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Regional Effects of Ovariectomy and Cadmium on Bone Mineral in Ribs from Aged Female Beagles.[1]

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Abstract

The purpose of this study was to evaluate effects of estrogen depletion and cadmium (Cd) on bone calcium and to determine if these effects were localized in specific regions of ribs. Fourteen female beagles (7-9 y old) with ^{45}Ca labeled skeletons were divided into four groups: sham controls (SO-); ovariectomized (OV-); shams exposed to Cd (SO+); ovariecomized exposed to Cd (OV+). Total Cd exposure period was 7 months, including 1 month by capsules and 6 months by drinking H_2O (15 ppm). Ribs were taken at necropsy from 12 of the 14 dogs, and each rib was quartered. Wet, dry, and ash weights, as well as total Ca and ^{45}Ca content, were determined for each quarter. Analysis of ribs from control animals demonstrated that a given rib is heterogeneous in composition. One end appears to be less mineralized and more metabolically active than other regions. The OV- and OV+ mid-rib regions had significantly lower dry and ash weights than SO- (mid-region avg. dry wt.: -32% and -37%; ash wt.: -33% and -39%, respectively). Total Ca contents of these same regions were also decreased in the OV- and OV+ (-36% and -46%). The only significant change in Ca/dry and Ca/ash was observed when comparing OV+ to SO- (mid-region avg. Ca/dry: -15%; Ca/ash: -12%). Analysis of treatment suggests that there are regional effects of ovariectomy and Cd on bone and that Cd exposure following ovariectomy increased the loss of bone mineral occurring as a result of ovariectomy.

Introduction

Osteoporosis. Osteoporosis, a debilitating bone disease, is marked by a significant, but often unnoticed, decrease in bone mass and strength, resulting in increased risk of fractures from minimal trauma. It afflicts 15-20 million people in the United States, at an estimated yearly health cost of $6.1 billion (1).

Cadmium. Two factors associated with the osteoporotic condition that are central to our research are estrogen depletion at menopause and cadmium (Cd) exposure. The effects of Cd exposure in combination with estrogen depletion are most evident in the etiology of those suffering from Itai-Itai, a severe osteoporotic-like disease in Japan (2). During 1967 and 1968, at least 95% of suspected cases in the Toyama prefecture of Japan occurred in postmenopausal women (49-89 years old). Cd was epidemiologically linked with the disease; a smelter mine upstream had polluted the river water of Toyama with Cd. Women of the area were exposed to high levels of Cd via drinking water and diet (2). Cd in soil and water is a by-product of many industrial processes. Bone loss has been observed in industrial workers chronically exposed to Cd (3). Cigarette smoking is a main contributor to non-occupational cadmium exposure. Such exposure, in combination with smoking-induced estrogen deficits, has been suggested as a factor causing decreased bone mineral content, increased incidence of fractures, and edentulism in female smokers (4-8).

This study was designed to examine the effects of ovariectomy and Cd on bone in an aged beagle model and determine if these effects occur in specific regions of the bone. To address the possible heterogeneous nature of the beagle rib bone, biochemical analysis of Ca and ^{45}Ca distribution by specific region was conducted.

Materials and Methods

Fourteen female beagles (7-9 y old) with ^{45}Ca labeled skeletons (100 μCi/kg body weight) were divided into four groups: sham controls (SO-; n=3); ovariectomized (OV-; n=4); shams exposed to Cd (SO+; n=3); ovariectomized exposed to Cd (OV+; n=4). Total Cd exposure period was 7 months: 1 month by capsules and 6 months by drinking H_2O (15 ppm) (16,23). Ribs were taken at necropsy from 12 of the 14 dogs, cleaned of extraneous tissue and visually oriented according to structural similarities. Wet weights of whole bones were recorded prior to cutting and were recorded a second time by weighing individual pieces to the nearest 0.01 mg. The bones were then placed in 70% ethanol (4 days) and defatted in chloroform-methanol (2:1; V:V) (3 days). Each defatted bone piece was transferred to a tared acid-cleaned scintillation vial and dried at 110°C (2 days). After 6 hours in a dessicator with dry N_2 gas, dry weights were taken. Dried bone pieces were then ashed in a muffle furnace at 525°C (4 days). Vials containing ash were then transferred to the dry N_2 gas dessicator for 6 hours prior to recording ash weights. Bone ash was dissolved in 10 ml of 6 N HCl and allowed to settle (24 hours). One mL aliquots were analyzed for ^{45}Ca activity. Background radiation was subtracted and values were corrected for radiodecay (533 days from the last isotope injection; decay factor=.0938) and converted to dpm/bone piece. Total bone calcium was ana-lyzed by flame atomic absorption spectrophotometry (sensitivity = 0.065 µg calcium). Duplicate 1-ml samples from each bone ash solution were diluted with 0.1% lanthanum/0.1 N HCl (1:2000). A standard curve (1,2,3 ppm Ca) was generated by diluting a stock 1000 ppm Ca solu-tion with 0.1% lanthanum/0.1 N HCl. A mean calcium con-centration per duplicate determinations was calculated. Calcium concentration (ppm) was converted to grams per bone.

The mean, standard deviation, and standard error were determined for each parameter calculated per group. Statistical comparisons were done using analysis of variance (ANOVA); significant differences were tested by Fischer's least significant difference test ($p<0.10$ or $p<0.05$).

Results and Discussion

Regional analysis of control ribs showed that end 2 was significantly lower (-36%) in ash weight than other portions of the same rib (Fig. 1a). ^{45}Ca analysis of control ribs showed that end 2 was significantly higher in ^{45}Ca/dry, ^{45}Ca/ash, and ^{45}Ca/Ca activity (+31%, +77%, +81%, respectively; Fig. 1b). The OV- and OV+ mid-rib regions had decreased dry and ash weights compared to SO- (dry wt.: -32% and -37%; ash wt.: -33% and -39%, respectively; Fig. 2a). Total Ca in the same regions were decreased in the OV- and OV+ (-36% and -46%; Fig. 2b). The only significant difference in Ca/dry and Ca/ash was a decrease in the mid-rib sections of OV+ compared to SO- (-15% and -12%, respectively; Fig. 2c). When comparing rib ends, no con-sistent changes were observed as a result of ovariectomy or Cd exposure.

Previous <u>in vivo</u> and <u>in vitro</u> experiments support the effects of ovariectomy and Cd on bone resorption (9,11-15,26-30). In this study, use of the aged beagle was important for two reasons. First, the beagle exhibits a similar bone remodeling pattern to the human (17,18). Second, the aged female beagles are already skeletally mature and in declining stages of reproductive function (few regular estrus cycles). Therefore, the results of our study can be more appropriately compared with the pathophysiology of accelerated bone loss observed postmenopausally. Another important characteristic of our study was quartering the ribs, since a previous study using whole bones yielded biochemical data that did not correlate with dual photon absorptiometric scanning or with serum and fecal ^{45}Ca data (published and unpublished data) (13,14,16,27-29). This study demonstrates the rib is heterogeneous in composition and that the effects of ovariectomy and Cd exposure are regional.

Regional Analysis. One end (end 2) was significantly lower in inorganic mineral content than other areas. This end appeared to be involved in rapid turnover (high radioisotopic activity) in comparison to the other regions. Evidence that trabecular bone remodels faster than cortical bone would suggest that this region is predominantly trabecular (19). Recently, Ninomiya et al. (15) suggested a difference in the biochemical composi-tion of cortical and trabecular bone. The view that regions of bone are heterogeneous in composition and metabolic activity is crucial when evaluating the effects of ovariectomy and subsequent Cd exposure.

Treatment Analysis. Ovariectomy in the aged beagle caused accelerated loss of bone mineral mid-rib. Parks et al. suggested that the mid-rib regions are predominantly cortical bone (24). Our study refutes studies (23,20) stating that ovariectomy does not affect cortical bone in dogs. Cd exposure in the ovariectomized animal also caused a mid-rib regional loss of bone mineral. The loss of bone mineral was greater than that observed due to ovariectomy alone. This study, in conjunction with bone scanning studies of these dogs (23,26), suggests that ovariectomy sensitizes cortical bone to the effects of Cd. One possible mechanism for accelerated bone loss due to Cd was suggested by Kjellstrom et al. (2). Bone loss was due to Cd-induced renal damage leading to urinary calcium loss in combination with nutritional deficiency (calcium and vitamin D). Cd may have inhibited the synthesis of $1,25(OH)_2$ Vitamin D by renal tubular cells and subsequently increased plasma parathyroid hormone (PTH) levels. Increased PTH would lead to accelerated bone resorption. However, renal and hormonal studies conducted on the dogs during our experiment revealed normal renal function and normal levels of PTH (27,30). This suggests that the mechanisms of Cd induced bone loss are either direct or involve mediators other than calciotropic hormones.

It is possible that bone substitutes the Cd ion for the Ca ion. The ionic radii of Ca and Cd are approximately equal (Ca^{2+}=0.99; Cd^{2+}=0.97) and in the case of some proteins, a binding site will accept either ion (21). Christoffersen et al. (22) suggests that a possible direct mechanism of Cd-induced bone loss involves the absorption of Cd into the crystal structure of the bone and disrupted normal resorption due to the dissolution resistance of the Cd-hydroxyapatite (Cd-HA) crystal. Acid produced for bone resorption may then penetrate into other areas of the bone structure, reacting with the normal Ca-HA crystals. Since end 2 is the most metabolically active area, it may contain a high percentage of the Cd-HA crystal relative to the other regions and be "protected" from resorption. The less metabolically active mid-rib regions could have increased mineral loss due to the resorptive factors released by the metabolically active end. End 1 may have been too distal for the resorptive factors to reach and thus no effect was observed. We have not ruled out that Cd-induced osteoclast-like cells (12) may have played a role in the decreased bone mass observed in this study.

In conclusion, the use of the aged dog presents a more appropriate model than young animals (dogs and rodents) with respect to postmenopausal women. Further studies are required to more clearly understand interactions between Cd exposure, ovariectomy, and bone loss. Our data suggest that Cd exposure from cigarette smoke or industrial pollution results in bone mineral loss such as in Itai-Itai disease. Furthermore, this study provides evidence that the multifactorial nature of osteoporosis and Itai-Itai may be linked with the heterogeneous nature of bone tissue.

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Fig. 1A So- Ash Weights

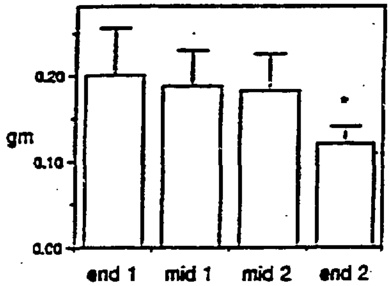


Fig. 1B So- 45Ca/Ash Ratios

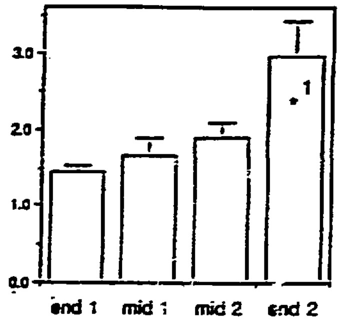


Fig. 2 A Mid 1 Ash Weights

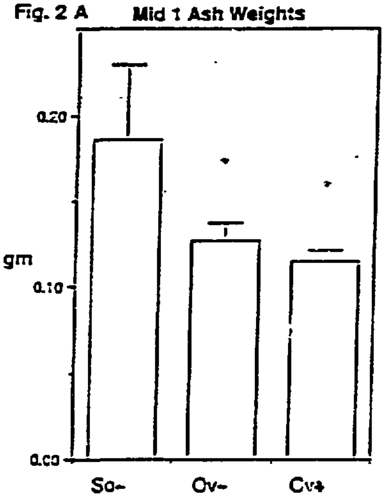


Fig. 2 B Mid 1 Total Ca

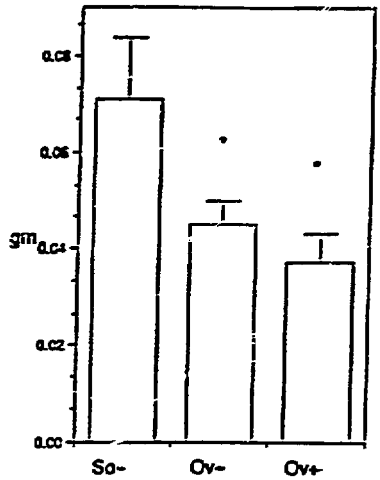
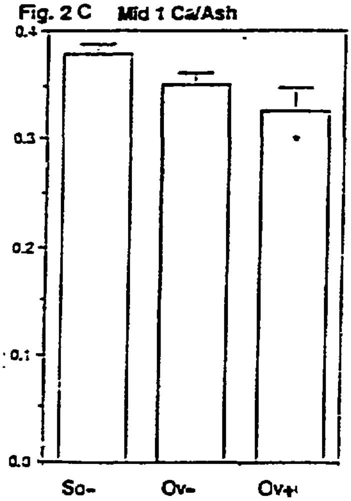


Fig. 2 C Mid 1 Ca/Ash



Figs. 1A–B) Rib heterogeneity shown by end 2 having significantly less inorganic mineral and more ^{45}Ca, suggesting a greater degree of bone turnover. Figs. 2A–C) Mid-rib showed significant bone mineral loss in OV+ (-39, -46, -12%) compared to SO-. Significant bone mineral and Ca loss was observed in OV- (-33 and -36%). Exposure to Cd following OV accelerated bone loss although the greater effect was due to OV alone. (*$p<0.10$; *[1]$p<0.05$)