Abstract

The hibernating bear is an excellent model for disuse osteoporosis in humans because it is a naturally occurring large animal model. Furthermore, bears and humans have similar lower limb skeletal morphology, and bears walk plantigrade like humans. Black bears (*Ursus americanus*) may not develop disuse osteoporosis during long periods of disuse (i.e. hibernation) because they maintain osteoblastic bone formation during hibernation. As a consequence, bone volume, mineral content, porosity, and strength are not adversely affected by annual periods of disuse. In fact, cortical bone bending strength has been shown to increase with age in hibernating black bears without a significant change in porosity. Other animals require remobilization periods 2–3 times longer than the immobilization period to recover the bone lost during disuse. Our findings support the hypothesis that black bears, which hibernate for as long as 5–7 months annually, have evolved biological mechanisms to mitigate the adverse effects of disuse on bone porosity and strength.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: Disuse osteoporosis; Bone mechanical properties; Bone remodeling; Black bear; Aging; Hibernation

1. Introduction

We have collected some evidence to support the hypothesis that black bears (*Ursus americanus*) have evolved biological mechanisms to mitigate or avoid the adverse effects of disuse on bone and/or have better compensatory mechanisms to more rapidly recover from disuse osteoporosis.

Others have provided additional evidence to support this hypothesis (Floyd et al., 1990; Pardy et al., 2004).

The hibernating black bear is an excellent model for disuse osteoporosis in humans because it is a naturally occurring large animal model. Furthermore, black bears and humans have similar lower limb skeletal morphology and bears walk plantigrade like humans. We have quantified age-related changes in the material properties, histology, mineral content, and whole bone bending strength of black bear cortical bone. We have used this data to make inferences about how bear bones are affected by disuse. The inferences we have made are based on the fact that other species require remobilization periods of 2–3 times the length of the disuse period to recover lost bone (Kaneps et al., 1997; Weinreb et al., 1997). In a study on long-term immobilization in dogs, 32 weeks of disuse resulted in significant relative bone loss in both young
and old dogs (Jaworski and Uhthoff, 1986). After 28 weeks of remobilization, young dogs recovered only 70% of the cortical bone lost during disuse and old dogs recovered only 40% of the lost cortical bone. These findings suggest that black bears, which hibernate for 5–6 months annually in northern regions (e.g., Michigan), should not have a sufficient recovery period to regain bone if they lose it at the same rate as dogs. We are trying to answer some important questions regarding hibernating bears: (1) if bears do lose bone during disuse, how can they recover sufficient bone when their active and disuse (i.e., hibernation) periods are approximately equal in duration and (2) if they are able to prevent or mitigate bone loss during disuse, what are the biological mechanisms? The purpose of this paper is to summarize the observations on bone metabolism and mechanics in hibernating bears and start to piece together a theory on how bone mass and strength are regulated by bears during lengthy periods of inactivity.

Disuse osteoporosis occurs in patients with spinal cord injuries, patients confined to prolonged bed rest, and astronauts exposed to microgravity during spaceflight (Leblanc et al., 1990; Garland et al., 1992; Collet et al., 1997; Dauty et al., 2000; Vico et al., 2000). During remobilization, the recovery of the bone lost during disuse is slow and may not be complete (Lindgren and Mattsson, 1977; Jaworski and Uhthoff, 1986; Leblanc et al., 1990; Vico et al., 2000). In fact, bone loss can continue during remobilization (Trebcz, 2001). Animal studies have shown that immediate rapid increases in bone resorption and sustained decreases in bone formation contribute to bone loss during limb immobilization by casting, tenotomy, or neurectomy (Weinreb et al., 1989; Rantakokko et al., 1999). Following 2 weeks of hind limb immobilization and 4 weeks of remobilization, the bending strength, apparent density, and degree of mineralization of rat femurs were significantly lower than in age-matched controls (Trebcz, 2001). In other studies where remobilization did restore the bone lost by immobilization, the recovery period was 2–3 times longer than the immobilization period (Kaneps et al., 1997; Weinreb et al., 1997). In hibernating ground squirrels, golden hamsters, and little brown bats bone is lost by reduced bone formation and increased bone resorption (Haller and Zimny, 1977; Steinberg et al., 1979; Steinberg et al., 1981; Steinberg et al., 1986; Kwiecinski et al., 1987). In black bears both bone resorption and formation surfaces increase in trabecular bone during hibernation relative to summer (Floyd et al., 1990), although bone mineral density and content, bone volume fraction, and trabecular thickness are not significantly different in the autumn (prior to hibernation) and spring (following hibernation) (Pardy et al., 2004).

2. What level of disuse do bears experience during hibernation?

Many animals hibernate in response to seasonal variations in food supplies, including bears which remain dormant for 3–7 months. Many hibernators periodically wake (every 4–10 days) to eat, urinate, and defecate; black bears do not. Black bear resting heart rate drops from 40 beats per minute to as low as 8 beats per minute during hibernation (Hellgren, 1998). Black bear body temperature only drops a few degrees Celsius during hibernation; however, the body temperature of other hibernators can drop below freezing (Harlow et al., 2004). Hibernating bears lose as much as 37% of their bodyweight during hibernation, primarily by the catabolism of fat stores (Hellgren, 1998). There is a significant decrease in skeletal muscle protein concentration during hibernation, although muscle cross-sectional area does not change (Tinker et al., 1998). Black bear muscle strength significantly decreases (23%) during hibernation (Harlow et al., 2001). However, this level of strength loss is much less than what was predicted to occur in humans for a disuse period equivalent in duration to hibernation. Bone formation, bone volume, and bone mineral content do not decrease during this period of inactivity (Floyd et al., 1990; Donahue et al., 2003a; Pardy et al., 2004). Clearly hibernating bears have evolved some unique biological mechanisms to survive long periods of limited food supplies and inactivity.

The captive black bears we have studied den in 4-foot diameter, 6-foot long culverts filled with straw. They are essentially inactive during the denning period. They have not been observed leaving the den although they do rearrange the straw to some extent. Wild black bears in Minnesota typically hibernate in dens too small to permit standing and do not leave the den over the course of hibernation (Rogers, 2004). The wild bears we have studied in Virginia hibernate for 4–4.5 months; most of them tightly fit into hollow trees. Thus, in many instances, if bears mechanically load their bones during hibernation they would have to do so by shivering or non-standing exercise (perhaps isometric muscle contractions). Shivering has been observed in black bears hibernating in northern Minnesota, using infrared cameras (Rogers, 2004). Harlow et al. (2004) suggested that bears use 3–4 daily bouts of skeletal muscle activity to mitigate muscle strength loss (Harlow et al., 2004). Pardy et al. (2004) suggested that skeletal muscle shivering in bears may be sufficient to maintain trabecular bone mass and microarchitecture by a mechanism involving low magnitude, high-frequency mechanical stimulation, because daily 10 min bouts of low magnitude, high-frequency mechanical stimulation prevented disuse-induced decreases in bone formation rate in hind limb suspended rats (Rubin et al., 2001).
However, the high-frequency stimulation was superimposed on the rats normal weight bearing activities; wild bears shiver while laying down because most dens are too small to permit standing (Rogers, 2004). Furthermore, high frequency, low magnitude (e.g., 5 microstrain) mechanical stimuli are only anabolic for trabecular bone, not cortical bone (Rubin et al., 2002), and our findings suggest that black bear cortical bone is not impaired by disuse (Donahue and Harvey, 2004; Harvey and Donahue, 2004; McGee et al., 2004).

During hibernation, captive grizzly bears also show short bouts (<0.2 s) of local muscle shuddering activity with episodes of periodic activation (3–10 s between bouts) lasting longer than an hour (Lin et al., 2004). Shuddering is described as less vigorous than shivering. It is unclear if shivering can provide sufficient mechanical stimulation to maintain bone mass in hibernating bears. Shivering is probably used to raise body temperature in response to decreasing ambient temperatures and not a mechanism to maintain bone mass, since more frequent shivering in hibernating bears is associated with colder ambient temperatures (Barnes, 2004).

We recently began studies on bone remodeling and mechanics in hibernating grizzly bears (Ursus arctos horribilis). Captive grizzly bears used in these studies hibernate in 3 m square concrete dens and have free access to 3 × 6 m outside runs. However, they are active for less than 20 min per day during hibernation. Females that gave birth averaged only 2.5 min of activity per day during the first 6 weeks of lactation. Otherwise, they were lay continuously while caring for and nursing the cubs. It is important to note that these captive bears are well fed during their active period and may not need to conserve as much energy during hibernation as wild bears as they are frequently fatter than wild bears. Hibernation is a survival response to food shortages, thus it would be counter-productive to survival to burn calories for exercise during hibernation to maintain muscle and bone strength. Even 20 min of daily activity in captive grizzlies represents a considerable reduction in activity level during hibernation; during the summer these bears are active (i.e., traveling and foraging) for about 15.2 h per day (Rode et al., 2001).

There is likely some low level of mechanical stimulation (e.g., due to shivering, rolling over, or grooming in the den) on the bones of hibernating bears (i.e., they are not in a complete state of disuse). But, is this level of mechanical stimulation sufficient to maintain bone mass? Astronauts lose significant bone mass in microgravity (i.e., during spaceflight) despite rigorous musculoskeletal conditioning exercises (Lang et al., 2004). Femoral neck bending strength index decreased 2.55%/month for spaceflights lasting 4–6 months (Lang et al., 2004). This was associated with endosteal expansion without periosteal apposition. Daily bouts of exercise (up to 1.5 h) were unable to prevent, and in some cases exacerbated, disuse-induced cortical thinning and mechanical property loss in rat tibiae and femora (Shaw et al., 1987). Given the results of studies on humans and other animals and the low levels of activity in hibernating bears, it seems likely that there are biological factors that regulate bone metabolism, mass, and strength in hibernating bears, either alone or in combination with low-level mechanical factors.

3. Serum markers of bone metabolism in hibernating bears

We have collected blood from captive and wild hibernating black bears in almost every month of the year. The serum was assayed for biochemical markers (i.e., type I collagen peptides) of bone resorption and bone formation. During bone formation, the carboxy-terminal propeptide of type I procollagen (PICP) is cleaved off and released into the circulation. The concentration of PICP in serum has been positively correlated with histomorphometric measurements of bone formation (Eriksen et al., 1993). During resorption of bone, cross-linked carboxy-terminal telopeptide (ICTP) fragments are released into the circulation. Serum ICTP has been positively correlated with bone resorption and negatively correlated with bone mineral density (Eriksen et al., 1993; Yasumizu et al., 1998). Our data on hibernating black bears suggest that bone resorption increases during disuse (Donahue et al., 2003b; Donahue et al., 2003a). This was expected since increased bone resorption is a common feature of disuse in many animals. However, probably our most

![Fig. 1. Mean serum PICP concentrations for the three study periods. Data are shown as mean value bar plots with standard error bars. Groups with the same letter are not significantly (p<0.05) different from each other. The PICP concentration was not significantly different during hibernation than during the pre-hibernation period. However, post-hibernation, PICP concentration was significantly higher than in the other two periods. Originally appeared in Donahue et al. (2003a).](image-url)
interesting finding is that the serum marker of bone formation did not decrease during disuse. This is contrary to other animal studies which show decreased bone formation during disuse (Dehority et al., 1999; Rubin et al., 2001). In both our studies we found that the mean formation marker levels (averaged over an entire period) were not significantly different between the pre-hibernation and hibernation periods, but significantly increased during the remobilization period (Fig. 1). In our time-course study of these markers in captive bears the formation marker actually increased shortly after the onset of disuse (Fig. 2B), possibly to maintain the normal balance between bone resorption (Fig. 2A) and formation (Donahue et al., 2003a). This finding supports the histological data of Floyd et al. (1990), which showed increased bone resorption and formation surfaces during hibernation. In both our wild and captive bear studies, the formation marker was significantly increased immediately upon remobilization (Donahue et al., 2003b; Donahue et al., 2003a).
Two possible interpretations for our data are that (1) bone is not lost during disuse because bone formation remains coupled to bone resorption even though the rate of turnover increases, or (2) some bone is lost during disuse because the increase in resorption is greater than the increase in formation, but the bone lost during disuse is rapidly recovered during remobilization following spring arousal. A possible explanation for the former interpretation is that bone resorption increases during disuse because of the lack of mechanical stimulation, but because bears do not urinate or defecate during hibernation and blood calcium concentrations remain stable (Floyd et al., 1990), bone formation may increase to prevent blood calcium concentration from becoming too high. Thus, the calcium regulatory hormones may help regulate osteoblastic activity to retain a normal balance between resorption and formation. An explanation for the latter is that there is some bone loss during disuse because there is an imbalance between resorption and formation (i.e., the increase in resorption is greater than the increase in formation), but during disuse the bone cells become accustomed to perceiving very low or absent levels of mechanical stimulation as normal, so that when mechanical loading resumes during remobilization the bone cells perceive the mechanical loading level as high and activate a bone formation response, in accordance with mechanostat theory (Frost, 1987), to rapidly recover the bone lost during disuse. Interestingly, MC3T3 osteoblastic cells cultured in black bear serum, show PGE2 levels with a seasonal pattern strikingly similar to the seasonal changes in the bone formation marker PICP (Fig. 1) (Donahue et al., 2004). Since PGE2 is a potent stimulator of bone formation in vivo (Jee and Ma, 1997), our findings suggest that seasonal variations in the concentrations of circulating molecules help regulate bone formation.

4. Age-related changes in bone strength

To assess the effects of annual periods of disuse on bone mechanical behavior we have tested cortical bone coupons, machined from black bear tibial diaphyses, in uniaxial tension and three-point bending. We have also loaded whole black bear tibias to failure in three-point bending. Tibiae were obtained from black bears that were killed late in their active period (September–October) by licensed hunters in the Upper Peninsula of Michigan. The ages of the bears were determined, by the Michigan Department of Natural Resources, from the dental growth layers in the teeth (Coy and Garshelis, 1992). Sex differences in bone strength (i.e., ultimate stress) were assessed using ANCOVA treating age as the covariant. There were no significant ($p = 0.38$) differences between males and females, therefore the data were pooled and regressed against age. The ultimate stress significantly ($p = 0.008$) increased with age for cortical bone coupons loaded in three-point bending (Fig. 3) (Harvey and Donahue, 2004). The rate of increase was similar to that of human cortical bone for the same relative age ranges (i.e., growth–maturity), although bear bone was stronger than human bone (Currey and Butler, 1975). The age-related increases in strength were probably due in part to age-related increases in mineral content (Fig. 4). Notably, black bear cortical bone porosity showed a trend towards an age-related decrease (Fig. 5), which contrasts the age-related increase in human bone porosity (Wang and Ni, 2003). These data are exceptional when one considers that these bears were inactive for 5–6 months every year.
and other animals lose significant bone mass during disuse and require recovery periods 2–3 times longer than the disuse period to recover the lost bone (Kaneps et al., 1997; Weinreb et al., 1997). Yet, black bears are able to increase bone strength at approximately the same rate as humans. We have also found that for whole bone bending, the ultimate stress did not significantly change with age, although the results approached a significant ($p = 0.051$) age-related increase in strength (McGee et al., 2004). Cortical bone tensile strength did not significantly change with age ($p = 0.16$) (Donahue and Harvey, 2004). Taken together our findings suggest that either (1) cortical bone strength is not adversely affected by disuse (possibly because osteoblastic activity and bone formation are not impaired by disuse), or (2) bears are able to recover bone lost during disuse much faster than other animals; serum markers of bone formation are significantly elevated during remobilization immediately after hibernation (Donahue et al., 2003b; Donahue et al., 2003a).

5. Histological and cross-sectional properties during hibernation

Osteopenia results from decreased osteoblastic formation and increased osteoclastic resorption, decreasing the total amount of bone in the skeleton after a prolonged period of disuse (Weinreb et al., 1989; Rantakokko et al., 1999). In a study on long-term immobilization in dogs, 32 weeks of disuse resulted in significant relative bone loss in both young and old dogs (Jaworski and Uhthoff, 1986). Bone loss was manifest as increased intracortical porosity and decreased cortex thickness. When turkey ulnar diaphyses were in a state of disuse for 4 weeks there was a significant decrease in cross-sectional area and a significant increase (4-fold) in intracortical pore size (Rubin et al., 1996). We recently measured intracortical pore areas in tibias from a 9-month old active grizzly bear and a 13-month old that had been hibernating for 17 weeks. The resorption cavity area was larger (1.7-fold) during hibernation, but not to the extent of the increase observed in other animals during disuse (i.e., 4-fold) (Rubin et al., 1996). However, there were fewer resorption cavities during hibernation, suggesting that intracortical porosity might not change, or even decrease, during hibernation (Fig. 6). Obviously these histological measures need to be more thoroughly investigated before any conclusions can be drawn, but our preliminary observations support
Table 1
Observations on bone remodeling, histology, mechanical behavior, and biology from studies on hibernating black bears

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone resorption and formation surfaces, and bone formation rate increase 3-4 fold during hibernation relative to summer with no change in bone volume (Floyd et al., 1990).</td>
<td>Resorption increases due to a lack of mechanical stimulation in accordance with mechanostat theory. Because bears do not urinate or defecate during hibernation and blood calcium concentration remains constant, calcium regulatory hormones (and possibly other hormones and growth factors) help maintain bone formation and bone mass.</td>
</tr>
<tr>
<td>Serum markers of bone resorption and formation indicate increased bone resorption during hibernation, and an increase or no change in bone formation (Donahue et al., 2003a,b).</td>
<td>There is a possible imbalance between resorption and formation during hibernation, even though both may be increased, resulting in a net bone loss. During hibernation bone cells may become accustomed to perceiving very low or absent levels of mechanical stimulation as normal, so that when mechanical loading resumes they perceive the mechanical loading level as high and activate a bone formation response.</td>
</tr>
<tr>
<td>During remobilization in the spring, the bone formation rate increases 7-fold over the hibernation value and bone resorption surface decreases (Floyd et al., 1990).</td>
<td>This suggests that bone resorption and formation are balanced during hibernation such that there is no net trabecular bone loss in the ulna and radius. It should be noted that the serum markers of bone turnover reflect cortical and trabecular bone turnover in the entire skeleton.</td>
</tr>
<tr>
<td>During remobilization, the bone formation marker increases several fold and the resorption marker returns to pre-hibernation values (Donahue et al., 2003a,b).</td>
<td>The ability to maintain bone formation during disuse and/or rapidly increase bone formation upon remobilization allows bear to increase bone strength with aging despite annual 6-month periods of disuse. Other species require remobilization periods 2–3 times longer than the disuse period to recover bone strength.</td>
</tr>
<tr>
<td>Trabecular bone mineral density and content, volume fraction, structural model index, and trabecular thickness are not significantly different in black bears killed in the spring compared to black bears killed in the autumn (Pardy et al., 2004).</td>
<td>This suggests that the serum molecules which regulate bone formation in vivo also regulate the release of a molecule (PGE2), associated with bone formation, from bone cells in vitro.</td>
</tr>
<tr>
<td>Cortical bone bending strength, stiffness, and ash fraction significantly increase with age and porosity is unchanged (Harvey and Donahue, 2004).</td>
<td></td>
</tr>
<tr>
<td>The ultimate stress in whole tibiae loaded in three-point bending shows a trend of increasing with age (McGee et al., 2004).</td>
<td></td>
</tr>
<tr>
<td>Black bear serum modulates PGE2 release in MC-3T3 osteoblastic cells with the same seasonal variation as the serum concentration of the bone formation marker PICP (Donahue et al., 2004).</td>
<td></td>
</tr>
</tbody>
</table>

Possible explanations for the observed phenomena are given in attempt to synthesize the data and form a theory on the regulation of bone mass and strength in hibernating bears.

the theory that hibernating bears do not develop disuse osteoporosis.

6. Summary

The existing evidence suggests that hibernating bears do not develop disuse osteoporosis. The evidence is summarized in Table 1 in an attempt to formulate a theory on how bears resist disuse-induced bone loss. Bone mineral density and content, bone volume fraction, and trabecular thickness are not significantly affected by hibernation (Pardy et al., 2004), probably because bone formation is not impaired by disuse (Floyd et al., 1990; Donahue et al., 2003a,b). As a consequence, bone strength is not adversely affected by annual periods of disuse (Donahue and Harvey, 2004; Harvey and Donahue, 2004; McGee et al., 2004). In fact, cortical bone bending strength has been shown to increase with age in hibernating black bears without a significant change in porosity (Harvey and Donahue, 2004). The level of activity exhibited by bears during hibernation is considerably less than what they exhibit during the summer and should not be sufficient to maintain bone formation, mass, and strength by itself. We hypothesize that seasonal changes in the concentrations of circulating molecules (e.g., hormones), in the absence of calcium excretion, are central to preserving bone formation, mass, and strength during disuse. It is likely that many molecules act in concert, and possibly interact with the low levels of mechanical stimulation (e.g., shivering) experienced during hibernation. Clearly much is yet to be learned about the biological mechanisms that regulate bone metabolism in hibernating bears. We are optimistic that understanding these mechanisms will contribute to the development of anabolic therapies for osteoporosis in humans.

Acknowledgments

Funding from the National Institutes of Health (NIAMS AR050420), NASA and the Michigan Space Grant Consortium, and Timothy Floyd, MD. Doug Wagner from the Michigan Department of Natural Resources allowed us to collect bones from hunter-killed bears.
References


Donahue, S.W., Harvey, K.B., 2004. Bone strength is not compromised with aging in black bears (Ursus americanus) despite annual periods of disuse (hibernation). American Society of Biomechanics 28th Annual Conference, Portland, OR.


Harvey, K.B., Donahue, S.W., 2004. Bending properties, porosity, and ash fraction of black bear (Ursus americanus) cortical bone are not compromised with aging despite annual periods of disuse. J. Biomech. 37, 1513–1520.


McGee, M.E., Harvey, K.B., Donahue, S.W., 2004. Whole bone bending properties of black bear tibiae are not compromised by annual periods of disuse. 2004 BMES Annual Fall Meeting, Philadelphia, Biomedical Engineering Society.


