Fatigue microdamage in the radial predilection site for osteosarcoma in dogs

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Objective—To evaluate whether body size and anatomic site influence the quantity of bone microdamage in dogs without osteosarcoma (OS).

Sample Population—Pairs of radii were collected from 10 small dogs (<15 kg) and 10 large dogs (>25 kg).

Procedure—Specimens were stained in basic fuchsin for bone microdamage. Transverse sections were cut from each proximal and distal radial metaphysis at 15 and 95% of bone length. The following variables were determined for each region: mean microcrack length (CrLe, µm), microcrack density (CrDn, microcracks/mm²), microcrack surface density (CrSDn, µm/mm²), and estimated activation frequency (Act, microcracks/mm²).

Results—Metaphyseal region did not significantly influence CrDn, CrLe, and CrSDn. The CrDn and CrSDn were influenced by body size, with microdamage being increased in large dogs, compared with small dogs. However, mean CrLe was not significantly influenced by body size. Act significantly decreased with age and was significantly decreased in large dogs and in the distal radial metaphysis, compared with small dogs and the proximal radial metaphysis, respectively.

Conclusions and Clinical Relevance—Our data did not reveal an increase in microdamage or remodeling at the OS predilection site (ie, the distal metaphysis of the radius), suggesting that induction of microdamage and an associated increase in bone remodeling are unlikely to be an important risk factor for induction of OS. (Am J Vet Res 2002;63:896–899)

Osteosarcoma (OS) is a common malignant bone tumor of humans and dogs. Osteosarcoma develops most often in long bone metaphyses with late-closing physes of limbs that receive the greatest stresses during the cyclic loading associated with daily activity, namely the pelvic limb in humans and the thoracic limb in dogs. Osteosarcoma is approximately 8 times as prevalent in dogs, compared with humans, with an annual incidence of 7.9/100,000 dogs. Although survival rates after treatment of OS have improved in recent years, preventative treatment is not used in humans or dogs, because the disease mechanism is unclear. Body size is a risk factor for OS in dogs, with large dogs weighing >25 kg being 185 times as likely to develop OS than small dogs weighing <15 kg. In dogs, the thoracic limb, which supports 60% of the body weight, contains the most important OS predilection site, namely the distal radial metaphysis. Ninety-six percent of OS of the radius develops in the distal radial metaphysis. The pattern of musculoskeletal neoplasia in small dogs weighing <15 kg is quite different, and OS is much less common than in large dogs weighing >25 kg. Taken together, these data suggest that site-specific bone injury in regions subjected to high strain may form part of the OS disease mechanism. Other risk factors for OS in dogs also have been studied, including familial incidence, preexisting bone defects, multiple cartilaginous exostoses, and fracture-induced trauma. Tissue repair responses to fracture, the presence of metallic implants, and bone infarction also have been implicated as risk factors for induction of OS.

Small in vivo microcracks, approximately 0.1 mm long, have been identified in canine and human compact bone from the long bones of weight-bearing limbs. Such microdamage is a normal physiologic consequence of the cyclic loading associated with daily activity. In the adult skeleton, osteonal remodeling is targeted to the repair of bone microdamage, suggesting that microdamage formation and subsequent bone adaptation by remodeling will be increased in regions subjected to high cyclic strains.

The purpose of the study presented here was to measure the density of microcracks and the associated remodeling in the metaphyses of the left and right radius in large and small dogs. We hypothesized that the quantity of bone microdamage and associated remodeling in dogs without OS may be significantly different in large dogs (>25 kg), compared with small dogs (<15 kg). We also hypothesized that the quantity of bone microdamage and associated remodeling in dogs without OS may be significantly different between radial metaphyseal sites.

Materials and Methods

Specimen preparation—Left and right radii were collected from pet or research dogs that had been euthanatized for reasons unrelated to the locomotor system and our study. Paired bones were collected from 10 small dogs (<15 kg) and 10 large dogs (>25 kg). Body weight groups were defined on the basis of current epidemiologic information. Bones were stored at −20°C for further processing. Age, weight, and sex of dogs were recorded. Three-centimeter-wide metaphyseal bone segments were excised and bulk-stained in 1% basic fuchsin in a graded series of alcohols (80, 90, 100%) under a vacuum of 20 mm Hg for 18 days, before being embedded in methylmethacrylate. This technique stains preexisting microcracks before histologic sectioning, thus allowing them to be differentiated from any damage introduced during the preparation.
of the bone sections. Transverse sections, 150 μm thick, were cut perpendicular to the long axis of the shaft of the radius from each proximal and distal radial metaphysis at 15 and 85% of bone length. These percentiles correspond to the characteristic O5 predilection site in the distal portion of the radius and the equivalent metaphyseal region in the proximal portion of the radius.

Morphometric analysis for bone microdamage—Standard morphometric analysis for bone microdamage was performed at 200X magnification by use of bright light and computerized image analysis. In these mineralized sections, microcracks were delineated as linear structures with basic fuchsin staining around the cracks. Diffuse damage, represented by areas of diffuse staining of the matrix, was not measured, and unstained cracks were considered artifact associated with tissue processing. The following histomorphometric variables were determined: bone area (BA, mm²); microcrack density (CrDn, microcracks/mm²); mean microcrack length (CrLe, mm), and microcrack surface density (CrSDn, µm²/mm²). The entire bone cortex was evaluated. A single observer (KLG) collected data.

Morphometric analysis for bone turnover—Activation frequency (Acf, microcracks/mm²/yr) was estimated by use of a standard algorithm as a marker of bone turnover. This method provides an estimate for Acf over an extended period. Mean tissue age was assumed to equal chronologic age. Mean osteon area (OnAr, mm²), on the basis of 10 complete osteons, was calculated, and the number of intact osteons (OnNInt, microcracks/mm² [> 90% of perimeter intact]) and number of fragmentary osteons (OnNFg, microcracks/mm² [> 10% of perimeter remodeled]) were determined in a field area at least 2.3 mm² within the cranial cortex of each metaphyseal region. The Acf was determined by the following equation:

Acf = (β [OnNInt + OnNFg])/t

where t = age (years) and β = (1 – [OnNInt + OnNFg]/[OnAr])⁻¹. Osteon counting was performed in at least 3 fields-of-view evaluated in bright light. A single observer (KLG) collected data.

Statistical analysis—Repeated-measures ANCOVA and a posthoc t-test (Tukey) were used to evaluate the effect of body size (large vs small dogs) and region (proximal or distal metaphysis) on CrLe, CrDn, CrSDn, and Acf. Data were averaged between left and right metaphyses before inclusion in the ANCOVA model. Metaphyseal region was analyzed as a repeated-measure. Age was analyzed as a covariate. Results are expressed as mean (± SD) values. Differences were considered significant at P < 0.05 for testing a null hypothesis of no difference.

Results

In our study, large dogs (> 25 kg) included 2 sexually intact males, 1 castrated male, 4 sexually intact females, and 3 spayed females with a mean age and body weight of 8.9 ± 4.3 years and 31.3 ± 7.0 kg, respectively. Small dogs (< 15 kg) included 1 sexually intact male, 1 castrated male, and 8 sexually intact females with a mean age and body weight of 3.3 ± 3.6 years and 11.0 ± 1.8 kg, respectively. Classic linear microcracks were readily identified in radial metaphyseal bone (Fig 1). Metaphyseal region did not significantly influence CrDn (P = 0.41; power = 0.8 at Δ = 50%), CrLe (P = 0.98; power = 0.8 at Δ = 35%), or CrSDn (P = 0.17; power = 0.8 at Δ = 127%; Table 1). CrDn and CrSDn increased with age but not signifi-

Figure 1—Photomicrograph of transverse mineralized bone sections of the canine distal radial metaphysis. Metaphyseal bone segments were bulk-stained with basic fuchsin before sectioning for histologic detection of in vivo bone microdamage. A—Bone section from a 1-year-old sexually intact female Beagle (small dog < 15 kg). B—Bone section from a 12-year-old castrated male Alaskan Malamute (large dog > 25 kg) containing several in vivo microcracks (black arrows). Notice the micropetrosis (loss of osteocytes; white arrows) and large central canals in the secondary osteons. Bars = 100 mm.

Table 1—ANCOVA summary for microdamage variables in dogs

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>CrDn (microcracks/mm²)</th>
<th>CrLe (µm)</th>
<th>CrSDn (µm²/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body size*</td>
<td>P = 0.01</td>
<td>P = 0.47</td>
<td>P = 0.02</td>
</tr>
<tr>
<td>Metaphyseal region†</td>
<td>P = 0.41</td>
<td>P = 0.98</td>
<td>P = 0.17</td>
</tr>
<tr>
<td>Age (y)</td>
<td>P = 0.08</td>
<td>P = 0.84</td>
<td>P = 0.08</td>
</tr>
</tbody>
</table>

Values of P < 0.05 are considered significant.

*Large dogs (> 25 kg) vs small dogs (< 15 kg). †Proximal portion of the radius versus the distal portion of the radius.

CrDn = Microcrack density. CrLe = Mean microcrack length. CrSDn = Microcrack surface density.
cantly decreased in large dogs ($P < 0.001$) and in the distal radial metaphysis ($P < 0.001$), compared with small dogs and the proximal radial metaphysis, respectively.

Table 2—Body size versus mean ($\pm$ SD) CrDn, CrLe, and CrSDn in dogs

<table>
<thead>
<tr>
<th>Variable</th>
<th>CrDn (microcracks/mm$^2$)</th>
<th>CrLe (µm)</th>
<th>CrSDn (µm/mm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large dogs</td>
<td>$0.25 \pm 0.28^a$</td>
<td>$56 \pm 10^a$</td>
<td>$14.5 \pm 15.9^a$</td>
</tr>
<tr>
<td>Small dogs</td>
<td>$0.07 \pm 0.05^b$</td>
<td>$60 \pm 31^a$</td>
<td>$5.19 \pm 3.94^a$</td>
</tr>
</tbody>
</table>

$^a,b$Values with different superscript letters within a column indicate significant differences ($P < 0.05$) between large and small dogs.

See Table 1 for remainder of key.

Figure 2—Microcrack density (CrDn) versus age in dogs. CrDn increased with age but not significantly.

Figure 3—Effect of body size and metaphyseal region on mean ($\pm$ SD) microcrack density (CrDn) in dogs. The CrDn was significantly ($P < 0.05$) increased in large dogs compared with small dogs.

Figure 4—Effect of body size and metaphyseal region on mean ($\pm$ SD) microcrack length (CrLe) in dogs.

Figure 5—Effect of body size and metaphyseal region on mean ($\pm$ SD) microcrack surface density (CrSDn) in dogs. The CrSDn was significantly ($P < 0.05$) increased in large dogs compared with small dogs.

Figure 6—Activation frequency (Acf) versus age in dogs. The Acf decreased significantly ($P < 0.05$) with increasing age.
large dogs, compared with small dogs. These data do not suggest that it is likely that a mechanically induced increase in bone remodeling is an important risk factor for induction of OS. Furthermore, use of preventative treatment with a drug, such as a bisphosphonate, that inhibits bone remodeling may exacerbate the accumulation of microdamage identified in large dogs and, in time, may increase the risk of bone fracture.18–21

References

Discussion
Our data did not reveal an increase in microdamage at the OS predilection site of the radius, although microdamage was increased in large dogs, compared with small dogs. Microdamage within the long bone mid-diaphysis of the weight-bearing limb accumulates with aging in humans’ and dogs,19 and a similar relationship with aging was detected for CrDn and CrSDn in our study. However, age alone did not explain all of the variation associated with CrDn, and body size also had a significant effect, with increased amounts of microdamage being identified in large dogs. Although accumulation of microdamage was detected in large dogs, microdamage did not accumulate in the distal radial metaphysis or OS predilection site, compared with the proximal metaphysis. The CrLe measured in our study were within the range that would be expected from physiologic loading of the skeleton.20 Interestingly, propagation of a microcrack after it has been initiated may not necessarily be affected by load, since the increased microdamage detected in large dogs was not associated with an increase in CrLe.

Normally, there is equilibrium between the rate of microdamage formation and the rate of bone repair by reparative osteonal remodeling, such that microdamage does not accumulate and lead to an increased risk of fracture.21 Development of fatigue fractures in humans is thought to be associated with site-specific accumulation of microdamage.19,22–24 However, damage accumulation may not exceed the maximal rate of reparative bone remodeling, even in regions known to be exposed to high strains, such as the second metatarsal bone.22 The precise mechanism by which microdamage activates remodeling is unclear.

Activation of basic multicellular units and increased remodeling is known to develop in more active dogs, and is twice as high in the thoracic limb, compared with the pelvic limb.22 Similarly, OS is twice as common in the thoracic limb, compared with the pelvic limb.1,3,5

However, our data did not indicate that remodeling is increased in the distal radial metaphysis or OS predilection site, compared with the proximal radius, although we did detect decreased remodeling in large dogs, compared with small dogs. Remodeling also decreased exponentially with age as has been previously described.23 Determination of bone remodeling often is achieved by tetracycline labeling, although this is not practical in client-owned dogs. We estimated turnover of osteonal bone indirectly by use of a standard algorithm,14 which can be applied to any canine bones of known age. We assumed that mean tissue age was the same as chronologic age. Therefore, our estimation of AfC is a theoretic minimum, as this assumes that there has been no turnover since birth. Therefore, our estimation of true AfC may be less precise in older dogs, as there has been a greater opportunity for remodeling events to have occurred over chronologic time in these dogs, such that mean tissue age may be less than chronologic age.

Although we did not detect increased microdamage or remodeling in the distal radial metaphysis, compared with the proximal radial metaphysis, we did detect increased microdamage and decreased remodeling in large dogs, compared with small dogs. These data do