Acta orthop. scand. *53*, 527–534, 1982

INFLUENCE OF AGE ON MECHANICAL PROPERTIES OF HEALING FRACTURES AND INTACT BONES IN RATS

ARNE EKELAND*, LARS B. ENGESÆTER*+ & NORVALD LANGELAND+

Institute for Surgical Research*, Rikshospitalet and Sophies Minde Orthopaedic Hospital+, University of Oslo, Norway

Mechanical properties of fractured and intact femora have been studied in young and adult, male rats. A standardized, closed, mid-diaphyseal fracture was produced in the left femur, the right femur serving as control. The fracture was left to heal without immobilization. At various intervals, both fractured and intact femora were loaded in torsion until failure.

The fractured femora regained the mechanical properties of the contralateral, intact bones after about 4 weeks in young and after about 12 weeks in adult rats. For intact bones, both the ultimate torsional moment (strength) and the torsional stiffness increased with age of the animals, whereas the ultimate torsional angle remained unchanged. For bone as a material, however, the ultimate torsional stress (strength) and the modulus of rigidity (stiffness) increased with age only in young rats, being almost constant in the adult animals.

The various biomechanical parameters of the healing fractures did not reach those of the contralateral, intact bones simultaneously. The torsional moment required to twist a healing femoral fracture 20 degrees (0.35 radians), a deformation close to what an intact femur can resist, proved to be a functional and simple measure of the degree of fracture repair in rats.

Key words: biomechanics; bone development; callus; femur, growth; modulus of rigidity; strain; stress, mechanical; torsional test

Accepted 28.x.81

Many experimental studies on bones are performed in growing animals. The properties of the bones may, however, be variously influenced by changes in animal age during the experimental period. This stresses the importance of a basic knowledge about age influences on bone physiology when studies on bones are planned.

Important properties of fractured and intact bones may be evaluated quantitatively by mechanical tests (Currey 1970, Reilly & Burstein 1974, Panjabi et al. 1977). During these tests, healing fractures and intact bones are strained, much like the conditions *in vivo*. This makes mechanical tests more functional than many other experimental methods, and quite relevant in orthopaedic research. In a previous biomechanical study, we found that the strength of intact rat femora increased with age in growing rats (Ekeland et al. 1981). Age may also influence fracture repair, and it is a well-known clinical experience that fractures heal faster in children than they do in adults (Moberg 1965). Similar findings have been reported from experimental studies in dogs (Robinson 1971, Editorial JAMA 1971).

The purpose of the present study was to investigate the influence of age on fracture repair in rats, and to extend our studies of age influences on mechanical properties of intact rat bones. Fractured and intact bones were both loaded in

0001-6470/82/040527-08 $02.50/0

torsion. This test method is probably the most functional of the common mechanical bone tests, since it evaluates the mechanical properties of the entire diaphyseal bone, indicating the weakest section (Burstein & Frankel 1971, Ekeland et al. 1981).

MATERIALS AND METHODS

Experimental animals

Fifty-eight young and 38 adult outbred, male Wistar/Af/Han/Mol SPF rats were used. The young rats were initially about 23 days old, and their median weight was 53 grams. The adult rats were initially about 100 days old, and had a median weight of 341 grams. Four to five rats were kept in each cage. They were given standard animal pellets containing 0.9 per cent calcium and 0.7 per cent phosphorus (Bl. nr. 3155, Möllesentralen i/s, Oslo, Norway) and water *ad libitum.*

Experimental fracture and mechanical testing

With the rats in ether anaesthesia, a standardized, closed, transverse fracture was made in the mid-shaft of all the left femora, using a special fracturing forceps (Ekeland et al. 1981). The first two rats from each group were killed immediately after the fracture. The fracture location was examined, and the forceps adjusted accordingly for each group to give fractures in the middle of the bone (Ekeland et al. 1981). The rats walked on the unimmobilized, fractured limb within a few days.

At various intervals (1–22 weeks) after the fracture, groups of 6–10 animals were weighed and killed with ether (Table 1). Both femora were immediately dissected free and stored in cooled, isotonic, Ringer's solution. Within 2 hours, both fractured and intact femora were tested in torsion until failure, as previously described (deformation rate 2.5 degrees per second, i.e. 0.04 radians per second) (Engesæter et al. 1978, Ekeland et al. 1981).

Biomechanical calculations

The *ultimate torsional moment* (T_u) (maximal torsional moment) and the corresponding *ultimate torsional angle* (θ_{T_u}) (ultimate torsional deformation) were obtained from the load-deformation curves (Ekeland et al. 1981). For healing femoral fractures, the torsional moment which was necessary to twist the bones 20 degrees (0.35 radians) was also read from the curves, as intact bones fail near to this torsional deformation (Ekeland et al. 1981). The *torsional stiffness* (K) was measured from the slope of the linear portion of the curves ($K = dT/d\theta$) (Figure 1) (Panjabi et al. 1973, White et al. 1974, Netz et al. 1978, Paavolainen 1978).

Table 1. Number of rats in the experimental groups subjected to torsional tests

Animals	Weeks after fracture 1	2	3	4	6	8	9	12	22	Total number
Young rats	10	10	10	8	6	6		6		56
Adult rats	6		6		6		6	6	6	36

A sliding caliper (accuracy of ±0.01 mm) was applied to measure the dimensions of the intact femora. Before the mechanical test, the femoral length was measured from the top of the caput to the distal end of the medial condyle. The major (2a) and minor (2b) diameters were measured in the middle of the bone. Immediately after the test, the thickness (t) of the ventral cortex was measured in the middle of the femur.

The ultimate torsional stress (τ_u) (maximal torsional stress) was then calculated for intact femora according to the formula (Roark & Young 1975, Engesæter et al. 1978):

$$\tau_u = \frac{T_u \times b}{I_p} = \frac{4 \times T_u \times b}{\pi\,(ab^3 + a^3b - (a-t)(b-t)^3 - (a-t)^3(b-t))} \quad (1)$$

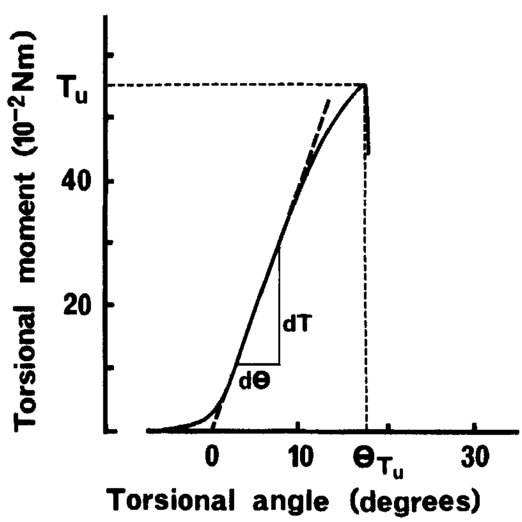

Figure 1. Load-deformation curve for an intact femur of an adult rat. The bone was tested in torsion 9 weeks after the start of the experiment. The ultimate torsional moment (T_u) *and the ultimate torsional angle* (θ_{T_u}) *are indicated. The torsional stiffness (K) is determined from the slope of the linear portion of the curve* ($K = dT/d\theta$).

where I_p is the polar moment of inertia, and the bone is considered as an elastic, isotropic, homogenous beam with a hollow elliptical cross section.

The *modulus of rigidity* (G), considering bone as an elastic, isotrope, homogenous material, is given by the formula (Frankel & Burstein 1970):

$$G = \frac{\tau}{\gamma} \qquad (2)$$

where τ is the stress for a given shear strain (γ) of the bone material. Strain depends on bone geometry, and to convert the torsional angle (θ) which occurs during torsional loading of a bone to strain, one should multiply by a factor equal to the average bone radius (approximately $\frac{a+b}{2}$) divided by the tested bone length (L) (Panjabi et al. 1973). Accordingly, strain is expressed by the equation:

$$\gamma = \frac{\theta\,(a+b)}{2 \times L} \qquad (3)$$

Within the elastic portion of the load-deformation curves, we have K = T/θ, and therefore θ = T/K. When this expression for θ is substituted in equation (3), a formula for the modulus of rigidity can be derived from the equations (2), (1) and (3):

$$G = \frac{\tau}{\gamma} = \frac{2 \times K \times b \times L}{I_p(a+b)} =$$

$$\frac{8 \times K \times b \times L}{\pi(a+b)\,(ab^3+a^3b-(a-t)\,(b-t)^3-(a-t)^3\,(b-t))} \qquad (4)$$

The stiffness of the bone material of intact femora was calculated according to equation (4).

Statistical analysis

Median with 0.25- and 0.75-fractiles were used to express the average and the dispersion of the measured values. The statistical significance probability was calculated by Wilcoxon's two-tailed test for pair differences (Diem & Lentner 1975). Differences were considered significant when $2P \leqq 0.05$.

RESULTS

Growth of the animals

The young rats had a seven-fold increase in body weight during the experimental period of 12 weeks, whereas the body weight of adult rats only increased about 40 per cent in this period (Figure 2A). The length of the intact femora increased by about 60 per cent in young, and by about 10 per cent in adult rats from the first to the twelfth week after the fracture (Figure 2B).

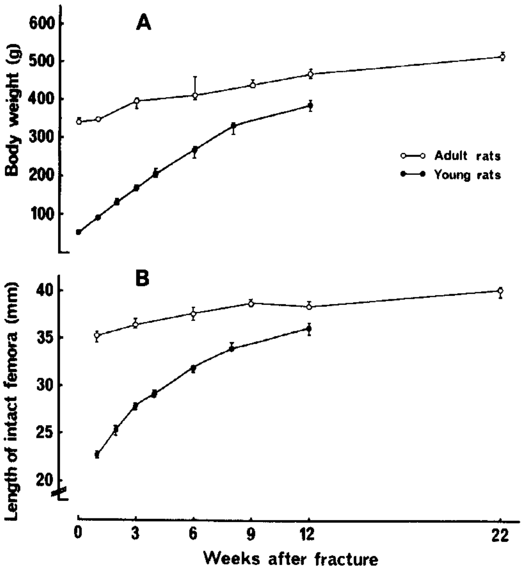

Figure 2. Body weight (A) and length of intact femora (B) in young and adult rats as a function of time following fracture of the contralateral bone. Six to ten animals in each group. (Median with 0.25- and 0.75-fractiles).

Mechanical properties of the femora

Young rats. Already 2 weeks after the start of the experiment, the ultimate torsional moment of the healing femoral fractures matched that of the contralateral, intact bones (Figure 3A). At this time none of the fractures were consolidated as evaluated manually, and the torsional stiffness and the ultimate torsional angle of the fractures did not reach the respective values of the contralateral bones until 4–6 weeks after the fracture (Figure 3B and C). The healing fractures were even transiently stiffer than the contralateral bones at 6 weeks after the fracture (Figure 3B).

The torsional moment which was necessary to twist the healing fractures 20 degrees (0.35 radians), merged in with the ultimate torsional moment for the contralateral, intact bones after 4 weeks (Figure 3A). At this time all the fractures were consolidated. The displacement of the healing fractures was small.

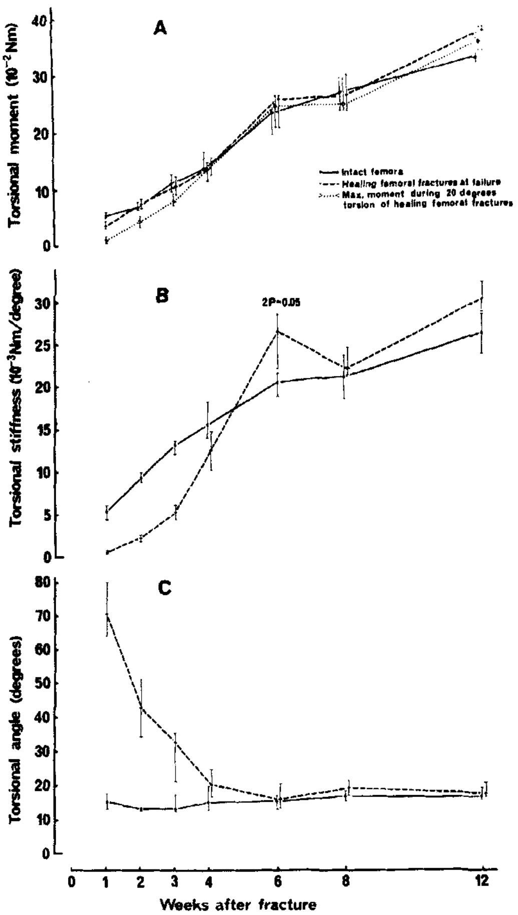

Figure 3. Young rats. *Age at fracture: 23 days. Ultimate torsional moment (A), torsional stiffness (B) and ultimate torsional angle (C) for fractured and intact femora as a function of time following the fracture. The torsional moment required to twist the healing fractures 20 degrees (0.35 radians) is also given (A). Six to ten animals in each group. Significance levels are only indicated when the biomechanical parameters of healing fractures significantly* exceed *those of intact bones. (Median with 0.25- and 0.75-fractiles).*

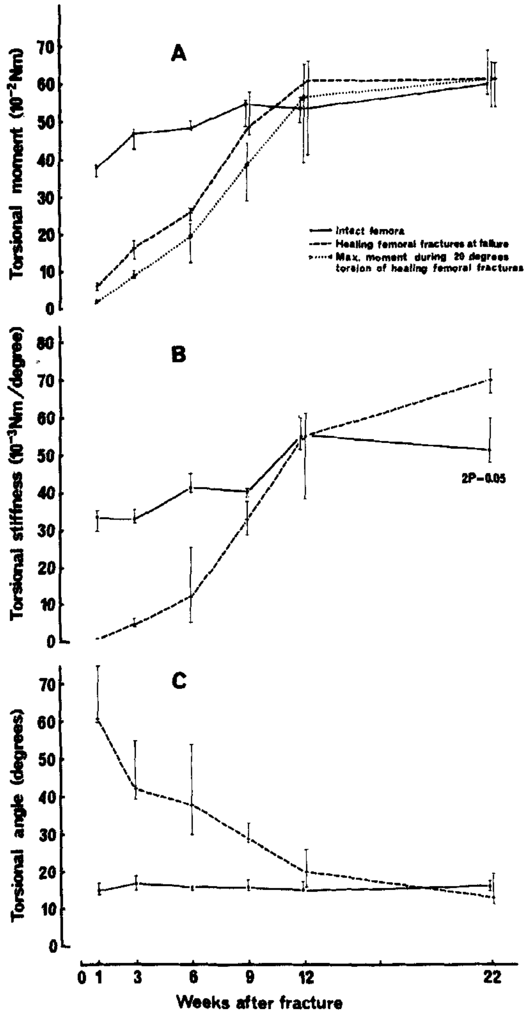

Figure 4. Adult rats. *Age at fracture: 100 days. Ultimate torsional moment (A), torsional stiffness (B) and ultimate torsional angle (C) for fractured and intact femora as a function of time following the fracture. The torsional moment required to twist the healing fractures 20 degrees (0.35 radians) is also given (A). Six to ten animals in each group. Significance levels are only indicated when the biomechanical parameters of healing fractures significantly* exceed *those of intact bones. (Median with 0.25- and 0.75-fractiles).*

Adult rats. The ultimate torsional moment, the torsional stiffness and the ultimate torsional angle of the healing femoral fractures reached the corresponding values of the contralateral, intact bones after 9–12, 12 and 12–22 weeks, respectively (Figure 4A, B and C). At 22 weeks after the fracture, the torsional stiffness was significantly higher ($2P = 0.05$) for healing fractures than for intact bones.

The torsional moment required to twist the healing fractures 20 degrees (0.35 radians), merged in with the ultimate torsional moment for the contralateral, intact bones at 12 weeks after the fracture (Figure 4A). Most of the fractures were consolidated after 9 weeks, and all after 12 weeks. Some of the fractures healed with angula-

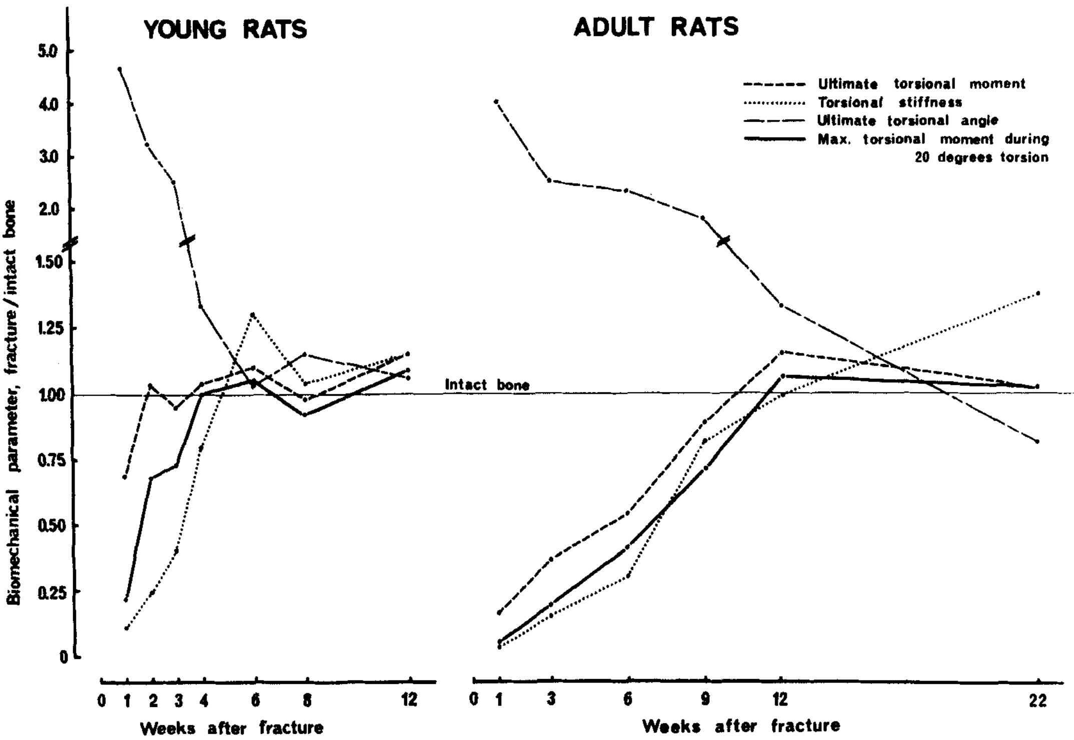

Figure 5. Biomechanical parameter ratios of fractured to intact bones from young and adult rats as a function of time following the fracture. The ratio of the torsional moment required to twist the healing fractures 20 degrees (0.35 radians) to the ultimate torsional moment of the contralateral, intact bones, seems to make up an average of the other ratios in indicating the degree of fracture repair. The ratios are calculated from the median values of the test data, and 6–10 rats were tested each time.

tions, and the displacement of the fractures was generally larger than observed in the young rats.

The ultimate torsional angle for intact bones was almost constant and of the same magnitude both in young and adult rats (Figures 3C and 4C). The median was 15 degrees (0.26 radians), and 97.5 per cent of the angles were below 20 degrees (0.35 radians).

During the mechanical tests, the healing fractures failed along a line which in the early phases of fracture repair passed through the callus transverse to the longitudinal axis of the bone. As the fractures became consolidated, this line adopted a more oblique course, first through the callus, later through the bone distal to the callus. Intact bones failed along a line at about 45 degrees (0.79 radians) to the longitudinal axis of the bone.

The mutual relationship of the biomechanical parameters from young and adult rats as a function of time is given in Figure 5.

Mechanical properties of the bone material

Strength and stiffness of the bone material (e.g. ultimate torsional stress and modulus of rigidity) increased with age in young rats. In adult rats, the corresponding values did not change significantly during the study. The graphs suggest a positive correlation between these two biomechanical parameters (Figures 6A and B).

DISCUSSION

The experimental fractures in the present study were estimated as healed when they had regained the mechanical properties (ultimate torsional moment, torsional stiffness, and ultimate tor-

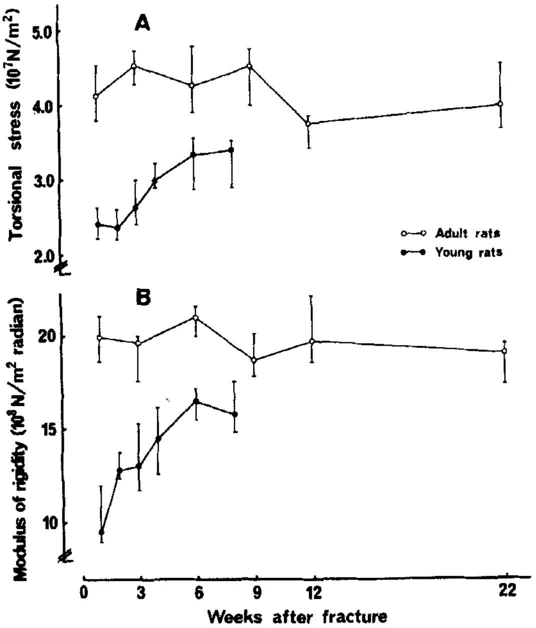

Figure 6. Ultimate torsional stress (bone material strength) (A) and modulus of rigidity (bone material stiffness) (B) of intact femora from young and adult rats as a function of time following fracture of the contralateral bone. The young rats were 23 days, the adult rats 100 days old at fracture. Six to ten animals in each group. (Median with 0.25- and 0.75-fractiles).

sional angle) of the contralateral, intact bones. Accordingly, unimmobilized femoral fractures were mechanically healed after about 4 weeks in young, and after about 12 weeks in adult, male rats.

Unlike our results, Sudmann et al. (1979) found a considerable number of non-unions in adult, male rats with unimmobilized femoral fractures. According to Mindell et al. (1971), the healing of unimmobilized, femoral osteotomies depends on intact periosteal attachments. That all the fractures in adult rats healed in the present experiment may be due to the small amount of damage inflicted on the periost and the soft tissues by our fracturing forceps.

The ultimate torsional moment is considered a suitable measure of strength of diaphyseal bones (Netz et al. 1978), and it is widely used in experimental work. The present study has, however, demonstrated that this expression of strength alone has shortcomings in the evaluation of fracture repair, especially in young rats. Thus, already 2 weeks after femoral fracture in these animals, the ultimate torsional moment for the healing fractures and for the contralateral, intact bones was almost equal (Figure 3A). The same fractures failed, however, after a twist of about 45 degrees (0.79 radians), whereas the intact femora could be twisted only about 13 degrees (0.23 radians) before failure (Figure 3C). This difference was also reflected in the torsional stiffness, which at this time was about four times higher in intact bones than in healing fractures (Figure 3B). These findings can be explained by the nature of the callus. In the early phases of fracture repair, the callus is composed mainly of fibrous and cartilaginous tissues (Rokkanen & Slätis 1964, Slätis & Rokkanen 1965). These tissues behave in a rubber-like manner, being elastic and deformable for small loads. The volume of callus is rather large, however, and after sufficient deformation, it may be quite strong (Rø et al. 1976, Ekeland et al. 1981).

The ultimate torsional angle of intact femora was almost constant during the study, and similar to the corresponding angular deformation previously observed in bending tests (Ekeland et al. 1981). This emphasizes the importance of this mechanical parameter, and suggests failure to depend more upon the extent of deformation than the strength of intact bones (Currey 1970, Sammarco et al. 1971). Accordingly, the torsional moment necessary to twist a healing fracture 20 degrees (0.35 radians), a deformation in the upper range of what an intact bone can resist, was found to provide a functional measure which related fracture strength to the degree of fracture repair. When the fracture healing was evaluated by this measure, the results seemed to make up an average of those obtained by the three other biomechanical parameters employed (Figure 5). Fracture strength has been calculated in a similar way in rabbits (Panjabi et al. 1977, White et al. 1977).

The torsional stiffness of the healing fractures exceeded that of intact bones after 6 weeks in young and after 22 weeks in adult rats. As observed by Northmore-Ball et al. (1980), this was transient in young rats. The increased stiffness was probably caused by the large polar moment of inertia and the increasing mineralization of the callus, rather than the callus tissue being stiffer than normal bone material. These properties of the callus may also explain why the line of failure during torsional loading changed as the fractures consolidated and strengthened. Finally the line passed through the bone distal to the callus, and the line of failure in healing fractures may therefore provide information both as to the healing phase as well as the fracture strength (White et al. 1977).

A new formula for the modulus of rigidity of rat femora was deduced and used in the present study. Both the modulus of rigidity and the ultimate torsional stress of intact femora increased with age in young rats, whereas corresponding values in adult rats did not change significantly. These results agree with those of a previous study (Ekeland et al. 1981), where both the ultimate bending and the ultimate torsional stresses increased to reach a plateau when the rats passed the initial age of adult rats in the present study (100 days). This may be related to the increase in calcium concentration with age in young rat femora (Engesæter et al. 1980a, Ekeland & Underdal 1982), as both strength and stiffness of bone material increase with increasing mineral content (Vose & Kubala 1959, Currey 1969a, b, Burstein et al. 1975). Also collagen may, however, contribute to these age-related changes in the mechanical properties of bone. Thus, Vogel (1979) has reported both the insoluble collagen content and the bending strength of rat femora to increase with age, whereas the soluble collagen content decreased. The latter has also been shown by Engesæter et al. (1980b). Decreased solubility of collagen is associated with an increasing amount of intra- and inter-molecular cross-links (Rasmussen & Bordier 1974, Uitto & Lichtenstein 1976, Bornstein & Traub 1979). Accordingly, the increase in strength and stiffness of the bone material from young rats with age may be a reflection of both increased cross-linking of collagen molecules, and enhanced bone mineralization (Lees & Davidsson 1977).

In conclusion, the torsional moment which was necessary to twist a healing femoral fracture 20 degrees (0.35 radians), proved to be a functional and simple measure for the degree of fracture repair in rats. Fractured femora healed about three times faster in young than in adult male rats. Both strength and stiffness of intact femora increased with age of the animals, whereas the ultimate deformation remained unchanged. Strength and stiffness for bone as a material, however, increased with age only in young rats, being rather constant in adult animals.

ACKNOWLEDGEMENTS

The authors thank Rolf Hauan, mechanical engineer, Bonde & Co., Engineers and James A. Ashton Miller, mechanical engineer, Biomechanical Laboratory, Sophies Minde Orthopaedic Hospital, for technical advice. Mrs. Trine Underdal is thanked for technical assistance, and The Norwegian Research Council for Science and the Humanities for partial sponsorship of this study.

REFERENCES

Bornstein, P. & Traub, W. (1979) The chemistry and biology of collagen. *The proteins* (Eds. Newrath, H. & Hill, R. L.) 3rd ed., volume 4, pp. 411–632. Academic Press, New York, San Francisco, London, 679 pp.

Burstein, A. H. & Frankel, V. H. (1971) A standard test for laboratory animal bone. *J. Biomech.* **4**, 155–158.

Burstein, A. H., Zika, J. M., Heiple, K. G. & Klein, L. (1975) Contribution of collagen and mineral to the elastic-plastic properties of bone. *J. Bone Joint Surg.* **57**-A, 956–961.

Currey, J. D. (1969a) The mechanical consequences of variation in the mineral content of bone. *J. Biomech.* **2**, 1–11.

Currey, J. D. (1969b) The relationship between the stiffness and the mineral content of bone. *J. Biomech.* **2**, 477–480.

Currey, J. D. (1970) The mechanical properties of bone. *Clin. Orthop.* **73**, 210–231.

Diem, K. & Lentner, C. (eds.) (1975) Documenta Geigy. *Scientific tables.* 7th ed., pp. 192–193. Ciba-Geigy Ltd., Basle, 810 pp.

Editorial (1971) Bone healing rates studied in young, old. Investigator explores relationship of age and healing, as well as effect of growth hormones on bones. *JAMA* **216**, 602–603.

Ekeland, A., Engesæter, L. B. & Langeland, N. (1981) Mechanical properties of fractured and intact rat femora evaluated by bending, torsional and tensile tests. *Acta Orthop. Scand.* **52**, 605–613.

Ekeland, A. & Underdal, T. (1982) Effects of salmon calcitonin on synthesis and mineralization of collagen in rats. *Acta Orthop. Scand.* (Submitted for publication).

Engesæter, L. B., Ekeland, A. & Langeland, N. (1978) Methods for testing the mechanical properties of the rat femur. *Acta Orthop. Scand.* **49**, 512–518.

Engesæter, L. B., Underdal, T. & Langeland, N. (1980a) Effects of oxytetracycline on mineralization of bone in young rats. *Acta Orthop. Scand.* **51**, 459–465.

Engesæter, L. B., Underdal, T. & Løvstad, R. (1980b) Effects of oxytetracycline on solubility and synthesis of collagen in young rats. *Acta Orthop. Scand.* **51**, 43–48.

Frankel, V. H. & Burstein, A. H. (1970) *Orthopaedic biomechanics*, pp. 40–76. Lea & Febiger, Philadelphia, 188 pp.

Less, S. & Davidsson, C. L. (1977) The role of collagen in the elastic properties of calcified tissues. *J. Biomech.* **10**, 473–486.

Mindell, E. R., Robard, S. & Kwasman, B. G. (1971) Chondrogenesis in bone repair. A study of the healing fracture callus in the rat. *Clin. Orthop.* **79**, 187–196.

Moberg, E. (1965) Allmänna synpunkter på frakturer och extremitetsskador. *Nordisk lærebog i kirurgi.* (Eds. Efskind, L., Husfeldt, E. & Romanus, R.) 8th ed., pp. 833–859. Munksgaard, København, 1051 pp.

Netz, P., Eriksson, K. & Strömberg, L. (1978) Torsional strength and geometry of diaphyseal bone. An experimental study in dogs. *Acta Orthop. Scand.* **49**, 430–434.

Northmore-Ball, M. D., Wood, M. R. & Meggitt, B. F. (1980) A biomechanical study of the effects of growth hormone in experimental fracture healing. *J. Bone Joint Surg.* **62**-B, 391–396.

Paavolainen, P. (1978) Studies on mechanical strength of bone. I. Torsional strength of normal rabbit tibiofibular bone. *Acta Orthop. Scand.* **49**, 497–505.

Panjabi, M. M., White III, A. A. & Southwick, W. O. (1973) Mechanical properties of bone as a function of rate of deformation. *J. Bone Joint Surg.* **55**-A, 322–330.

Panjabi, M. M., White III, A. A. & Southwick, W. O. (1977) Temporal changes in the physical properties of healing fractures in rabbits. *J. Biomech.* **10**, 689–699.

Rasmussen, H. & Bordier, P. (1974) *The physiological and cellular basis of metabolic bone disease*, p. 78. The Williams & Wilkins Company, Baltimore, 364 pp.

Reilly, D. T. & Burstein, A. H. (1974) The mechanical properties of cortical bone. *J. Bone Joint Surg.* **56**-A, 1001–1022.

Roark, R. J. & Young, W. C. (1975) *Formulas for stress and strain.* 5th ed., pp. 61–69, 286–323. McGraw-Hill Kogakusha Ltd., Tokyo, 624 pp.

Robinson, R. A. (1971) Healing of bone discontinuities in puppies and dogs. The 38th Annual Meeting of The American Academy of Orthopaedic Surgeons, San Francisco, U.S.A. March 6–11, 1971. *J. Bone Joint Surg.* **53**-A, 1017.

Rokkanen, P. & Slätis, P. (1964) The repair of experimental fractures during long-term anticoagulant treatment. *Acta Orthop. Scand.* **35**, 21–38.

Rø, J., Sudmann, E. & Marton, P. F. (1976) Effect of indomethacin on fracture healing in rats. *Acta Orthop. Scand.* **47**, 588–599.

Sammarco, G. J., Burstein, A. H., Davis, W. L. & Frankel, V. H. (1971) The biomechanics of torsional fractures: The effect of loading on ultimate properties. *J. Biomech.* **4**, 113–117.

Slätis, P. & Rokkanen, P. (1965) The normal repair of experimental fractures. A histo-quantitative study of rats. *Acta Orthop. Scand.* **36**, 221–229.

Sudmann, E., Dregelid, E., Bessesen, A. & Mørland, J. (1979) Inhibition of fracture healing by indomethacin in rats. *Eur. J. Clin. Invest.* **9**, 333–339.

Uitto, J. & Lichtenstein, J. R. (1976) Defects in the biochemistry of collagen in diseases of connective tissue. *J. Invest. Dermatol.* **66**, 59–79.

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

Vose, G. P. & Kubala, A. L. (1959) Bone strength – its relationship to X-ray-determined ash content. *Hum. Biol.* **31**, 261–270.

White III, A. A., Panjabi, M. M. & Hardy, R. J. (1974) Analysis of mechanical symmetry in rabbit long bones. *Acta Orthop. Scand.* **45**, 328–336.

White III, A. A., Panjabi, M. M. & Southwick, W. O. (1977) The four biomechanical stages of fracture repair. *J. Bone Joint Surg.* **59**-A, 188–192.

Correspondence to: Arne Ekeland, Institute for Surgical Research, Rikshospitalet, Oslo 1, Norway.