In vivo strain measurements in rat femur

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INTRODUCTION

Determination of mechanical response of bone *in vivo* provides a basis for understanding and quantifying cortical bone strain history. When coupled with bone physiological and metabolic studies, this provides additional insight into the factors governing regulation of bone metabolism. Strain gauges have been used to analyze the mechanical response of bone during normal locomotion. Several authors have described techniques for strain gauge preparation and implantation in large animals like dogs, sheep, and horses (*Lanyon* 1973, *Cochran* 1974, *Rybicki et al.* 1977, *Carter et al.* 1980, *Caler et al.* 1981). To our knowledge only two papers have been published concerning multidirectional surface strain measurements in bones in smaller animals, like rat femora (*Keller & Spengler* 1982) and chick tibiotarsus (*Biewener et al.* 1986). If the method is to be applied in smaller animals e.g. rats, aspects concerning distribution of strain around the bone, influence of walking speed on strain recordings, and reproducibility of strain recordings over time have to be evaluated. Implantation of strain gauges in smaller animals is, for obvious reasons, technically more difficult. However, this could make larger series and more controlled conditions possible. The aim of the present study was to evaluate femoral dynamic strain in groups of living rats. Methodological aspects were studied, i.e. deterioration of the strain gauge, effect of coating, and the influence of proper position of gauges on strain recordings.

MATERIAL AND METHODS

Experimental design

In order to record *in vivo* strain in rats, strain gauges were implanted in the femora. Twelve 8 weeks old male Wistar rats, weighing from 232 to 261 g received single gauges (group A). In addition, three 52 weeks old male Wistar rats weighing from 570 to 605 g (group B) were used, since femora from grown-ups were required to receive 3 gauges. The animals were kept in separate cages (45 cm × 25 cm × 20 cm) and given water and standard animal pellets (WOS R3, standard diet) ad libitum. Laboratory conditions were approximately: temperature, 24°C; relative humidity, 60°C; nyctohemeral cycle, 12h/12h).

The animals were killed seven days after the implantation. In order to evaluate methodological aspects, *in vitro* stiffness measurements were performed on 10 excised femora from group A and on acrylic rods.

Strain gauge unit and measuring equipment

The strain gauge unit consisted of a strain gauge element, two wires and a connector. The overall dimension of the gauge was 3.2 × 5.0 mm. The reistance grid measured 0.6 × 1.0 mm. The resistance was 120.5 ohms, and the k-factor 1.87 (0.6/120LY11, Hottinger Baldwin Messtechnik, Darmstadt, FRG). The transverse sensitivity of the gauges was about 0.1 per cent, making corrections unnecessary. The connecting wires were made from 0.6 mm, polyvinylchloride-coated cable. The wires were soldered to the connector, which was embedded in acrylic resin designed to be exteriorized at the neck.

Following soldering and finishing, the soldered junctions and the backing of the gauge were covered with a resin coating (Enamel Bond System, 3M, St.Paul, USA).

During measurements, the strain gauge was included in a full bridge circuit, connected to a multi-channel DC amplifier (MGT, 231, Hottinger Baldwin Messtechnik, Darmstadt, FRG) (Fig. 1). Voltage applied to the gauge was 1.5 V. The output from the amplifier was recorded on a DC tape recorder (Model 3964A, Hewlett-Packard, USA), as well as with a three channel

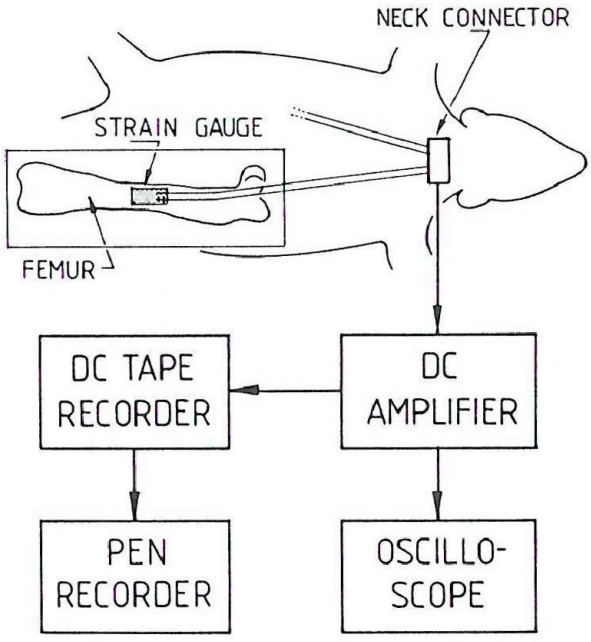

Figure 1. Experimental arrangement for recording of strain data of rat femora in vivo.

pen recorder (Model 314, W + W Electronic AG, Basel, Switzerland). The equipment was calibrated every day.

Surgical technique

Following a subcutaneous injection of 0.75 mg/100 g of fentanyl/fluanisone (Hypnorm vet, Leo, Copenhagen, Ballerup, Denmark), both femoral shafts were subperiosteally exposed through lateral, longitunidal incisions. The bone surface at the gauge implantation site was washed with Ringer solution and dried slightly with argon. In order to enhance adhesion, the bone surface was pre-treated with an adhesion promotor designed for dental use (Scotchbond, 3M, St.Paul, USA). The grids of the gauges were positioned parallel to the long axis of the femur, and glued to the bone by a methylmethacrylate-based adhesive (X 60, Hottinger Baldwin Messtechnik, Darmstadt, FRG). Excess adhesive at the soldered junctions were glued to the proximal femur, and served as strain relief for the cables. The strain gauge and the soldered junctions were coated with a resin (Enamel Bond System, 3M, St.Paul, USA). The fascia and skin were sutured with Polyamid, (Dexon, Davis + Geck, Inc., Manati, USA). A 5 mm incision was made in the skin of the rat's neck, just distal to the ears. The strain gauge was passed through a subcutaneous tunnel made from the neck to the implantation site. The connector was fixed to the skin by suturing.

Group A: Identical operative procedures were carried out on both legs. The upper, lateral corner of the gauge was positioned at the anterior surface where the trochanteric edge merges with the shaft.

Group B: Only the right femoral shaft was exposed. Three strain gauges were fixed to the femur at a middiaphyseal level. One gauge was comented to the anterior, one to the medial, and one to the posterior surface. The anterior gauge had an identical position as in group A.

In vivo measurements

Group A: Strain measurements were made on rats placed in a treadmill running at a speed of 6.0, 10.2, and 14.7 m/min. The treadmill was like a conveyor belt, onto which the rat was caged during the registration period. From day 2 through 7, measurements were performed daily at all three speeds. Each walking period lasted for one minute with three minutes of rest between the three walking speeds.

Group B: *In vivo* strain measurements were obtained at a speed of 10.2 m/min.

The strain level of each gauge was obtained prior to the recordings during gait, with the animals at rest. Absolute change in dynamic strain during gait is reflected by the peak-to-baseline value of a walking cycle. The peak-to-baseline strain value of 30 walking cycles was measured from each recording (Fig. 2), and the average values calculated as the arithmetic mean. This value has been chosen to represent strain during walking, and is termed dynamic strain. Each peak was measured from an individual baseline, thus long-wave variations in strain were excluded (Fig. 2).

Verification of the strain recordings

In order to reveal the influence of possible non-strain signals generated in the measuring chain by animal movement, the following test was carried out at day 3: The strain gauge on the

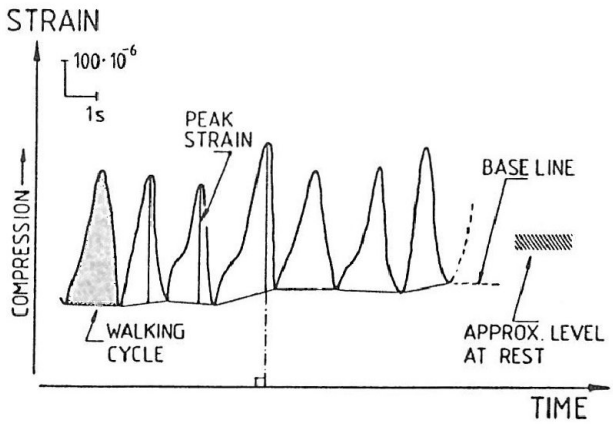

Figure 2. Interpretation of strain curves. Peak strain is measured perpendicularly to the electrical base-line (X-axis). Approximate strain level at rest is indicated.

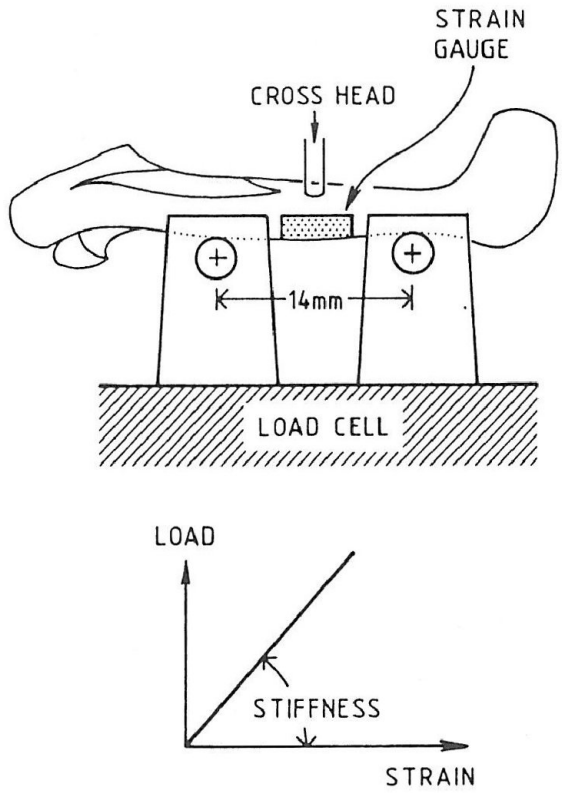

Figure 3. Three point loading system with specimen in position. The strain gauge is positioned opposite the cross head, on the anterior surface of the femur. The stiffness of the bone is the slope of the schematic load-strain curve.

right femur of two animals were exposed. The grid of the gauge was cut and a resin coating filled in so that infinite resistance was obtained. The wound was closed in the same manner as described earlier. A 120 ohm resistor, simulating the strain gauge resistance, was coupled to the neck connector.

Following a recovery period of 24 hours, recordings were carried out at a treadmill speed of 10.2 m/min. Measurements from these animals were excluded from the series.

In vitro measurements

The excised femora were subjected to three-point bending in a test jig which was attached to the load cell of a mechanical testing machine (Instron 1193, High Wycombe, England). Deformation was measured by the strain gauge. The femur was positioned in the jig with the crosshead opposite the grid of the strain gauge, causing maximum tensile strain at the site of the gauge (Fig. 3). Bending was applied at a speed of 0.5 mm/min up to a load of 5.0 N, which is well within the elastic range of the bone (Mølster 1986).

The implanted strain gauges were removed after testing and new strain gauges were glued in the same position. Stiffness measurements were repeated, both prior to and after application of the insulating resin.

Stiffness was determined from the resulting load/strain curves and expressed as the slope of the initial, nearly linear part of the curve and presented as $mN/10^{-6}$ (Fig. 3).

The potential effect of severe malposition of the strain gauges on strain recordings was evaluated by testing circular acrylic rods of 4 mm in diameter. Strain and deflection recordings were performed simultaneously on the same specimen. Strain gauges were cemented in the same manner as for bones. Recordings were obtained from gauges with their grids positioned a: parallel, b: rotated 25 degrees, c: with a lateral displacement of 1.7 mm to the long axis of rods.

To check the accuracy of the strain measuring system, an electronic extensometer (Instron G-51-15M) was clamped across the strain gauge which was glued to an acrylic rod. Tension was applied and the signals from the two strain measuring devices were recorded.

Statistics

In this study strain gauges were considered as independent units, and recordings from left and right femora were therefore pooled, except when the two sides were compared. Differences between the two sides were tested by the Wilcoxon one-sample test. Groups of data were compared by the Kruskal-Wallis test, and each group of data was tested against each of the others by the Wilcoxon two-sample test. Differences were considered significant when $p<0.05$.

RESULTS

Group A: The animals tolerated the strain gauge implantation well. They regained normal gait on the first postoperative day. At a walking speed of 6.0 m/min the animals lost all interest in walking, whereas a speed of 14.7 m/min induced fast walking, and turned the animals easily out of breath.

One unit was excluded from the study, due to failed fixation. No sign of mechanical loosening of the strain gauge units was observed at the time of sacrifice, 7 days postoperatively. At this time, 13 of the 21 strain gauges included in the study functioned. Remaining gauges revealed electrical disturbance or no signals at all. In five animals both gauges were intact.

Recorded signals showed variations synchronous to the limb movements of the animal. The approximate strain level at rest is shown in Figure 2. During each run there was a long-waved baseline variation, which was independent of the limb movements. These fluctuations became more pronounced over time. Furthermore, a short-waved electrical noise gradually appeared, particularly at day 6 and 7. These disturbances did not have any influence on interpretation of the strain data. Pulling the cables did not affect the recorded signal.

The two strain gauges with intentionally broken grids showed only minor noise signals, which were not synchronous with walking.

The individual absolute peak strain values varied between the extremes of $195 \cdot 10^{-6}$ and $785 \cdot 10^{-6}$ with respect to animal, time after operation and walking speed. The strain was compressive relative to the chosen baseline. The paired differences in strain values between left and right femora, of day 2 through day 7, varied from $-78 \cdot 10^{-6}$ to $67 \cdot 10^{-6}$, for all 3 speeds. These differences were not statistically significant. The Kruskal-Wallis test revealed that the measurements at day 2 through 7 were not statistically different (Figure 4). Neither were strain values at different walking speeds statistically different.

Group B: A limp appeared following a walking period of about 30 to 40 s on the second day postoperatively. This group was therefore terminated on ethical grounds after three rats had

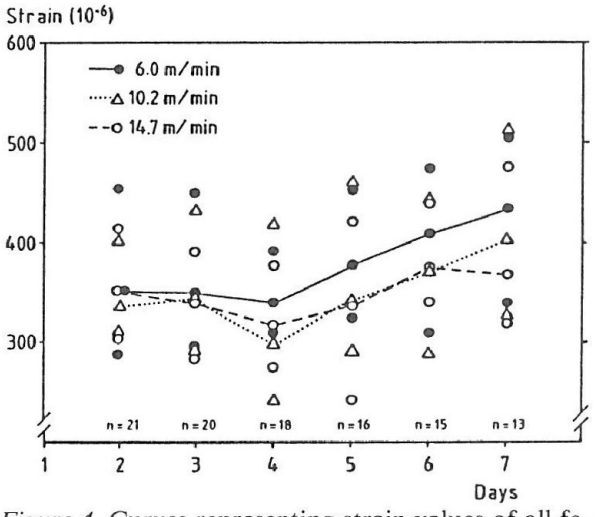

Figure 4. Curves representing strain values of all femora, and at three different speeds (left and right sides pooled). Median values with upper and lower quartiles indicated by the same symbol dots as used for the curves. n = number of intact gauges.

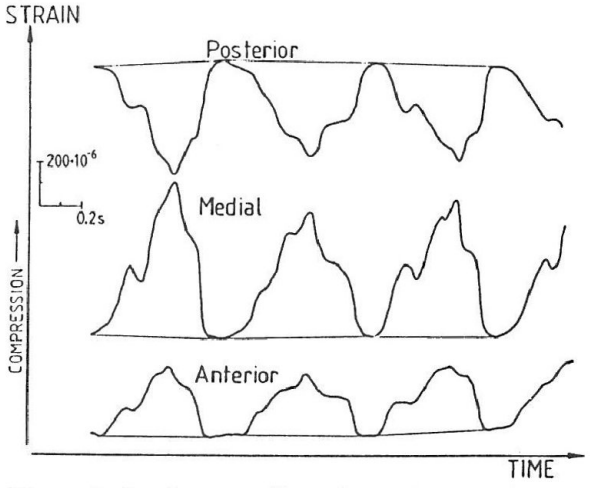

Figure 5. Strain recordings from the anterior, the medial, and the posterior aspects at the middiaphyseal level during walking (group B).

been entered into the protocol. Recordings obtained after these two days showed that peak strain was compressive at the anterior and the medial aspects, while the peak strain was tensile on the posterior aspect during walking (Fig. 5). Strain values recorded at the three aspects at day one are shown in Fig. 6.

In vitro results: Recordings from replacement gauges on excised femora (Group A) corresponded within five per cent with chronically implanted gauges. The Kruskal-Wallis test revealed that there were no statistically discernible differences between measurements from the original, the uncoated-replaced and the coated-replaced strain gauges (Fig. 7).

Rotating the strain gauges 25 degrees to the longitudinal axis of the acrylic rods decreased the median strain/deflection values by 8 per cent ($p = 0.008$). Furthermore, a 1.7 mm lateral displacement decreased the strain/deflection values by 12 per cent ($p < 0.005$) (Table 1). Parallel measurements of strain gauges and the calibrated extensometer differed by less than 2 per cent.

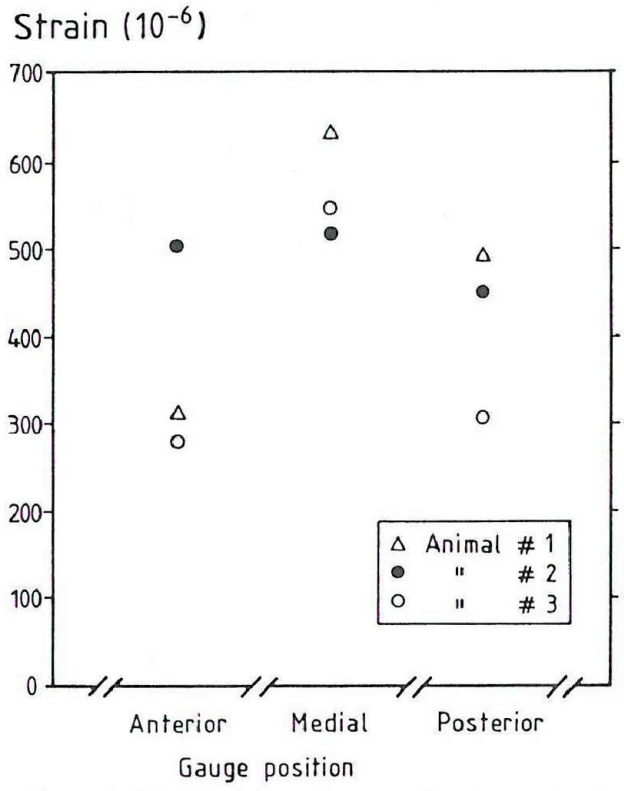

Figure 6. Strain values at 3 aspects for three animals (group B) at day one. Walking speed 10.2 m/min.

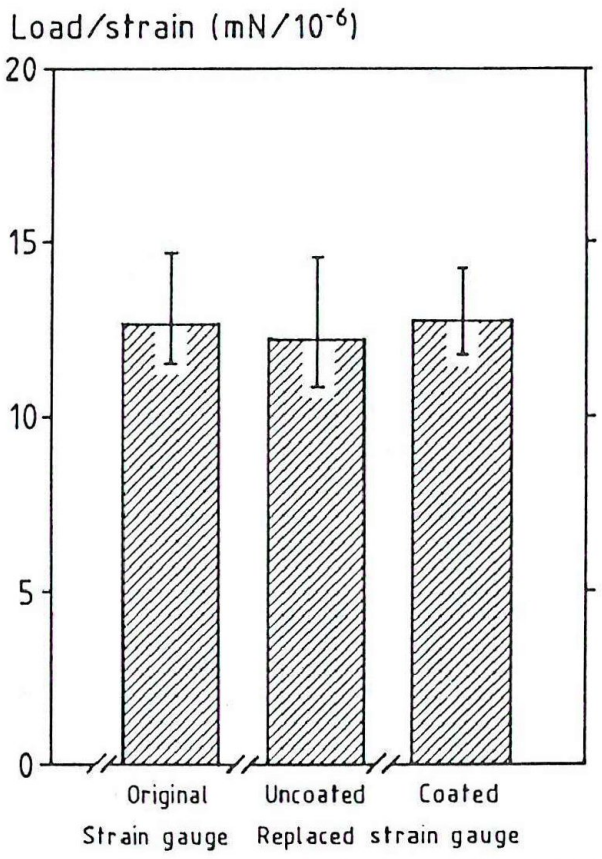

Figure 7. Bars presenting strain gauge stiffness of femora measured with original and replaced strain gauges. Median values with vertical lines representing upper and lower quartiles are given.

Table 1
Strain/deflection results

Parallel	Rotated	Laterally displaced
2463 (2437-2480)	2290 (2177-2342)	2117 (2073-2203)

Median values (10^{-6}/mm) from gauges with grids positioned parallel, rotated 25 degrees, or with a lateral displacement of 1.7 mm to the long axis of acrylic rods (lower-upper quartiles).

DISCUSSION

In this study, strain gauges were successfully attached to rat femora, and *in vivo* strain measurements were obtained for 7 days in half of the implanted gauges. Small difference in strain values between the right and left femora throughout the study indicates that the measuring technique is reproducible, assuming that similar mechanical factors act on the two sides.

The small variations in strain between the 3 speeds during this study, is interesting, since strain at the calcaneus of sheep and human tibiae during walking, increase as the walking speed increases (*Lanyon* 1973, *Lanyon et al.* 1975). Walking speeds between 6.0 and 14.7 m/min could be outside the range where speed influences strain values. It is also possible that the femoral surface strain in rats during walking is independent of changes in speed, due to the rat's gait. Moreover, the modest variations in strain values of day 2 through day 5 concerning all 3 speeds (Fig. 4), indicate the validity of strain recordings chosen at random during that observation period.

Cementing one gauge to the femoral shaft did not seem to interfere with physiological walking (group B), the surgical trauma seemed too vigorous and strain recordings during longer periods of walking would probably not be reliable. Earlier studies on canine femora have shown that the antero-lateral surface is tensile and the posterior surface compressive during walking (*Carter et al.* 1981). The anterior femoral curvature found in most species, reflects a similar loading configuration. On the other hand, the compression of the antero-medial femoral aspects shown by us, may be explained by the more horizontal position of the rat femur during walking. Magnitude of strain found in the present study corresponds with earlier recordings from rat femora (*Keller & Spengler* 1982). However, values obtained from the sheep radius (*Lanyon & Baggott* 1976), the radius of dogs (*Cochran* 1974, *Carter et al.* 1980) and the human tibiae (*Lanyon et al.* 1975) during walking are generally greater. We found, as have other researchers (*Lanyon* 1973, *Carter et al.* 1980), that it is somewhat difficult to determine the absolute value of zero strain for *in vivo* bone strain gauges. However, the aim of the present study was to evaluate dynamic strain during walking. The peak-to-baseline value of a walking cycle was, therefore, found to reflect the absolute change in dynamic strain during walking.

Thirteen out of 21 gauges functioned at the time of sacrifice. In a previous study on rat femora, good recordings were obtained from two gauges 12 days after implantation (*Keller & Spengler* 1982). Other authors have reported obtaining recordings for longer periods of time in dogs and sheep (*Cochran* 1972, *Lanyon* 1976, *Carter et al.* 1980), but were not dealing with an animal model with as rapid growth rate as the rat. The reliability of the system was best during the first 5 days, as the electrical noise increased markedly from day 5 through 7. For gauges showing no signals at all, the reason was most probably fracture of wires of the soldered joints at the gauge. The short-waved and the long-waved disturbances, observed at the later stages of the experiment, were probably caused by electrolyte leakage. However, moderate electrical disturbances did not interfere with the strain measurements, as variations synchronous to walking were easily identified. The strain relief seemed adequate, as the signals were not affected by pulling the cables.

The small difference found between recordings of the gauge and the extensometer, shows that our strain gauge measurements were accurate. In the present study, the application of a resin to coat the gauges did not affect the measurements to such a degree as has been reported by earlier investigators (*Keller & Spengler* 1982). Moreover, the agreement between recordings obtained with replacement and original gauges, demonstrates the reproducibility of our method and indicates that the strain gauge unit does not deteriorate during implantation for one week.

Our study on acrylic rods showed that minor changes in gauge position may alter the relation to the neutral axis. However, the implanted gauges were found to be positioned within the limits examined.

A previous study has shown that the directions of principal compressive strains in long bones are aligned within an angle of 25 degrees of the bone's long axis (*Goodship et al.* 1979). Throughout the period of deformation the angle of the principal compressive strain to the long axis of the bone has been found to remain relatively constant (*Lanyon & Smith* 1970, *Lanyon* 1973, *Lanyon et al.* 1975, *Lanyon & Baggott* 1976). Thus, unidirectional gauges seems to be appropriate when measuring strain in

limb segments which contain only one single long bone.

In conclusion a standardized *in vivo* strain gauge technique can be established in rats over one week, with a success rate of 50 per cent. The requirement of reproducibility over time seems fulfilled for 5 days. Changes in walking speed witin the reported range have no influence on strain values. Peak strain at the anterior and the medial aspects of the rat femur are compressive, while peak strain at the posterior aspect is tensile during walking.

Summary

The purpose of the present study was to evaluate strain in the femora of rats during walking. Uni-directional strain gauge units were implanted. Peak strain at the anterior and medial aspects of the rat femur was compressive, while peak strain at the posterior aspect was tensile during walking. Median strain values at the anterior mid-diaphyseal aspect varied between $297 \cdot 10^{-6}$ and $434 \cdot 10^{-6}$. There were no statistical differences between recordings at different times after implantation as evaluated by daily measurements for one week. Neither was there any statistically significant difference in strain values between the walking speeds of 6.0, 10.2, and 14.7 m/min, or between values for right and left femur. *In vitro* measurements on replaced gauges corresponded within 5 per cent with results from bones where gauges had been implanted for one week. Waterproofing the strain gauge unit with a resin did not significantly affect the measurements. The method, therefore, enables studies of strain behavior of bone from small species *in vivo* under physiological and pathophysiological conditions.

Sammendrag

Hensikten med undersøkelsen var å bedømme formendringer i rottefemora under gange. Strekklapper som registrerte forandringer i én retning ble implantert. Under gange viste forandringene anteriort og medialt seg å være kompressive, mens det posteriort ble registrert strekk. Anteriort midt på diafysen ble det målt relative forkortninger med medianverdier som varierte mellom $297 \cdot 10^{-6}$ og $434 \cdot 10^{-6}$. Bedømt etter daglige målinger gjennom en uke var der ingen statistisk signifikante forskjeller mellom registreringer på forskjellige tider etter implanteringen. Heller ikke ble det funner noen statistisk signifikant forskjell mellom formendringene for ganghastigheter på 6.0, 10.2, og 14.7 m/min. eller mellom høyre og venstre femur. *In vitro* målinger med nye strekklapper avvek ikke mer enn 5% fra verdiene fra knokler hvor strekklappene hadde vært implantert i en uke. Det hadde ingen vesentlig betydning for målingene at strekklapper var blitt dekket av en resin. Metoden gir således muligheter for å studere deformasjonsmønsteret i knokler hos små arter *in vivo* under fysiologiske og patofysiologiske forhold.

Yhteenveto / K. Pelkonen

Tutkimuksen tarkoituksena oli arvioida rotan reisiluuhun kohdistuvaa kuormitusta kävelyn aikana. Luuhun implantoitiin yksisuuntaiset kuormitusmittausanturit. Huippukuormat rotan reisiluun etu- ja keskiosassa olivat puristuskuormia. Reisiluun takaosassa kävelyn huippukuorma oli jännitystä. Keskimääräiset kuormitusarvot vaihtelivat etuosassa (anterior mid-diaphyseal aspect) $297 \cdot 10^{-6}$ ja $434 \cdot 10^{-6}$ välillä. Viikon ajan implantoimisen jälkeen suoritetuissa päivittäisissä mittauksissa ei ollut keskinäisiä tilastollisesti merkitseviä eroja. Eri nopeuksilla suoritetun kävelyn välillä (6.0, 10.2 ja 14.7 m/min) ja vasemman ja oikean reisiluun välillä ei myöskään ollut eroja. In vitro-mittaukset uusilla antureilla ja luuhun viikon ajan implantoiduilla antureilla täsmäsivät 5% tarkkuudella. Anturin tiivistäminen vedenpitäväksi hartsilla ei merkittävästi vaikuttanut mittauksiin. Menetelmä soveltuu näinollen pienillä lajeilla in vivo-tehtävään luun kuormituksen mittaamiseen sekä normaaleissa fysiologissa että patofysiologisissa tilanteissa.

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