ANALYSIS OF BONE HISTOLOGY, COMPOSITION, AND MECHANICAL PROPERTIES OF BLACK BEAR TIBIAS IN RELATION TO DISUSE OSTEOPOROSIS

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INTRODUCTION

Osteoporosis is a disease characterized by low bone mass and structural deterioration of bone tissue, which leads to bone fragility and increased fracture risk. The major factor contributing to the structural deterioration of cortical bone is increased porosity caused by bone remodeling. Consequently, the strength and stiffness of bones are reduced. For prolonged bed rest, immobilization, and space flight, disuse atrophy of bone is prevalent and increases fracture risk when normal bone loading is resumed.[1, 2] Disuse osteopenia occurs in many animals including hibernating ground squirrels, rats, roosters, sheep, cats, dogs, monkeys, and humans. On the contrary, black bears may not develop osteopenia during long periods of disuse (i.e., hibernation) to the extent that other animals do.[3, 4] Additionally, black bears may have a mechanism for more rapid and complete bone recovery from disuse than other animals do.[3] The specific aims of the current study are to evaluate the histological, compositional, and mechanical characteristics of tibias from annually denning black bears to gain a better understanding of how black bear bones adapt to annual periods of disuse. More specifically, we are correlating bone remodeling and composition parameters with mechanical behavior (strength, stiffness, and fracture energy). Because black bears are inactive during annual periods of disuse, and osteoclastic resorption increases during this time, we hypothesize that bear bone is remodeled at a faster rate than in other animals, as indicated by Haversian canal accumulation. However, because osteoblastic function does not decline during annual periods of disuse and it is elevated during remobilization periods,[3] we hypothesize that age-related changes in bone histology, composition, and mechanical properties are not different in black bears than in humans, despite annual 6 month periods of disuse in black bears.

METHODS

Samples

One tibia was removed from each of eight black bears (*Ursus Americanus*) that were killed late in their active period (September-October) by licensed hunters in the Upper Peninsula of Michigan. Of the eight tibias, 5 were from male bears (ages ranged from 1 to 3 years), and 3 were from females (ages ranged from 3 to 13 years). Cortical bone histology, composition, and mechanical properties of mid-shaft sections of each tibia are being examined.

Histology

Histological analyses of the midshaft cross-section were conducted for all specimens (n = 8). The histological specimens were refrigerated in 70% ethanol for 48 hours, and then stained en bloc as described previously.[5] Briefly, the bones were stained under vacuum in 1% basic fuchsin (EM Science, Gibbstown, New Jersey) in 70%, 80%, 90%, and 100% ethanol for 16 hours at each concentration and then rinsed with 100% ethanol. The samples were air dried and embedded in methyl methacrylate (Lang Dental Mfg. Co., Inc., Wheeling, Illinois). Cross-sectional wafers 250 microns thick were cut from each specimen on an Isomet 1000 Precision Saw (Model 11-2180, Buehler, Lake Bluff, Illinois) and ground down to a thickness of approximately 150 microns. The specimens were mounted on glass slides and labeled corresponding to their anatomical orientation. The Haversian canals, resorption cavities, and refilling osteons in the cross-section were counted at x100 magnification using a grided eyepiece with a transmitted light microscopic. An image of the cross-section was obtained using a digital camera (Spot Insight QE, Diagnostic Instruments, Inc., Sterling Heights, Michigan) and accompanying Spot Advanced software was used to calculate the area of the cross-section. The remodeling parameters for each specimen were normalized by the cross-sectional area of the specimen to obtain density values for each parameter. Porosity was also calculated for each specimen using the previous method of Martin and Boardman.[6] Porosity and the normalized remodeling parameters were regressed against age for male and female specimens combined.

Composition

The bone samples for composition analysis were placed in a beaker of water until thawed and then gently patted dry to remove surface water. The samples were weighed and then put in a furnace (Model 1619, Jelrus Technical Products Corp., New Hyde Park, New

York) at 100°C for 24 hours to remove the water, and then at 600°C for 48 hours to remove the collagen, leaving only hydroxyapatite mineral. The samples were reweighed between each heating cycle to find the relative composition, by weight percentage, of water, collagen, and mineral. Mineral content was regressed against age for male and female specimens combined.

Mechanical Testing

The mechanical testing is currently in progress (will be completed by March 2003). The specimens will be milled into 2mm x 3.5mm x 30mm rectangular bars with the longest dimension corresponding to the longitudinal axis of the bone (Figure 1). The specimens will be tested in three-point bending on a fixture designed according to American Society for Testing and Materials Standard D5934-96. The specimens will be loaded to failure at a displacement rate of 1 mm/sec. The elastic modulus, ultimate stress, and fracture energy will be calculated for each specimen and correlated with age, gender, mineral content, and the histological parameters.

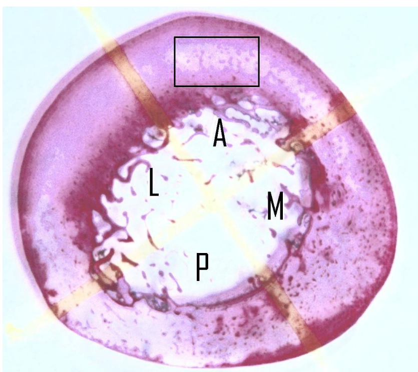

Figure 1. Tibial cross-section illustrating the location from which the beam will be extracted for mechanical testing.

RESULTS

The cross-sectional area (CSA) of the black bear tibias significantly ($p = 0.037$) decreases with age. Porosity ($p = 0.175$), mineral content ($p = 0.905$), Haversian canal density ($p = 0.580$), resorption cavity density ($p = 0.162$), refilling osteon density ($p = 0.133$), and BMU density ($p = 0.506$) do not significantly change with age. Additionally, CSA ($p = 0.017$) and porosity ($p = 0.011$), were significantly lower in females than in males.

DISCUSSION

This study demonstrated that black bears maintain normal bone mineral composition with age, which may be attributed to the fact that osteoblastic function does not decline during annual periods of disuse and is elevated during remobilization periods, thus minimizing bone loss during disuse and allowing full recovery during the active period. Bone porosity in black bears does not increase with age as in other species, which may also be attributed to osteoblastic formation during disuse and full recovery during the active period. Conversely, humans experience increased bone porosity with age which ultimately leads to osteoporosis and a greater likelihood for bone fracture. In general, women experience the greatest amount of bone loss, which often leads to bone fractures that are both painful and debilitating. Additionally, in black bears, the normalized remodeling parameters do not change with age suggesting that the ability of bear bone to recover from disuse is not compromised with aging. Despite the fact that female bears give birth and nurse their cubs during hibernation, females had significantly lower porosity than males, suggesting that they may have an additional protective mechanism against osteoporosis. Taken together, these findings suggest that black bears possess some osteoregulatory mechanism, not known to any other species, which allows them to minimize bone loss during hibernation and completely recover lost bone during their remobilization, with females likely having more sophisticated bone maintenance capabilities than males. These characteristics seem to prevent black bears from accumulating bone loss due to annual periods of disuse, thus avoiding the progression of osteopenia with age. The results of the mechanical testing will be needed to further support our hypothesis that black bears have a biological mechanism to prevent the negative effects of disuse osteoporosis on the mechanical competence of bone. Even though black bears experience decreasing CSA with age, which is likely to reduce uniaxial mechanical properties of the bone, increases in the cross-sectional moment of inertia may compensate by maintaining bending properties. With bending being the most common loading experienced by the tibia,[7] this potential structural compensation may be helpful in avoiding fracture.

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