Conservation of the elastic and flexural moduli of osteopenic femoral cortical bone in experimental inflammatory arthritis in the rabbit*

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Experimental inflammatory arthritis (EIA) produced by carrageenan injection provokes a rapid bone remodeling state with cortical and cancellous bone loss. The objective of this study was to determine whether changes in cortical mechanical properties and/or geometry occur in long bones, either near or remote to the site of inflammation. EIA was induced in the right tibio-femoral joint of rabbits over 56 days. The right humerus and right femur from 15 normal and 25 arthritis group animals were excized. Semi-cylindrical specimens of the medial cortical shaft were subjected to non-destructive four-point bending tests. Transverse sections at the four contact sites of the loading jig were photographed and digitized to obtain average cross-sectional area (A) and moment of inertia (I). Moment of inertia and slope of the load/deflection curve permitted calculation of modulus of elasticity (E) for each specimen. Load/time curves were also used to calculate per cent stress remaining in relaxation experiments. Per cent stress remaining, E, A, I and $\sqrt{I/A}$ (radius of gyration) were examined for differences by bone (humerus, femur) and by treatment (N,A) using two way ANOVA. The induction of inflammatory arthritis did not significantly alter the modulus of elasticity in either the femur or humerus; however, arthritis reduced the moment of inertia from $34.54 \pm 2.88 \times 10^{-12}$ m^4 to $25.06 \pm 1.80 \times 10^{-12}$ m^4 (mean $\pm$ SEM, $p < 0.05$). This was observed in the femur (near the arthritic joint), but not in the humerus (remote from arthritic joint). Analysis of area and ratio I/A demonstrated that this geometric effect of treatment was due to reduced area without gross cross-sectional shape changes. Per cent stress remaining in the femur (but not in the humerus) was higher in the arthritis specimens than in the normal specimens (N: $80.86 \pm 0.97\%$; A: $83.25 \pm 0.71\%$, $p < 0.05$). Thus, in this arthritis model, the principal mechanical or geometric effect on cortical bone was reduction of the cross-sectional area and moment of inertia. The viscoelastic relaxation response of bone was also altered, perhaps due to loss of water or collagen degradation.

1. Introduction

Fracture risk is increased in patients who have rheumatoid arthritis compared to the general population [1–3]. Differences in bone structure and remodeling kinetics have been shown in inflammatory arthritis [4–10] but the nature of changes in the mechanical properties of bone remains ill–defined.

In a rabbit model of experimental inflammatory arthritis (EIA) affecting the tibio-femoral joint, the cross-sectional area of the femoral metaphysis and diaphysis is reduced by endosteal resorption, despite increases in bone formation [6, 8]. Bone resorption rates were calculated to be elevated at least fourfold in this model of experimental inflammatory arthritis (EIA) [6]. *Ex vivo* torsional testing of femora from arthritic knee joints shows that EIA is associated with reduced fracture strength and fracture toughness [11].

In order to create an approach for prophylaxis and treatment, our long-range purpose is to determine how inflammatory arthritis produces critical weakening of bone leading to fracture. This study focused on changes in mechanical properties of the diaphysis of long bones in

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rabbits subjected to EIA of the tibio-femoral joint. Both femoral and humeral specimens were examined in order to investigate the possibility of changes in cortical bone both local to, and remote from the arthritic site. In order to measure changes in both bone geometry and material properties, we applied non-destructive *in vitro* mechanical testing of machined half-cylinders of bone (load/deflection testing in four-point bending with stress relaxation tests), as well as measurement of three geometric properties (moment of inertia, cross-sectional area, and the radius of gyration $(\sqrt{I/A})$) [12]. We also calculated the flexural modulus of the bone samples, thus combining material and geometric information.

2. Materials and methods

Experimental inflammatory arthritis was induced in the right tibio-femoral joint of 25 New Zealand White (NZW) rabbits (A group) as previously described [6], using intra-articular injection of 0.3 ml of a sterile 1% solution of the sulfated mucopolysaccharide carrageenan (Satiagum B; Sugro, Basel, Switzerland), 14 times over 56 days. Sixteen NZW rabbits from the same breeding stock comprised the normal group (N group), and received no injections. All animals were mature and initially weighed 3.6 to 4.5 kg. The animals were housed in standard cages with food and water *ad libitum*. All procedures were conducted with the approval of the Animal Care Committee of the Wellesley Hospital Research Institute.

After euthanasia by anaesthetic overdose, the right humerus and right femur of each animal were immediately excized and frozen wet at $-20\,^{\circ}C$. Before mechanical testing, the bone specimens were thawed at $4\,^{\circ}C$ and were kept hydrated in saline at room temperature during further processing. Under continuous saline irrigation, a low-speed saw (Buehler Isomet) with a diamond wafering blade was used to cut each bone to produce the *in vitro* test samples. Each bone was initially cut transversely at the proximal and distal one quarter length points (Fig. 1). The remaining central diaphyseal shaft was then cut in half sagittally to yield semi-cylindrical specimens of the medial and lateral shaft. Humeral specimens were approximately 4 cm long, and femoral specimens 5 cm long. The medial samples were held in saline-soaked gauze until mechanical testing (maximum 4 h after thawing).

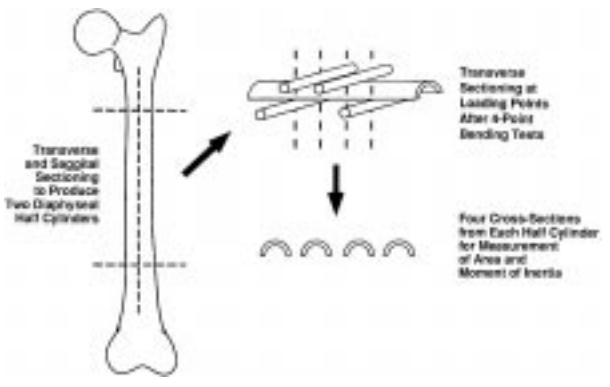


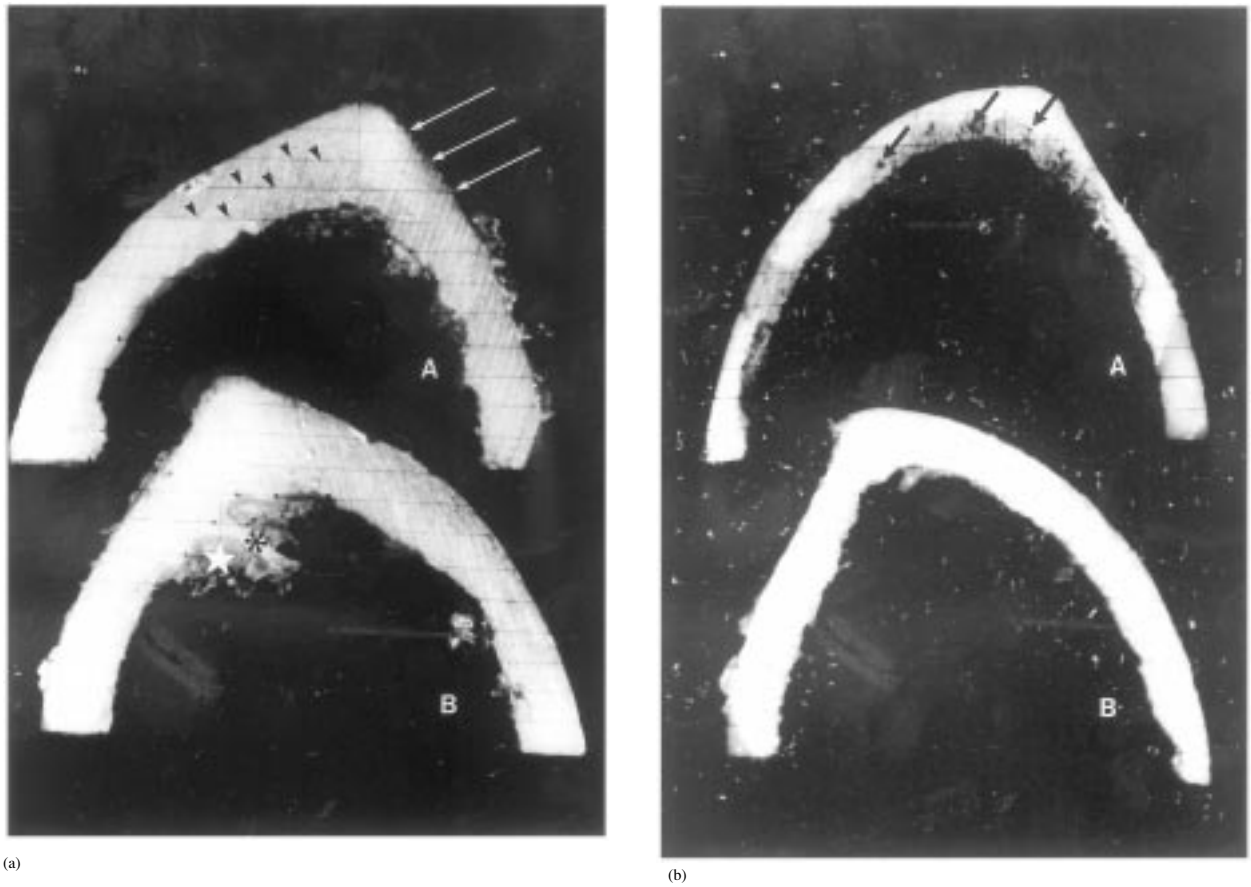
Figure 1 Specimens for mechanical testing were cut from the centers of the right rabbit femur or humerus. The central half-length was removed by transverse sectioning and the medical half-cylinder was obtained by sagittal sectioning. After mechanical testing by 4-point bending, four thin specimens were cut transversely at the load points to provide cross-sectional views of the central medial diaphysis. The distance from the center of the bone length to the outer load points (and cuts) was ~ 10.5 mm; for inner load points it was ~ 3.5 mm. Each cross-sectional specimen was photomicrographed and the image digitized and analyzed to obtain the cross-sectional area and moment of inertia.

Mechanical testing was performed on an Instron electromechanical testing system (Model TT–CM), using a custom-built four-point bending jig with roller specimen supports for unconstrained loading. Load was measured using a 50 kg Instron load cell attached to the two central loading points. Deflection at the central loading points was measured using a 5 mm DC linearly variable differential transformer (LVDT, SE Labs). Data acquisition was accomplished using a computer equipped with a 12-bit analog to digital conversion board (Omega) and custom-written software.

Mechanical tests were non-destructive to allow measurement of geometric parameters of each bone specimen after testing (see below). With the specimen placed so that its endosteal surface faced the jig's outer two supports, a single load to a maximum of 1 kg was applied at a constant deflection rate of 2 mm/min. For these bone segments, a 1 kg load was well into the linear portion of the load-deflection curve. Once 1 kg load was attained, this deflection was then maintained, and the decay in load was recorded for 100 s. Load/deflection/time information was gathered by the computer during the entire loading and relaxation period.

After mechanical testing, each specimen was cut at the locations of the four loading points to produce four cross-sections (Fig. 1). Photographic prints (20.5 × magnification, Wild-Leitz M400 Photomacroscope) of each cross-section and corresponding scale markers were digitized using an Apple IIe computer and digitizing tablet (Kurta Series Two). A prescribed grid of 50 points was drawn on each photograph using lines parallel to the sagittal plane of each cross-section (Fig. 2). For digitizing, trabecular struts and porosities open to the endosteal surface were excluded. Errors inherent in estimating the soft/hard tissue boundary were minimized by sampling each specimen at four sites, and by having the same author do all microscopy, photography, and digitization. Observer bias was minimized by having the observer blinded to the treatment condition (arthritis or normal) and preliminary observations of mechanical testing; however, it was not possible to blind for the site (humerus or femur) from which the specimen was taken since the geometry of the two bones is different.

Average cross-sectional area and moment of inertia were calculated from the matrix of digitized points for each specimen by finite approximation. Each finite area element was either trapezoidal or triangular and its corresponding area was calculated directly and summed. The moment of inertia was calculated about the sagittal plane (the support plane during bending) under the assumption that the centroid height was identical with the neutral axis in bending: i.e. assuming that the material was isotropic and homogeneous. The moment of inertia and cross-sectional area for each test sample were calculated as the means of the values obtained from



(a) (b)

Figure 2 Using prints magnified 20.5 ×, area and moment of inertia data were obtained by digitizing points of intersection of grid lines (black arrowheads) and endosteal or periosteal bone surface (white arrows). Each figure shows two of the four sites: A, an outer load point, and B, an inner load point (Wild-Leitz photomacroscope, 15 ×). (a) Normal group specimen. Cross-section shows uniform surface. Trabecular struts (black asterisk) were excluded from analysis. Soft tissue is shown at white asterisk. (b) Arthritis group specimen. Shape is similar to that of the normal specimen, but cortex is thinner and exhibits porosity (black arrows). Porosities were included as part of area digitized unless they extended to either bone surface.

each of the four cross-sections. Since changes in moment of inertia could be due to either (i) changes in cross-sectional area, or (ii) changes in cross-sectional shape, we endeavored to separate these effects by calculating the radius of gyration $(\sqrt{\mathrm{I/A}})$ [12].

Once the geometric data for each sample had been calculated, the modulus of elasticity (E) for the material in each bending sample was calculated from the load/deflection/time information which was stored to disk during each experiment. Modulus was calculated from the load/deflection information during loading using the equation for a beam in simple four-point bending [13]:

$$E = \frac{a\Delta P(3L^2 - 4a^2)}{48\Delta DI} \qquad (1)$$

where E is the modulus, ΔP is change in applied load, L is the span length between the outermost supports of the test jig, a is the distance between the loading points, ΔD is the change in deflection, and I is the calculated moment of inertia. In the present jig, the nominal value for L was 21 mm and for a was 7 mm. Since this modulus is a property of the bone material only, we also calculated the flexural modulus for each material as $E \cdot I$. This parameter incorporated both material and geometric contributions to bending stiffness.

Stress relaxation was quantified as the per cent stress remaining at 100 s. Since the geometry of the sample remained nearly constant during the relaxation experiment, this parameter was merely calculated as:

$$\%\ \text{stress remaining} = 100 \times \frac{P(100)}{P(0)} \qquad (2)$$

where $P(100)$ is the load remaining at 100 s and $P(0)$ is the load at time zero (taken to be the load at maximum: approximately 1 kg).

Modulus, per cent stress remaining, flexural modulus, moment of inertia, cross-sectional area, and radius of gyration were examined statistically. A two-way analysis of variance with Fisher's least significant difference test for multiple comparisons was applied (StatView 4.0, Abacus Concepts). The two experimental variables used were: (i) site (humerus, femur), and (ii) treatment group (normal, arthritis). Additionally, in each of the four groups, we explored the notion that the flexural modulus $E \cdot I$ might be preserved in each group. Therefore, the relationship between the elastic modulus (E) and the inverse of the moment of inertia ($1/I$) was studied by regression analysis. For each type of specimen (humerus, femur), variation of geometry with sampling site (A, B, C or D) was also analyzed using a one-way analysis of variance. Lastly, since animals with arthritis experience weight loss, the relationship of per cent weight loss to the mechanical properties was studied by determining Pearson correlation coefficients for all major endpoints. All data are expressed as the mean $\pm$ the standard error of the mean (SE).

TABLE I Mechanical properties of compact bone near the joint affected by experimental inflammatory arthritis: machined specimens of rabbit femoral diaphysis

	Material properties		Geometric properties			Bending behavior
	Elastic modulus (GPa)	% stress remaining at 100 s	Moment of inertia (10^{-12} m^4)	Area (mm^2)	Radius of gyration (mm)	Flexural modulus (Pa-m^4)
Treatment						
Normal ($n = 13$)	1.66 (0.14)*	80.86 (0.97)	34.54 (2.88)	11.23 (0.45)	1.74 (0.05)	0.054 (0.003)
Arthritis ($n = 21$)	2.15 (0.16)	83.25 (0.71)	25.06 (1.80)	8.10 (0.31)	1.74 (0.04)	0.050 (0.002)
p-value	NS	< 0.05	< 0.05	< 0.05	NS	NS

*Mean (S.E.)

TABLE II Mechanical properties of compact bone remote from the joint affected by experimental inflammatory arthritis: machined specimens of rabbit humeral diaphysis

	Material properties		Geometric properties			Bending behavior
	Elastic modulus (GPa)	% stress remaining at 100 s	Moment of inertia (10^{-12} m^4)	Area (mm^2)	Radius of gyration (mm)	Flexural modulus (Pa-m^4)
Treatment						
Normal ($n = 14$)	5.00 (0.51)*	84.43 (2.23)	8.91 (0.93)	7.65 (0.24)	1.05 (0.05)	0.040 (0.002)
Arthritis ($n = 23$)	4.58 (0.28)	87.42 (0.47)	10.02 (0.82)	7.66 (0.19)	1.12 (0.03)	0.042 (0.001)
p-value	NS	NS	NS	NS	NS	NS

*Mean (S.E.)

3. Results

After loss of specimens during arthritis induction or processing, 13 normal femora, 14 normal humeri, 21 treated femora, and 23 treated humeri were available for analysis. The average length of the machined femoral specimens was 4.8 cm; the average length of machined humeral specimens was 3.9 cm.

As previously described [6, 8], the tibio-femoral joints of arthritis group animals were severely affected by EIA. Dissection of affected joints showed effusion, synovitis, gross cartilage destruction and femoral bone defects in some samples. At the articular ends of the humerus, these gross arthritic changes were not seen.

Results of bending tests for femoral cortical specimens from normal and arthritis specimens are shown in Table I and Fig. 3. In the femoral cortex, effects of arthritis were predominantly geometric. Cross-sectional area ($p < 0.05$) and moment of inertia ($p < 0.05$) were significantly reduced. Importantly, the radius of gyration ($\sqrt{I/A}$) was unchanged, indicating that the cross-sectional shape of the bone was not altered. The flexural and elastic moduli were similar in both groups but stress relaxation was diminished in arthritis, with $83.3 \pm 0.7\%$ stress remaining at 100 s compared to $80.9 \pm 1.0\%$ in the normal bone ($p < 0.05$).

Experimental arthritis caused no changes in measures of bone geometry or material properties at the remote site examined: the humerus (Table II).

The femora and humeri of the rabbits showed the expected anatomic differences. All geometric and mechanical parameters calculated varied significantly with site, regardless of treatment ($p < 0.0001$, Table III). The calculated elastic modulus for all humeral specimens was greater than twice that calculated for the equivalent femoral specimens ($p < 0.0001$). In stress relaxation, the humeral specimens were slightly more elastic, showing $86.34 \pm 0.87\%$ stress remaining at 100 s versus $82.34 \pm 0.60\%$ in the femora ($p < 0.0001$). The differing geometry of the two bones was reflected in

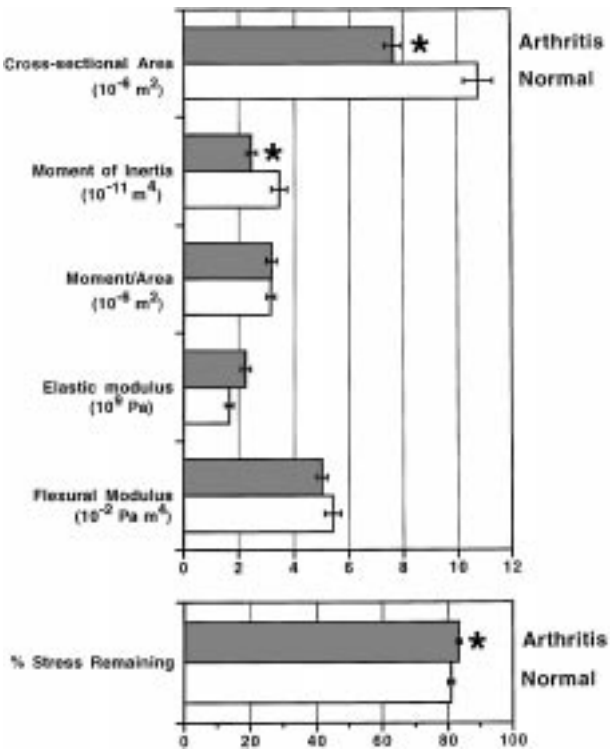


Figure 3 Calculated parameters for 4-point bending of normal and arthritic rabbit femora. Geometric (cross-sectional area, moment of inertia, radius of gyration), material (elastic modulus, % stress remaining), and composite (flexural modulus) properties are shown. Arthritis significantly reduced the cross-sectional area and moment of inertia of the femur, but left the radius of gyration (and hence, the shape of the bone) unchanged. The arthritic bone was also slightly less viscoelastic (% stress remaining increased). * = Difference between normal and arthritis groups significant with $p < 0.05$.

TABLE III Mechanical properties of machined compact bone specimens from two sites in the rabbit: humeral and femoral diaphysis

	Material Properties		Geometric Properties			Bending Behavior
	Elastic modulus (GPa)	% stress remaining at 100 s	Moment of inertia (10^{-12} m^4)	Area (mm^2)	Radius of gyration (mm)	Flexural modulus (Pa-m^4)
Site						
Humerus ($n = 37$)	4.74 (0.26)*	86.34 (0.87)	9.60 (0.62)	7.66 (0.15)	1.10 (0.03)	0.041 (0.001)
Femur ($n = 34$)	1.97 (0.12)	82.34 (0.60)	28.68 (1.73)	9.29 (0.37)	0.74 (0.03)	0.052 (0.002)
p-value	0.0001	0.0006	0.0001	0.0001	0.0001	0.0001

*Mean (S.E.)

lower values in the humerus for all measured geometric parameters–including the composite parameter, flexural modulus.

For the purpose of the calculation of the elastic modulus, it was assumed that the cross-sectional bone area was constant along the specimen test length. In fact, one-way analysis of variance showed there was slight variability in specimen cross-section at the four points of jig contact, in the humerus (A, 7.65 ± 0.19; B, 7.29 ± 0.16; C, 7.65 ± 0.16; D, 8.06 ± 0.18; $p < 0.02$), but not in the femur (A, 9.53 ± 0.48; B, 9.22 ± 0.41; C, 9.16 ± 0.32; D, 9.18 ± 0.27, N.S.). Regression analysis of plots of the calculated elastic modulus (E) versus the inverse of the moment of inertia ($1/I$) showed that the flexural modulus ($E \cdot I$) of the femora and the humeri from the rabbits examined was nearly constant (equal to the slopes of the regression lines in Fig. 4a and b). We note that arthritis had a negligible effect on the regressions obtained–an observation supported by the lack of significant change in mean flexural moduli shown in Tables I and II.

While normal rabbits averaged 13% weight gain during the course of the experiments, treated animals averaged 11% weight loss ($p < 0.0001$). There was only one substantial relationship between per cent weight loss and the mechanical properties as studied by Pearson correlation coefficients for the major parameters shown in Tables I and II. For the affected arthritic femur, the Pearson correlation coefficient for cross-sectional area was 0.69 ($p < 0.001$, in linear regression analysis). For all other parameters, the coefficient ranged from 0.06 to 0.41. For the humerus, the correlation describing per cent weight loss and cross-sectional area gave $r = 0.03$. For all other parameters, the coefficient ranged from 0.05 to 0.31.

3.1. Summary of results

In the femur, proximal to inflammatory arthritis of the tibio-femoral joint, the diaphyseal cortex had reduced cross-sectional area and moment of inertia, but no change in shape. There was no change in the modulus of elasticity of the bone material, but stress relaxation was slightly decreased. In the humerus, remote from the site of inflammatory arthritis, no change was found in any property measured. The geometry and mechanical properties of the femora and humeri differed significantly. Regardless of site, the flexural modulus was unaffected by treatment.

4. Discussion

This experiment produced an unexpected finding: two fundamental properties of bone, the flexural modulus and the elastic modulus, appear to be conserved despite the bone loss, porosity and rapid bone turnover associated with a pathological state: experimental inflammatory arthritis [6, 8–10, 14].

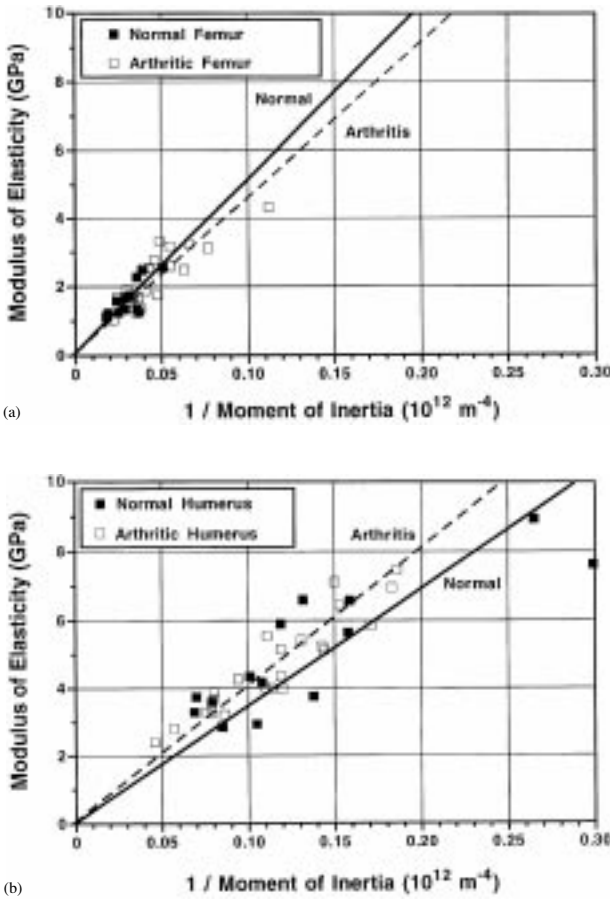


Figure 4 The flexural modulus ($E \cdot I$) for the rabbit femora and humeri were nearly constant within each experimental group. Least-squares linear regression plots of E versus $1/I$ are shown for each of the four experimental groups. Regression was forced through the origin (equation $E = m/I$; in each case, a better fit was obtained than if an intercept was permitted: equation $E = m/I + b$). The slope $m = E \cdot I$, the flexural modulus. (a) Regression for normal and arthritic femora. Normal: $m = 5.22 \pm 0.29 \times 10^{-2}$ Pa m^4, $r = 0.97$, $p < 0.0001$; arthritis: $m = 4.64 \pm 0.22 \times 10^{-2}$ Pa m^4, $r = 0.98$, $p < 0.0001$. There was no significant difference between the slopes for the normal and arthritic samples at the $p < 0.05$ level; however, the decrease with arthritis approached significance ($p < 0.10$). (b) Regression for normal and arthritic humerus. Normal: $m = 3.44 \pm 0.23 \times 10^{-2}$ Pa m^4, $r = 0.97, p < 0.0001$; arthritis: $4.00 \pm 0.10 \times 10^{-2}$ Pa m^4, $r = 0.99$, $p < 0.0001$. The slope for the arthritis group is increased slightly ($p < 0.05$, t-test).

Gross porosity, cortical thinning and reduced cross-sectional area occurred without grossly altering bone shape, as evidenced by the similarity in the radii of gyration for the two groups. Reduced area – rather than alteration of shape – is responsible for the lower moment of inertia in arthritic specimens.

Although normal and arthritis treatment groups differed in geometric properties, reduced stress relaxation was the only type of material behavior induced by EIA. This may have resulted from changes in the collagen content, hydration, or arrangement [15–17], or from other effects of arthritis on bone matrix [18].

Two other types of material behavior, the elastic modulus and the flexural modulus, were not affected by EIA. The elastic modulus for various types of bone is approximately proportional to the cube power of density, calcium content, and volume fraction of bone material (equivalent to 1 – porosity) [19–22]. While mineralization and porosity were not formally evaluated in this study, nor accounted for in calculation of the geometric parameters, our previous work demonstrated that hypomineralization of bone occurs in this model [23], and increased porosity is grossly evident in most arthritis group specimens [9, 10]. We had therefore expected an effect of hypomineralization or porosity on elastic behavior. Reconsidering the hypomineralization data, we find that the calculated ash weight is less than 63% [23]: a level below which the direct relationship between modulus and ash content may revert from linear to asymptotic. Therefore, even substantial mineralization change might result in a modulus change which was not measurable in these experiments [24, 25]. We have shown that porosity of the metaphysis is substantially increased in arthritis, compared to normal specimens [14]. In the diaphysis, the values are smaller, 8% vs 5% respectively [9], and possibly insufficient to permit our documenting a corresponding change in elastic modulus. Animal to animal scatter may have also limited our ability to detect changes in elastic modulus (see for example, Fig. 4a and b).

Bending behavior (flexural modulus) was also conserved: both from animal to animal within a treatment group and between treatment groups. Data for regression of the elastic modulus against inverse moment of inertia (Fig. 4) showed a strong relationship which was not altered by arthritis. Within groups it appears that, as bones enlarge in diameter, the modulus achieved is reduced such that the product $E \cdot I$ is maintained. Bone cross-sectional shape is also conserved in the femur or humerus. These constraints, which likely reflect functional demands, also appear to bear during the remodeling associated with experimental arthritis. In normal growing rats, a similar correlation of elastic modulus with moment of inertia has been reported [26]. The concepts of conservation of the flexural modulus and of the interaction of material and structural properties of bone through altered bone cell activity, are coherent with the bone ''mechanostat'', a homeostatic feedback mechanism proposed by Frost (as cited in Turner [27]) and developed further by Ferretti *et al*. [26].

Comparing the humerus and femur, the differences observed are typical of the wide variation of mechanical properties found across species and sites [22, 25, 28]. From bone to bone within the same species, differences in geometric properties are grossly apparent and often appear as specimen stiffness differences in whole-bone testing [29]; however, bone to bone comparisons of purely material properties using the same test methodology have infrequently been reported. Currey [25] cites different values of E in the deer femur vs antler. As well, Burstein *et al*. [16] found that human tibial and femoral cortices differ in longitudinal modulus.

The possibility of systemic or distant effects of experimental monoarticular arthritis upon other bones has been considered [30]. There was a significant increase in bone formation in the opposite untreated femur which could not be simply attributed to a shift in loading from one limb to the other, because *total* bone volume fraction in the same specimens was not also increased. These data are consistent with the hypothesis that an inflammatory effect exists but is diminished remote from the site of inflammation. If such an effect existed in the current study, its influence on mechanical or geometric properties was not detectable in the humerus.

Differences in body weight between the control and treatment groups should also have exerted effects at both bone sites; again, no effect was seen in the humeri. Generally, the Pearson correlation coefficients relating the major endpoints to per cent weight loss, were so low as to suggest only a weak influence of weight on the measured properties. The exception to this was the cross-sectional area of the femoral diaphysis, which appeared to be significantly related to weight loss ($r = 0.69$; $p < 0.001$). However, this relationship was not shown for the humerus, again suggesting that weight change was not an important factor determining mechanical properties in this study.

Specimen size and shape, strain rate, mode of loading and method of support can all affect mechanical data [31]. We adopted the method of non-destructive, bending testing of diaphyseal half-cylinders for several reasons. First, arthritic rabbit femora were considered to be too small, fragile and structurally inhomogeneous to allow machining of a uniform beam for classical materials testing. Second, bending tests of whole bones were impractical because of their irregular shapes, with risk of specimen misalignment, imprecise strain measurement and inadequate length to cross-section ratio of specimens [31, 32]. We therefore chose to lightly machine specimens. A sagittal cut was used, dividing the samples into equal medial and lateral specimens. These relatively uniform semi-cylinders, slightly curved in the plane of the supports, could be treated consistently in the materials testing machine. Third, we opted for non-destructive testing to allow for digitization of individual specimen cross-sections and accurate determination of moments of inertia and elastic moduli. Subsequent calculations used standard engineering formulae for pure bending – including assumptions of isotropy and homogeneity.

A few other details deserve comment. First, during testing, the bending lengths (the constants L and a in Equation 1) were fixed. Second, strain rate was not controllable since it depended on specimen geometry; however, the deflection rate was held constant. Third,

uniform geometry along the length of specimens taken from the femur (although not the humerus) was demonstrated by one way ANOVA comparing cross-sections at sites A, B, C and D. The mechanical parameters calculated were obtained using mean values for cross-sectional geometry and thus blur the effects of the changing humeral geometry. We note that no assumption of cylindrical geometry was used here. Rather, finite calculations from digitized images were used to establish geometric parameters.

Fourth, the bending test jig included rolling supports but did not restrain splaying of the sides of the half-cylinders which may have occurred during loading. Indeed, restraint was impractical since only the ends of the sample could have been constrained and splaying in the center remained possible. In our analysis, all deformation was therefore taken to have occurred in bending along the long axis of the sample. In a few extra samples loaded to failure, there was no indication of outright splaying or flattening of the specimens; nonetheless, it is likely that some contribution to deformation occurred in this mode and a reduction in the observed elastic modulus values is expected. This may explain some of the modulus differences observed between femora and humeri, where the varying anatomy of the two bones could be translated into differences in apparent modulus. Lastly, local deformation around the sites of contact of the loaders can result in a measured displacement that is greater than the true flexural displacement of the entire sample [33]. It is very unlikely, however, that these effects would invalidate the comparisons between groups presented here.

The above technical factors offer plausible explanations for the obtained values of 1.6 to 5 GPa for the elastic modulus of rabbit femoral bone in bending: values which are low compared to other reports of modulus for cortical bone. In the rabbit bone, other studies have reported the stiffness of whole bone specimens in bending or torsion [29, 34] without explicit calculation of modulus. Of three available reports citing moduli, one gives the elastic modulus of rabbit cortical bone in antero-posterior bending as 14.6 and 11.6 GPa for the femur and humerus respectively [28, 35]. The methodology used by Shono [35] is not given; however, it probably differed from ours in both the direction of bending (ours was medio-lateral) as well in the use of lightly machined specimens. A second report, gives a purely tensile elastic modulus of 15–27 GPa for pieces of compact bone from rabbit tibia [36]. While the latter two studies yielded higher modulus values, the present modulus data (in bending) are similar to the shear moduli obtained in torsion of intact rabbit femora: 3.9 and 4.4 GPa for normal and arthritic groups respectively [11]. While methodological issues are likely important, the lower values of E reported here may be due to an actual difference in mineralization in this species. Currey [22] ascribes his own findings of an inter-species range of E from 4 to 33 GPa to altered bone microstructure in response to functional differences. Indeed an elastic modulus of ~ 6 GPa has been reported for 3-point bending of machined deer antler cortical specimens [25], and for 4-point bending of swine femoral cortex [37].

In contrast to the conservation of elastic behavior and of bending behavior found in this non-destructive testing, we have shown elsewhere that fracture behavior is altered in EIA; both fracture strength and fracture toughness (energy per unit volume) are lower in treated femora [11]. Other work shows that the number and area of intra-cortical defects are increased in arthritis [9, 10]. Since fracture toughness normalizes for the low cross-sectional area of treated specimens, the current results together with the work of Bellingham *et al*. [11] and of Pysklywec *et al*. [9, 14] suggest that other structural factors, such as porosity, are prime factors contributing to the decreased fracture toughness of bone in experimental inflammatory arthritis and increased fracture risk in human inflammatory arthritis [2, 14, 38].

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