of variation for the 10 different pQCT-measurements were as follows: 0.35% (cortical area), 0.41% (BMD), 0.60% (BMC), 1.12% (periosteal circumference), 1.56% (cortical thickness), 1.88% (endosteal circumference) and 2.26% (CSA).

Biomechanical Testing

Three-point bending was used to measure bone strength (electromechanical material testing machine, Avalon Technologies, Rochester, MN) using a span length of 15 mm between the support bars and a loading speed of 1 mm/sec. The load was applied to the mid-diaphyseal area of the femur using a wedge-shaped metal bar that was directly connected to the load cell. The load and displacement were sampled with a frequency of 50 Hz and stored digitally. Displacement at failure (the total amount of movement of the load in mm from the moment of contact with the bone surface until the bone fractured) and the load applied at the moment when the bone fractured were recorded in newtons (N). The data accumulated during each test were used to construct a load-displacement curve (Figure 1). The area under the curve defines the amount of energy absorbed until failure (breakage) measured in joules (J). The maximum slope of the load-displacement curve is defined as the bone's stiffness to failure (N/mm). The same equipment and methodology was used in several studies (Lind et al., 2000; Lundberg et al., 2006, 2007) with slight adjustments to accommodate the specific bones being tested.

FIG. 1. Load-displacement curve achieved when subjecting the bone specimen to three-point bending. The four variables used to describe the mechanical properties of the bone were load at failure (N), displacement at failure (mm), energy to failure (J), and stiffness (N/mm).

Statistics

Colonies were combined by lake, and grouped as Great Lakes (GLs), freshwater reference (Lake Winnipeg), and marine reference (Bay of Fundy). All colonies were also pooled and then divided into high 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity equivalent (TCDD-TEQs > 500 pg/g) or low TEQ (TCDD-TEQs < 500 pg/g), based on their TCDD-TEQ concentrations derived from congener-specific PCBs, polychlorinated dibenzo-*p*-dioxin (PCDD), and polychlorinated dibenzofuran (PCDF) concentrations in their livers using World Health Organization

toxic equivalency factors (Van den Berg et al., 1998) for birds. Similarly, they were also grouped as high PCBs (Σ PCBs > 9500 ng/g) or low PCBs (Σ PCBs < 9500 ng/g) based on the sum of 42 congeners. These concentration groupings have no toxicological basis but were based on the natural separation of locations when ordered by concentration. See Fox et al. (2002) for details of the chemical analyses. The influence of the independent variables sex, body mass, bone length, and location on variation in the dependent variables body mass, bone length, and the various pQCT and biomechanical variables was assessed by analysis of covariance (ANCOVA). Thereafter, differences in continuous dependent variables between the locations or organohalogen groupings were analyzed using ANCOVA incorporating the appropriate independent variables of sex, body mass, and bone length, as necessary, to statistically control for their influence. Significance was assessed with the Bonferroni-Dunn test. Differences were considered significant at $p < .05$ (StatView, version 5.0; SAS Institute, Inc., Cary, NC).

RESULTS

Sex, Body Mass, Femur Length, and Location as Sources of Variability

The influence of the independent variables sex, body mass, bone length, and location on the variation in the dependent variables body mass, bone length, and the pQCT and biomechanical variables differed among the dependent variables (Table 2). Sex was a larger contributor to the variation in body

TABLE 2
Influence of the Independent Variables Sex, Body Mass, Bone Length and Location on Variation in the Different Dependent Variables Body Mass, Bone Length, and pQCT and Biomechanical Variables of Herring Gull Femurs, Based on ANCOVAs with Colonies Grouped as Great Lakes, Freshwater, and Marine

Dependent variables	Independent variables			
	Sex	Body mass	Bone length	Location
Body mass	405.2	—	—	6.4
Bone length	33.0	2.9	—	6.0
Periosteal circumference	0.7	8.2	9.0	3.3
Endosteal circumference	0.0	2.1	3.6	1.4
Total cross-sectional area	0.7	8.4	8.4	3.2
Cortical area	4.3	16.0	6.9	2.9
Cortical thickness	2.2	4.2	0.7	0.3
Cortical bone mineral content	4.4	12.9	8.8	5.2
Cortical bone mineral density	0.2	0.1	4.5	11.5
Displacement at failure	0.4	1.2	0.3	5.2
Load at failure	1.2	1.9	1.6	1.9
Energy required for failure	0.0	2.3	0.1	4.2
Stiffness to failure	1.9	0.1	2.9	2.3

Note. ANCOVA provides a numerical value for the shared variance. Values shown are F values ($F > 3$, $p < .05$). This F value together with the degrees of freedom is used to calculate the p value.

mass and bone length than was location. Sex also contributed significantly to cortical bone mineral content and cortical area. Bone length was the largest contributor to variance in total area and both periosteal and endosteal femoral circumference. Bone length also contributed significantly to the variance in cortical bone mineral content, cortical bone mineral density, total cross-sectional area, and cortical area. Body mass was the largest contributor to variance in cortical bone mineral content, cortical area, and cortical thickness and contributed equally with bone length to the variance in total cross-sectional area. Body mass also contributed significantly to the variance in periosteal circumference. Location was the largest contributor to the variance in cortical bone mineral density, displacement at failure, and energy required for failure, and also contributed significantly to body mass, bone length, total cross-sectional area, cortical bone mineral content, and periosteal circumference.

Body Mass and Bone Length

After controlling for sex, body mass of individuals from Great Lakes colonies was 4.4% lower than that of individuals from the freshwater reference colony (Table 3). Body mass was also lower (2.5%) in gulls from the high-TEQ colonies than in gulls from the low-TEQ colonies (Table 3).

When controlled for sex and body mass, femurs were shorter in individuals from Great Lakes colonies than those of individuals from the freshwater (2.9%) and marine (2%) reference colonies. Femur length did not differ between individuals from the freshwater and marine reference colonies, between the high- and low-TEQ colonies or between individuals of the high- and low-PCB colonies (Table 3).

pQCT Measurements

All pQCT variables were controlled for sex, body mass, and bone length. Cortical bone mineral density was significantly higher in gulls from the marine colony than in gulls from Great Lakes (2%) and the freshwater colony (2.6%) and was the only pQCT measure that differed between the two reference colonies (Table 3). The periosteal circumference (girth) of the femurs of Great Lakes gulls was smaller than those of gulls from the freshwater group (2.4%) and the marine reference colony (3%). The femurs of the Great Lakes gulls had smaller total cross-sectional areas than those of gulls from the freshwater group (5.4%) and marine (6.6%) reference colony. The femurs of the Great Lakes gulls had a markedly lower cortical bone mineral content (8.1%) than femurs of gulls from the marine reference colony. Cortical area, cortical thickness, and

TABLE 3

Body Mass, Bone Length and Measurements Obtained from Quantitative Computed Tomography (pQCT-Analysis) of Femurs of Adult Herring Gulls Collected in 1991 and 1993 From Great Lakes and Marine (Bay of Fundy) and Freshwater (Lake Winnipeg) reference colonies

Variable	Marine reference	Freshwater reference	Great Lakes 10 colonies	Low TEQ <500 pg/g	High TEQ >500 pg/g	Low PCB <9500 ng/g	High PCB >9500 ng/g
Sample size	19	19	102	85	55	80	60
Body mass (g)	1070 ± 125	1079 ± 121	1034 ± 129^{aa}	1055 ± 128	1030 ± 128^{ccc}	1047 ± 132	1043 ± 123
Bone length (mm)	59.9 ± 2.4	60.4 ± 2.4	58.7 ± 3.1^{aaab}	59.1 ± 3.0	59.1 ± 2.9	59.0 ± .0	59.2 ± 3.0
Periosteal circumference (mm)	17.2 ± 0.8	17.1 ± 0.9	16.7 ± 0.8^{aabbb}	16.9 ± 0.9	16.7 ± 0.8	16.8 ± 0.9	16.8 ± 0.8
Endosteal circumference (mm)	13.7 ± 0.8	13.7 ± 0.9	13.3 ± 0.9	13.4 ± 0.9	13.3 ± 0.9	13.4 ± 0.9	13.4 ± 0.9
Total cross-sectional area (mm ²)	23.7 ± 2.2	23.4 ± 2.4	22.2 ± 2.3^{aabbb}	22.7 ± 2.5	22.4 ± 2.1	22.6 ± 2.5	22.6 ± 2.2
Cortical area (mm ²)	8.7 ± 0.8	8.4 ± 1.1	8.1 ± 0.9	8.3 ± 1.0	8.2 ± 0.9	8.3 ± 1.0	8.2 ± 0.9
Cortical thickness (mm)	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
Cortical bone mineral content (mg/mm)	12.0 ± 1.2	11.4 ± 1.6	11.1 ± 1.3^{bbb}	11.3 ± 1.4	11.2 ± 1.3	11.3 ± 1.5	11.1 ± 1.3
Cortical bone mineral density (mg/cm ³)	1388 ± 26	1353 ± 27^{bbb}	1361 ± 25^{bbb}	1364 ± 27	1363 ± 27	1366 ± 27	1360 ± 27

Note. Colonies were also grouped by TCDD-TEQ and ΣPCB concentrations in liver. Differences between groups were evaluated by ANCOVA and controlled for sex, body mass, and bone length (except the variable body mass, which was only adjusted for sex). Values are mean ± SD, measured at the diaphyseal measure point located at a distance of 40% of the total bone length measured from the distal tip.

^aSignificantly lower than the freshwater reference (a, $p < .05$, aa, $p < .01$, aaa, $p < .001$).

^bSignificantly lower than the marine reference (b, $p < .05$, bb, $p < .01$, bbb, $p < .001$).

^cSignificantly lower than the high-TEQ group (ccc, $p < .001$).

endosteal circumference did not differ significantly between Great Lakes colonies and either the freshwater or marine reference colony (Table 3).

Although the body weight of the individuals of the high-TEQ colonies was significantly lower than that of the low-TEQ colonies, there were no significant differences detected in bone composition between individuals of the two TEQ groups or between the low- and high-PCB colonies (Table 3).

Biomechanical Variables

All biomechanical variables were controlled for sex, body mass, and bone length. There were no significant differences in biomechanical variables between the freshwater and marine reference colonies (Table 4). Femurs of gulls from the Great Lakes had significantly greater displacement at failure (34%) and energy required for failure (44%) than gulls from the freshwater reference (Table 4). Femurs of Great Lakes gulls had significantly lower (16%) stiffness to failure than those from the freshwater reference (Table 4). There were no significant differences in biomechanical properties between individuals of the high- and low-PCB colonies. However, gulls in the high-TEQ group had significantly increased displacement at failure (11.3%), load at failure (10.5%), and energy required for failure (18%) compared to gulls in the low-TEQ colonies (Tables 4).

DISCUSSION

Significant differences occurred in 9 of 13 variables between gulls from Great Lakes colonies and the freshwater and/or marine reference colony (Tables 3 and 4). In contrast, only cortical bone mineral density differed significantly between gulls from freshwater and marine reference colonies. Femurs

of gulls from Great Lakes colonies were smaller (in length, girth, and cross section), and had lower bone mineral density, bone mineral content, and stiffness to failure than femurs from gulls from the freshwater and/or marine reference colony (Tables 3 and 4). In contrast, the femurs of Great Lakes gulls had a greater displacement at failure and required more energy for failure than did the femurs of gulls from the freshwater reference colony. The gulls from Great Lakes colonies and Lake Winnipeg can freely mix, but are genetically isolated from gulls from the Bay of Fundy (Weseloh, 1984; Yauk & Quinn 1999). This would suggest that these differences in bone size, composition and strength are unlikely to be due to differences between the freshwater and marine environments or genotype, and more likely associated with sex, body size, or one or more localized factors such as diet and nutrition, and pollutant exposure.

Sex

Sex was greater contributor to variation in body mass and femur length than was location (Table 2), consistent with the genetic determination of body size in this sexually size dimorphic species. Sex was also a significant contributor to the variation in periosteal circumference (bone girth) and cortical area and mineral content, consistent with genetic and hormonal influences on phenotype and differences in the dynamics of bone in male and female birds.

Bone Length and Body Mass

Femur length is a reflection of body size, whereas body mass reflects both body size and tissue mass and is therefore an integrated measure of body condition and sensitive to localized

TABLE 4

Biomechanical Properties of Femurs of Adult Herring Gulls Collected in 1991 and 1993 from Great Lakes and Marine (Bay of Fundy) and Freshwater (Lake Winnipeg) Reference Colonies and when Grouped By TCDD-TEQ and ΣPCB Concentrations in Liver

Variable	Marine reference	Freshwater reference	Great Lakes (10 colonies)	Low TEQ <500 pg/g	High TEQ >500 pg/g	Low PCB <9500 ng/g	High PCB >9500 ng/g
Sample size (n)	19	19	102	85	55	80	60
Displacement at failure (mm)	1.12 ± 0.26	0.94 ± 0.36	1.26 ± 0.41^{aa}	1.15 ± 0.39	1.28 ± 0.41^c	1.15 ± 0.36	1.26 ± 0.45
Load at failure (N)	143.1 ± 27.9	130.5 ± 39.2	144.7 ± 36.3	136.9 ± 35.5	151.3 ± 34.8^{cc}	139.6 ± 37.6	146.6 ± 33.0
Energy required for failure (J)	127.1 ± 47.7	100.8 ± 67.8	145.6 ± 68.1^{aa}	128.0 ± 65.6	151.1 ± 67.7^c	148.3 ± 44.5	147.4 ± 46.8
Stiffness to failure (N/mm)	152.7 ± 35.0	170.5 ± 51.1	142.8 ± 44.9^{aa}	148.0 ± 45.0	147.8 ± 46.1	128.5 ± 63.1	148.3 ± 71.1

Note. Values are mean ± SD, measured at the diaphyseal measure point located at a distance of 40% of the total bone length measured from the distal tip. Differences between groups were evaluated by ANCOVA and controlled for sex, body mass, and bone length. Values in bold are significantly different from the freshwater reference (a) or contrasting concentration grouping (c).

environmental factors. Variations in cross-sectional and cortical area, periosteal and endosteal circumference, and cortical bone mineral content and density were significantly influenced by bone length (Table 2). Body mass significantly influenced variability in cross-sectional and cortical area, periosteal circumference, cortical thickness, and cortical bone mineral concentration (Table 2). These findings reflect the influence of body mass and length on the physical stresses on bones and their influence on the remodeling process. Bone adapts to mechanical stresses largely by changing its size and shape (Turner 2006).

Location

Location was a significant contributor to variability in body mass, bone length, cross-sectional and cortical area, and cortical bone mineral content and density, and in the strength properties displacement and energy at failure (Table 2).

Small increases in bone mineral density can markedly improve bone strength (Turner, 2006). Cortical bone mineral density was the only variable that differed significantly between the freshwater and marine reference sites (Table 3). The reasons for this observation remain to be clarified. Altered bone mineral density was reported in studies of additional species of free-ranging wildlife, including the skulls and bacula of East Greenland polar bears (*Ursus maritimus*) (Sonne et al., 2004, 2006), the jaws and radius of Baltic gray seals (*Halichoerus grypus*) (Lind et al., 2003), and the tibiae and femurs of American alligators (*Alligator mississippiensis*) (Lind et al., 2004). Cortical bone mineral content of femurs of gulls from Great Lakes colonies was significantly lower than that of gulls from the marine reference colony. Total cross-sectional area and cortical area of femurs of gulls from the Great Lakes were significantly smaller than those of gulls from both the freshwater and marine reference colonies.

Location was the only measured variable that contributed significantly to the variance in biomechanical properties (Table 2). While there were no significant differences in these properties between the freshwater and marine reference sites, the displacement at failure and the energy required for failure were significantly greater for the femurs of gulls from the Great Lakes relative to the freshwater reference colony (Table 4). Displacement at failure and energy required for failure were 34 and 31% greater, respectively, overall, while stiffness at failure was 16% less. Therefore, gulls from the Great Lakes colonies had softer and more plastic bones than those of gulls from the freshwater reference colony. While there were no significant decreases in length and girth, cross-sectional area, and mineral concentration or density between high-PCB and low-PCB colonies or high-TEQ and low-TEQ colonies, the femurs of the high-TEQ colonies had significantly higher displacement at failure, energy required for failure, and load at failure than those from gulls from the low-TEQ colonies.

Effects of PCB (Andrews, 1989; Hoffman et al., 1996; Lind et al., 1999, 2000; Lundberg et al., 2006) and PCDD (Jämsä et al., 2001; Miettinen et al., 2005) on bone tissue include decreased length and cross-sectional area, alterations in mineral density, and decreased strength following controlled exposure. The mineral density of skull bones of East Greenland polar bears was negatively related to PCBs, polybrominated diphenyl ethers (PBDEs), and other organohalogen pollutants (Sonne et al., 2004). The length and mineral density of the penis bone (baculum) of these polar bears decreased significantly with increasing levels of several organohalogen pollutants in their adipose tissue (Sonne et al., 2006). In a similar manner, organohalogen pollutants may have impaired bone growth and mineralization in Great Lakes gulls, producing altered bone growth and mineralization. Bone consists of a collagenous organic matrix with embedded hydroxyapatite crystals [$\text{Ca}_5(\text{PO}_4)_3(\text{OH})$]. Without sufficient mineralization, bones will plastically deform under load (Turner, 2006). The measured plasma concentrations of Ca and P in the gulls show that individuals from the Great Lakes area had significantly lower concentrations compared to gulls from the freshwater reference (Fox et al., 2007b). Plasma calcium concentrations were significantly lower in the gulls from Great Lakes relative to the gulls from the freshwater reference, and were lower in gulls from the high-PCB colonies than in those from the low-PCB colonies (Fox et al., 2007b). In addition, plasma inorganic phosphorus concentrations were significantly lower in the gulls from the Great Lakes relative to the gulls from the freshwater reference (Fox et al., 2007b). Effects on plasma P has also been reported in other species. Smits et al. (2007) reported decreased plasma inorganic phosphate concentrations in breeding colonies of white stork (*Ciconia ciconia*) in southwestern Spain accidentally exposed to large amounts of acidic and metal-rich water. Serum phosphorus concentrations were significantly reduced at low doses and significantly increased in the highest dose of a complex mixture of organohalogen pollutants and lead and cadmium in orally exposed male rats (Wade et al., 2002). Magnesium is another important constituent of bone mineral; a deficiency in Mg inhibits the osteoblastic activity (Palacios, 2006). Plasma magnesium concentrations were significantly lower in Great Lakes gulls than in the freshwater reference and were significantly lower in the high-PCB colonies than the low-PCB colonies (Fox et al., 2007a). Plasma magnesium concentrations were inversely associated with non-ortho-PCB and TCDD-TEQ concentrations in individual livers (Fox et al., 2007a). Plasma magnesium concentrations in loggerhead sea turtles (*Caretta caretta*) from North Carolina were inversely associated with Σ PCBs and TCDD-like PCB concentrations in blood (Keller et al., 2004).

Since steroid hormones are needed to maintain the equilibrium between the formation and destruction of bone, the interference of a pollutant displaying anti-estrogenic/anti-androgenic or estrogenic/androgenic properties might alter bone size and composition. The mechanisms underlying altered

bone composition might involve interference with the androgen/estrogen receptor (AR/ER) signaling pathway, the aryl hydrocarbon receptor (AhR) pathway, or cross-talk between these pathways. Published studies suggest some environmental pollutants do disrupt estrogen homeostasis (Lind et al., 2000; Lundberg et al., 2006). Moreover, these effects may be developmental and/or induced later in life, since the gulls are exposed in ovo through adulthood. Median EROD activities (in the AhR pathway) in the livers of the gulls from the various Great Lakes colonies were approximately 3- to 10-fold greater than gulls from the freshwater reference (Fox et al., 2007b). EROD activity was significantly greater in the high-PCB colonies than in the low-PCB colonies. EROD activity was significantly associated with Σ PCDDs in pooled livers and lead in pooled kidneys (Fox et al., 2007b).

The hormone thyroxine also plays an important role in the regulation of bone growth and chondrocyte differentiation (Ren et al., 2007). Although plasma thyroxine concentrations were not significantly lower in the iodine-deficient freshwater reference colony and the iodine-sufficient marine colony, concentrations in gulls from lakes Erie, Ontario, and Huron and the Detroit River were markedly lower than those from both the reference colonies (Fox et al., 2007b). Median plasma thyroxine concentrations of gulls in the high-PCB colonies were significantly lower than those of the low-PCB colonies and were inversely associated with Σ PCB concentrations in liver (Fox et al., 2007b). PCBs, PCDD, and PBDE are persistent bioaccumulative organohalogen pollutants that are known to affect thyroid function and to which herring gulls are heavily exposed (Hebert et al., 1999a; Norstrom et al., 2002).

Vitamin D₃ is a major hormone involved in the regulation of calcium metabolism and skeletal remodeling. Osteomalacia, a condition characterized by soft and demineralized bones (Shoback 2007), can result from vitamin D deficiency. The most consistent abnormality observed in vitamin D₃ deficiency-induced osteomalacia in humans is hypophosphatemia, which is not always accompanied by hypocalcemia (Catto, 1985). Long-Evans rat dams and their offspring showed reduced serum levels of 1,25-dihydroxyvitamin D₃ following exposure to a mixture of PCBs (Lilienthal et al., 2000). Unfortunately, the vitamin D or D₃ concentrations were not measured in these gulls.

In conclusion, the effects on bone size, mineral content, and strength observed in Great Lakes herring gulls may be less likely attributed to genetic than local environmental factors. The relative influence of nutrition and toxic pollutants is unclear, although several pollutants to which birds are chronically exposed have been implicated as bone toxins. Many potential mechanisms for adverse effects exist. However, since the gulls were exposed to different compounds/concentrations at different life stages and over time, it is difficult to draw any certain conclusions as to which specific toxicant(s) induced the observed alterations in bone tissue and whether the effects are primary or secondary. Our findings further enlarge the suite of

environmental diseases observed in this collection of gulls and add to the growing body of evidence that place of residence, in this case the Great Lakes, affects the well-being of individuals.

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