Cortical bone dynamics, strength, and densitometry after induction of emphysema in hamsters

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THERE IS A GROWING BODY of evidence relating chronic obstructive pulmonary disease (COPD) to osteopenia and osteoporosis (4, 7, 8, 11, 15). Low bone mineral density (BMD) has been measured at the forearm, leg, spine, and whole body of COPD patients (4, 7, 8, 11, 15). One possible explanation for the association between COPD and low bone mass is the high level of corticosteroid use among individuals suffering from COPD (9). Corticosteroid use is known to decrease BMD and is also associated with an increase in fracture risk (9, 25, 27). However, recent investigations have demonstrated low BMD in COPD patients not receiving corticosteroids (4, 8, 15). Therefore, the decreased BMD observed in individuals with COPD may be related to the underlying lung disease and not necessarily the treatment regimen.

The pathophysiological pathway connecting COPD to bone loss is unknown. However, numerous characteristics of COPD patients, in addition to the high incidence of corticosteroid use, may contribute to the decreased BMD observed in this patient population. For example, reduced physical activity levels in COPD patients may be important (11), because skeletal underloading is associated with bone loss (2, 3). Other characteristics of individuals with COPD associated with bone loss are cigarette smoking, hypercapnia, respiratory acidosis, vitamin D deficiency, and hypogonadism (4, 7).

Pulmonary emphysema, characterized by air space enlargement, is a major component of COPD. Several animal models have been utilized to gain a better understanding of the mechanisms leading to the development of COPD and emphysema and their sequelae (6, 20). Elastase-induced emphysema is commonly used as a model to investigate events downstream of the initial development of the disease (6, 13, 14, 18, 19). Previous research has shown that there are normally no alterations in activity levels with this model (14). Therefore, the model controls for corticosteroid use, as well as activity level, two factors known to influence skeletal metabolism. Because bone loss is associated with COPD in the patient population, it is the goal of the present investigation to determine whether there are any changes to the cortical bone in the elastase-induced emphysematous hamster model.

MATERIALS AND METHODS

The protocols were approved by the University of Utah Institutional Animal Care and Use Committee. In all respects, they conform to the Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85-23, revised 1985, Office of Science and Health Reports, Bethesda, MD].

Animals. After a 1-wk acclimation period, 20 adolescent male Syrian Golden hamsters (9 wk old, 100–118 g body wt) were divided randomly into control (Con) and emphysema (Emp) groups. Under deep ketamine-xylazine anesthesia

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(150 and 7.5 mg/kg im, respectively), saline (Con) or porcine elastase [Emp; 25 IU/100 g body wt (Sigma Chemical, St. Louis, MO) in 0.3 ml of normal saline] was instilled intratracheally using a 27-gauge hypodermic needle, as previously described (14, 19). Calcein (10 mg/kg body wt ip; Sigma Chemical), a fluorochrome used for histomorphometric measurement of bone formation, was administered at 11 and 4 days before necropsy.

**Tissue preparation.** At 3 wk after elastase injection, the hamsters were euthanized, and the lungs and femurs were removed. A saline-displacement technique was used to measure excised lung volume at 0 cmH2O airway pressure (14, 19). The right femurs were wrapped in saline-soaked gauze and placed in a −20°F freezer for subsequent densitometry and mechanical testing. The left femurs were fixed in 10% neutral buffered formalin and used for structural and dynamic histomorphometric measurements.

**Densitometry and mechanical testing.** The right femurs were completely thawed at room temperature and then scanned using a peripheral dual-energy X-ray absorptiometer (Norland, Medical Systems, Fort Atkinson, WI; coefficient of variation = 0.6%) adapted for small-animal research to assess bone mineral content (BMC) and BMD of the whole femur and the middiaphyseal shaft.

After densitometry measurement, the thawed femurs were placed in a materials testing machine (MTS, Eden Prairie, MN) equipped with a 5-kN load cell and then loaded to failure in three-point bending at a rate of 10 mm/min (1, 10, 24). Breaking load, flexural rigidity, and work to fracture were measured from the load-deformation curve (10).

**Histomorphometry of cortical bone.** The left femurs were dehydrated in graded ethanols and embedded undecalcified in methyl methacrylate (Fisher, Los Angeles, CA). Cross sections of the middiaphysis were cut on a low-speed bone saw (Isomet, Buehler, Lake Bluff, IL), mounted on plastic slides, and ground to ~30-μm thickness.

A digitizing tablet, a microcomputer (Apple SE, Apple Computer, Cupertino, CA), a fluorescence microscope (Nikon, Tokyo, Japan), and histomorphometry software (KSS Scientific Consultants, Magna, UT) were used to measure cortical bone formation and resorption indexes. Measurements included total surface perimeter, perimeter of single- and double-labeled surface, and interlabel width on the periosteal and endocortical surfaces. The perimeter of eroded surface was measured on the endocortical surface. From these measurements, percentage of single-labeled, double-labeled, and mineralizing surface, and mineral apposition rate, surface-referent bone formation rates, and eroded surface were calculated and expressed by use of standardized procedures and nomenclature (17). Additionally, cortical cross-sectional area and average and minimum cortical widths were measured. All measurements were made by one investigator and reviewed by a second. Both investigators were blinded.

**Table 1. BMD and BMC of femurs**

<table>
<thead>
<tr>
<th></th>
<th>Emp Group</th>
<th>Con Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femur BMD, g/cm²</td>
<td>0.119 ± 0.006</td>
<td>0.123 ± 0.004</td>
<td>0.162</td>
</tr>
<tr>
<td>BMD, g</td>
<td>0.150 ± 0.012</td>
<td>0.162 ± 0.008</td>
<td>0.026</td>
</tr>
<tr>
<td>Middiaphysis BMD, g/cm²</td>
<td>0.108 ± 0.006</td>
<td>0.113 ± 0.005</td>
<td>0.406</td>
</tr>
<tr>
<td>BMD, g</td>
<td>0.013 ± 0.001</td>
<td>0.014 ± 0.001</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Values are means ± SD. BMC, bone mineral density; BMC, bone mineral content; Emp, emphysema; Con, control.

**Statistics.** Differences between groups were tested for significance by a two-tailed nonparametric Wilcoxon’s two-sample test (JMP, version 5.0). Values are means ± SD and were considered significant at P < 0.05.

**RESULTS**

**Body weight and lung volume.** There was no statistical difference in starting weight (110.1 ± 4.7 and 113.1 ± 6.3 g for Emp and Con, respectively; P = 0.517) or final weight (122.5 ± 7.5 and 129.9 ± 8.6 g for Emp and Con, respectively; P = 0.120) between the Emp and Con groups. The saline-displacement lung volume of the Emp group was 25% greater than that of the Con group (1.2 ± 0.4 vs. 0.9 ± 0.1 g; P = 0.05). This finding is consistent with that of Snider and Sherter (21), who utilized the same model.

**BMD and mechanical testing.** The BMC of the whole femur (P = 0.026) and the middiaphysis (P = 0.045) were less in the Emp than in the Con group, with no statistical differences in BMD at either site (Table 1). The load at which fracture occurred was 6% lower in the Emp than in the Con group (1.2 ± 0.5 and 1.3 ± 0.4 N; P = 0.05). There were no differences in the measurement of flexural rigidity (P = 0.165) or work to fracture (P = 0.121; Table 2).

**Structure and dynamics of cortical bone.** The structural properties of the middiaphyseal cortical bone are presented in Table 3. The cortical bone area of the Emp group was smaller than that of the Con group (P = 0.013). However, no difference was found in average cortical widths (P = 0.513). The periosteal perimeter was smaller in the Emp than in the Con group (P = 0.034), although there were no differences in the endocortical perimeter (P = 0.713) or medullary area (P = 0.967).

Bone formation indexes for the periosteal and endocortical middiaphyseal surfaces are presented in Table 4. The mineral apposition rate was 27% less in the Emp than in the Con group at the periosteal surface (P = 0.045), with no differences in mineral apposition
rate on the endosteal surface ($P = 0.786$). There were no statistical differences in the percentage of single-labeled, double-labeled, and mineralizing surface or bone formation rates between the two groups on the periosteal and endocortical surfaces. The endocortical eroded surface was 93% greater in the Emp than in the Con group ($P = 0.015$).

**DISCUSSION**

The present investigation demonstrated that, by 3 wk after induction of emphysema in a hamster model, the femoral and middiaphysis BMC and middiaphyseal cortical bone area were lower in the Emp than in the Con group. Differences in BMC and cortical bone area between the two groups were also reflected in the mechanical properties of the femur, with the Emp group having a lower femoral strength than the Con group. The lower cortical bone areas observed in the Emp animals may be attributed to smaller periosteal mineral appositional rates and larger endocortical bone eroded surfaces in the Emp than in the Con animals. It is not possible in the present investigation to determine when the eroded surfaces occurred, because this was a cross-sectional study. However, the lack of significant differences in endocortical bone formation rates between the two groups suggests that the difference may be the result of an increase in bone resorption in the Emp group. This is not the only inflammation model that results in rapid alterations to bone structure and dynamics. For example, thermal injury in mice also results in rapid and dramatic suppression of bone formation with concomitant increases in resorption of the cortical bone by 10 days after injury (16).

Lower femoral BMD and whole body BMD have also been observed in patients with COPD, independent of treatment (8). The present investigation measured a lower femoral and middiaphyseal BMC in the Emp group. The lower femoral BMC in the Emp group supports the hypothesis that the decreased BMD in COPD patients is due in part to the underlying disease and not the treatment regimen per se.

Physical inactivity in COPD patients may contribute to the reduced BMD observed in this patient population, because skeletal underloading is associated with a reduction in BMD (2, 3). However, in a previous study, essentially no differences in activity levels were reported between Emp and Con groups in the elastase-induced emphysematous hamster model (14). The hamsters in the present investigation were mobile after the procedure, and there were no obvious differences in their mobility during the study. Additionally, there were no statistical differences in the initial and final weights of the two experimental groups, suggesting that differences in body weights and, thus, the skeletal load could not account for the marked differences in skeletal structure and strength observed in the Emp group compared with the Con group. There was a 5.7% difference in the final mean weights between the two experimental groups. The difference in the final weights was not statistically significant, but, given a larger sample size, there may be weight differences that account for some of the measured differences. However, because the final weights were not statistically different, it is unlikely that body weight and, thus, skeletal loading account for all the observed differences. Thus the lower BMC, cortical area, femoral strength, and periosteal mineral apposition rate and higher endosteal eroded surface observed in the Emp group are likely the direct result of the elastase-induced emphysema.

Another possible explanation for the different bone measures observed between the Emp and Con groups is a direct effect of the injected elastase on bone. However, in previous studies using radiolabeled porcine elastase, a majority of the elastase was isolated in the lung within alveolar macrophages or excreted in an inactive state in the urine after being processed in the liver (22, 23). Other investigators reported an increase in the number of macrophages in the lung after intratracheal injections of elastase, supporting the role of macrophages in isolating the elastase (12, 26). Additionally, the enzymatic activity of the elastase in the lungs is diminished to very small amounts by 4 days (23). However, small amounts of enzymatically active elastase remained in the lungs for up to 4 mo (22). Therefore, it appears from these investigations that minimal amounts of active elastase remain in the hamster after a few days with this model; consequently, elastase is unlikely to account for the structural and mechanical skeletal differences observed in the present investigation.

Systemic inflammatory cytokines are another possible contributing factor to the deleterious changes in skeletal tissue in COPD and emphysema. Interleukin-1, interleukin-6, and tumor necrosis factor-α, all of which are elevated in the serum of COPD patients, play important roles in skeletal metabolism (7). Respiratory acidosis, a decrease in blood pH due to an
increase in \( P_{\text{CO}_2} \), may also be a contributing factor, because there is a positive correlation between arterial \( p\text{H} \) and BMD in COPD patients (7). Chronic respiratory acidosis in the rat is also associated with a decrease in normalized cortical bone area and an increase in the number of osteoclasts (5). Thus numerous factors, including inflammatory cytokines, respiratory acidosis, cigarette smoking, and skeletal loading, may contribute to the low BMD observed in COPD patients.

In conclusion, the elastase-induced emphysematous hamster exhibited lower femoral BMC, cortical bone area, and femoral strength within 3 wk after elastase instillation. These differences in structural and mechanical properties in the Emp compared with the Con group occurred without the use of corticosteroids. Finally, the depressed indexes of bone formation with larger eroded surface observed in the present investigation may help explain why patients with COPD and other inflammatory conditions are prone to osteopenia.

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DISCLOSURES

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REFERENCES


