Age-dependent mechanical properties of rat femur

Measured in vivo and in vitro

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Strain gauges were implanted on the anterior surface of the femoral diaphysis of rats aged 6, 12 or 52 weeks. Strain was recorded while the rats were running on a treadmill. The peak strain was of the same magnitude at different animal ages, although there was a somewhat lower value for 52-week-old animals. Stiffness calculated from in vivo strain measurements and from a 3-point bending test on excised femora was correlated: stiffness increased with age. Maximum bending stress increased from 6–12 weeks of age, but then there was no further increase. Ultimate load, on the contrary, increased steadily over the entire 52-week period. This indicates that higher load-bearing capacity with increased age in adult rats is due to increased dimensions of the bone rather than its material properties. The present study demonstrates that conventional in vitro measurements of mechanical properties of bone correspond to measurements of strain during physical activity, and that both are valid measurements of physical properties of bone.

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External forces exert changes on the mechanical properties of the skeleton during growth. Knowledge about the structural and material qualities of bone during normal activity at different ages is therefore needed to better understand the mechanical demands in clinical situations. Methods for measuring the surface deformation of bones in vivo in large animals with implanted strain gauges have been described in several reports (Evans 1953, Lanyon and Smith 1969, Cochran 1972, Rybricki and Mills 1977, Carter 1980), and a few studies in small animals have also been presented (Keller and Spengler 1982, 1989, Biewener 1986, Husby et al. 1988). Changes in mechanical properties of rat femora during growth have been reported after in vitro mechanical testing (Vogel 1979, Ekeland 1982, Keller et al. 1986), and recently also by in vivo measurements (Keller and Spengler 1989).

The present investigation was planned to further explore the correlation and relevance of in vivo and in vitro mechanical variables, to study the changes in mechanical qualities that occur during growth and aging, and to determine the correlation of such changes to evolving mechanical demands with age.

Material and methods

A total of 24 male Wistar rats (Mol:WIST, Møllegaard Breeding Center, Ejby, Denmark) were used. There were three age groups with 8 rats each: 6, 12, and 52 weeks old with median body weights respectively of 199 (178–216), 324 (302–334), and 495 (455–530) g. The animals were given a standard maintenance rat diet (RM1 expanded, Special Diets Services, Witham, England) and water ad libitum.

Strain measurement in vivo

A single-element strain gauge (0.6/120 LY 11, Hottinger Baldwin Messtechnik, Darmstadt, Germany) was implanted on the anterior surface of the femoral diaphysis of each rat. Construction of the strain-gauge units, implantation, and recording of strain have been described earlier in detail (Husby et al. 1988). The animals were operated on under anesthesia with a mixture of fentanyl/fluanisone and midazolam. Postoperative analgesia was managed with buprenorphine-hydrochloride injected subcutaneously.

Strain in the axial direction was recorded from the second postoperative day and for 6 days while

Table 1. Body weight, femur length, and area moment of inertia for the different age groups. Median and 0.25–0.75 fractiles

Age (weeks)	6	12	52
Body weight (g)	199 (178–216)	324 (302–334)	495 (455–530)
Femur length (mm)	30 (29.7–30.5)	35 (34.5–35.2)	40 (39.2–40.4)
Area moment of inertia (mm^4)	4.3 (4.0–4.7)	5.5 (5.0–6.5)	11.9 (10.5–12.4)

All the variables increased during growth ($P < 0.001$).

Table 2. Peak strain (maximum deformation in vivo, µstrain) during running on the seventh postoperative day. Median and 0.25–0.75 fractiles

Age (weeks)	6	12	52
Peak strain	270 (181–347)	329 (219–392)	230 (183–256)

Strain was reduced for older animals ($P < 0.05$).

the rats were running on a treadmill at a speed of 10 m/min. Peak values of strain (maximum deformation) of 30 walking cycles were measured, and the mean value was calculated. Stride frequency was measured as well. The rats were killed on the last day of strain recording. In vivo stiffness was estimated from the body weight/strain ratio, because actual load on each femur during running was unknown.

Geometry

Excised femora were mechanically cleansed of soft tissue, and the femur length was measured with a sliding caliper. The cross-sectional area at the location of the strain gauge was estimated from measurements of the outer and inner anteroposterior and mediolateral diameters (Engesæter et al. 1978). The area moment of inertia (I) was calculated using the equation applicable for a hollow ellipse (Roark and Young 1976):

$$(1)\ I = \pi/4\ (R_0 r_0^3 - R_i r_i^3)$$

where R_0 and R_i are the outer and the inner mediolateral radius, and r_0 and r_i the outer and the inner anteroposterior radius.

Bending tests

The excised femora were kept moist, and 3-point bending tests were performed within 3 hours with an Instron® 1193 machine (Husby et al. 1988). Each femur was deflected at a speed of 10 mm/min until fracture occurred. Stiffness was calculated from the linear portion of the load-deflection curves. Ultimate load was recorded. Maximum bending stress (σ) was calculated from the load data using the equation for an elastic, isotropic, homogeneous beam with a hollow elliptical cross-section (Roark and Young 1976):

$$(2)\ \sigma = M \times r_0 / I$$

where M is the bending moment defined for this test as 1/4 (load × length) where load is ultimate load and length is the distance between the supporting bars of the jig (14 mm); r_0 is the distance from the neutral axis (1/2 external anteroposterior diameter of the femoral diaphysis); and I is the area moment of inertia (1). Maximum bending stress was taken as the strength of the bone substance.

Statistical analysis

Median values with 0.25 and 0.75 fractiles were used to express the average and the variance of the measurements. The Kruskal-Wallis test was used to test differences in medians between groups (Minitab, Ryan et al. 1985), and was considered significant when $P < 0.05$. The Kendall rank correlation procedure was applied to test for correlation between two variables (SPSS, 1976).

Table 3. Stiffness expressed as the body weight/in vivo strain ratio (N/μstrain × 0.001) and the conventional load/deflection ratio (N/mm). Median and 0.25–0.75 fractiles

Age (weeks)	6	12	52
Body weight/in vivo strain ratio	6 (5–7)	9 (7–11)	21 (17–24)
Load/deflection ratio	202 (183–206)	434 (357–505)	715 (600–803)

There was increment in stiffness with age when estimated both ways ($P < 0.005$).

Table 4. Maximum bending stress (MPa) and ultimate load (N) for the different age groups. Median and 0.25–0.75 fractiles

Age (weeks)	6	12	52
Maximum bending stress	121 (104–130)	199 (165–232)	220 (188–248)
Ultimate load	95 (86–103)	195 (184–224)	389 (337–425)

Results

Body weight, femur length, and area moment of inertia increased from 6 to 12 weeks by factors of 1.6, 1.2, and 1.3, respectively. During the following period until 52 weeks, the figures were 1.5 for body weight, 1.1 for femur length, whereas area moment of inertia increased by a factor of 2.2 (Table 1).

On the seventh postoperative day, 2 out of 24 strain gauges were excluded because of electrical malfunction. Peak strain was of the same magnitude for all the age groups, although reduced for older animals ($P < 0.05$; Table 2). The median stride frequency was 1.7 (1.5–1.8) stride/s, and there were no significant differences between the age groups, but there was a tendency towards a higher stride frequency for younger animals.

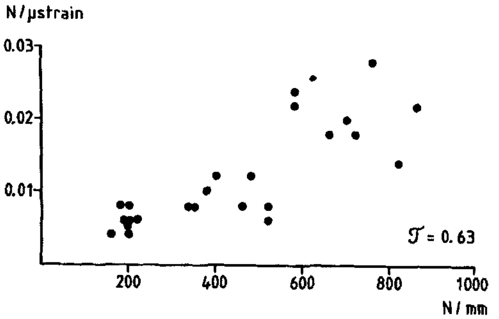

Figure 1. Stiffness calculated as body weight/in vivo strain (N/μstrain) and load/deflection (N/mm) plotted. Kendall's tau = 0.63 ($P < 0.001$).

In vivo stiffness expressed as body weight/in vivo strain ratio was correlated with the more conventional load/deflection ratio (Kendall's tau = 0.63, $P < 0.001$; Figure 1). Stiffness increased with age ($P < 0.005$; Table 3).

Maximum bending stress showed an increase from 6 to 12 weeks of age ($P < 0.001$), whereas the difference from 12 to 52 weeks was insignificant. Ultimate load, on the other hand, increased steadily from 6 to 52 weeks of age ($P < 0.005$; Table 4).

Discussion

The present study demonstrates a correlation between the body weight/in vivo strain and in vitro load/deflection measurements of stiffness. Peak strain during normal activity was lower in the femora of older animals, whereas stiffness, ultimate load, and maximum bending stress were higher.

The validity of our in vivo recordings was confirmed by correlation between body weight/strain and load/deflection used for stiffness calculations. Previous studies have shown that strain-gauge recordings correspond to recordings from replacement gauges and extensometer readings within 2–5 percent (Husby et al. 1988). It should be emphasized, however, that body weight/strain measurements refer to stiffness at the gauge implantation site only.

A single-element gauge recording of strain in the axial direction was used in the present study. The gauge was designed to accept a bending radius down to 0.3 mm, which is well below the smallest radius of the bones. Strain distribution remains unknown; such information requires the implantation of rosette gauges around the diaphyseal circumference. This might cause extensive trauma in the small rat femur (Husby et al. 1988), and the calculation of strain is complex because the curvature of the bone must be taken into account. Another consideration is that the strain-gauge recordings are not corrected for transverse sensitivity or Poisson's ratio. This is, however, considered a systematic error that does not preclude comparison between the different age groups.

Strain during running exercise in the present study remained remarkably stable at different animal ages. This observation is in agreement with Wolff's law, and implies that the various mechanical characteristics of bone are well balanced during growth and optimized on the basis of the functional demands. Reports from a variety of species also suggest that strain is nearly independent of age (Lanyon et al. 1979, Biewener et al. 1986, Keller and Spengler 1989). That the strain value was somewhat reduced in older animals may be due to a lower stride frequency, as suggested by Keller and Spengler (1989); but it may also be taken into account for the existence of a minimum effective strain as a determinant of bone architecture (Frost 1983). Higher strain in younger animals cause modeling of the bone to meet the increasing loads during growth. During maturation, the bones are not fully adapted, only nearly adapted.

Our maximum strain result for the anterior mid-diaphyseal rat femur came close to that of Keller and Spengler (1989). Biewener et al. (1986), recording strain from the tibiotarsus of exercised chicks, reported about a 2–4 fold higher strain values for the proximal and distal midshaft. Higher strain values in their paper probably reflect species differences, gauge location, gauge type, or running speed.

Increased bone stiffness with age was to be expected when testing the bone as a composite structure (Torzilli et al. 1981, 1982, Ekeland et al. 1982, Jonsson et al. 1984, Keller et al. 1986). Increased stiffness was also demonstrated when calculated from strain-gauge recordings. Structural stiffness depends on both geometry and material properties. Increased area moment of inertia clearly shows that bone dimensions are important in this respect. The approximations used for calculations of area moment of inertia is a well-tested model to study mechanical properties of rat femora (Engesæter et al. 1978, Ekeland et al. 1981), although more accurate estimates may be obtained from complex mathematical models and computer software (Nagurka and Hayes 1980).

An interesting finding in the present study was that ultimate load continued to increase from 12 to 52 weeks of age, whereas bone strength (maximum bending stress) did not reveal any significant change during this period. A constant level for bone strength has been reported at about 4 months of age in rats (Vogel 1979, Ekeland et al. 1981, Keller et al. 1986). Thus, the increase in load bearing capacity (e.g., the strength of bone as a structure) with age in adults rats must be a result of larger cross-sectional dimensions. Consequently, this indicates that young bones rely upon elastic deformation to avoid fractures, whereas old bones are more dependent upon size and shape of the bone. This interpretation accords with tests of human femora, where a lower stiffness, lower bending strength, and greater deflection before fracture have been reported for children when compared with adults (Vinz 1970, Currey and Butler 1975).

In conclusion, peak strain values were of the same magnitude at all ages, although somewhat lower in older animals. There was a correlation between in vivo and in vitro recordings of mechanical properties. Although the in vivo recordings primarily refer to the measuring point, the correlation with in vitro measurements indicates a general validity of the results.

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References

Biewener A A, Swartz S M, Bertram J E. Bone modeling during growth: dynamic strain equilibrium in the chick tibiotarsus. *Calcif Tissue Int* 1986; 39 (6): 390–5.

Carter D R, Smith D J, Spengler D M, Daly C H, Frankel V H. Measurement and analysis of in vivo bone strains on the canine radius and ulna. *J Biomech* 1980; 13 (1): 27–38.

Cochran G V. Implantation of strain gages on bone in vivo. *J Biomech* 1972; 5 (1): 119–23.

Currey J D, Butler G. The mechanical properties of bone tissue in children. *J Bone Joint Surg* (Am) 1975; 57 (6): 810–4.

Ekeland A, Engesæter L B, Langeland N. Mechanical properties of fractured and intact rat femora evaluated by bending, torsional and tensile tests. *Acta Orthop Scand* 1981; 52 (6): 605–13.

Ekeland A, Engesæter L B, Langeland N. Influence of age on mechanical properties of healing fractures and intact bones in rats. *Acta Orthop Scand* 1982; 53 (4): 527–34.

Engesæter L B, Ekeland A, Langeland N. Methods for testing the mechanical properties of the rat femur. *Acta Orthop Scand* 1978; 49 (6): 512–8.

Evans F G. Methods for studying the biomechanical significance of bone form. *Am J Phys Anthrop* 1953; 11: 413–35.

Frost H M. A determinant of bone architecture. The minimum effective strain. *Clin Orthop* 1983; 175: 286–92.

Husby O S, Indrekvam K, Gjerdet N R, Engesæter L B, Langeland N. In vivo strain measurements in rat femora. *Scand J Lab Anim Sci* 1988; 15: 159–70.

Jonsson U, Netz P, Strømberg L. Solid mechanics and strength of bone in young dogs. *Acta Orthop Scand* 1984; 55 (4): 446–51.

Keller T S, Spengler D M. In vivo strain gage implantation in rats. *J Biomech* 1982; 15 (12): 911–7.

Keller T S, Spengler D M, Carter D R. Geometric, elastic, and structural properties of maturing rat femora. *J Orthop Res* 1986; 4 (1): 57–67.

Keller T S, Spengler D M. Regulation of bone stress and strain in the immature and mature rat femur. *J Biomech* 1989; 22 (11–12): 1115–27.

Lanyon L E, Magee P T, Baggott D G. The relationship of functional stress and strain to the processes of bone remodelling. An experimental study on the sheep radius. *J Biomech* 1979; 12 (8): 593–600.

Lanyon L E, Smith R N. Measurements of bone strain in the walking animal. *Res Vet Sci* 1969; 10 (1): 93–4.

Nagurka M L, Hayes W C. An interactive graphics package for calculating cross sectional properties of complex shapes. *J Biomech* 1980; 13 (1): 59–64.

Roark R J, Young W C. Properties of plane area. Beams; flexure of straight bars. In: *Formulas for stress and strain* (Eds. Roark R.J, Young W.C). 5th ed. McGraw Hill, Tokyo 1976: 61–69: 89–112.

Ryan B F, Joiner B L, Ryan T A Jr. Correlation and regression. Nonparametric statistics. In: *Minitab handbook* (Eds. Ryan B.F, Joiner B.L, Ryan T.A.Jr). 2nd ed. Duxbury Press, Boston 1985: 218–59: 276–300.

Rybicki E F, Mills E J. In vivo and analytical studies of forces and moments in equine long bones. *J Biomech* 1977; 10 (11/12): 701–5.

SPSS Inc. *Nonpar corr. SPSS user's guide.* 2nd ed. McGraw Hill, New York 1976: 801–8.

Torzilli P A, Takebe K, Burstein A H, Heiple K G. Structural properties of immature canine bone. *J Biomech Eng* 1981; 103 (4): 232–8.

Torzilli P A, Takebe K, Burstein A H, Zika J M, Heiple K G. The material properties of immature bone. *J Biomech Eng* 1982; 104 (1): 12–20.

Vinz H. Die Änderung der Festigkeitseigenschaften des kompakten Knochengewebes im Laufe der Altersentwicklung. *Gegenbaurs Morphol Jahrb* 1970; 115 (2): 257–72.

Vogel H G. Influence of maturation and aging on mechanical and biochemical parameters of rat bone. *Gerontology* 1979; 25 (1): 16–23.