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THE EFFECTS OF MUSCLE FATIGUE ON BONE STRAIN

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Summary

There is anecdotal evidence that bone strains may increase to the point that bone becomes susceptible to rapid failure when muscles become fatigued. To determine whether neuromuscular response could be a factor in accelerating bone failure, we tested the hypothesis that muscle fatigue causes a significant increase in peak principal and shear strains in bone. Ten adult foxhounds were subjected to rigorous exercise that caused muscular fatigue while myoelectrical activity of the quadriceps and hamstrings and strain on the distal tibia were monitored simultaneously. Ground reaction forces on the dog hindlimbs were measured before and after strain gauges had been applied to the tibia. The data show a significant shift to lower median myoelectrical frequencies in the quadriceps, indicating muscular fatigue, following the 20 min exercise period. In conjunction with this shift, peak principal and shear strains increased on both compressive and tensile cortices of the tibia and shear strain on the tensile cortex increased significantly (P=0.02). The largest changes were along the anterior and antero-lateral surfaces of the tibia, where peak principal strain increased by an average of 26–35 % following muscular fatigue. The cross-sectional strain distribution was calculated at the gauge site at peak strain at the beginning of the exercise period and at peak strain after 20 min of exercise. These data show a change in strain distribution when muscle becomes fatigued. Strains on the posterior cortex of the bone showed the greatest change. Correlation analysis demonstrated a significant inverse association between median myoelectrical frequency and bone strain after 20 min of exercise (Spearman r^2=1.00; P=0.05). These data show that muscle fatigue may be associated with increased bone strain.

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Introduction

Stress fractures are over-use injuries (Markey, 1987; Matheson *et al.* 1989; Taimela *et al.* 1990; Bovens *et al.* 1989; Stanish, 1984) that result when the skeleton adapts too slowly to the stresses and strains imposed on it (Devas, 1975; Greaney *et al.* 1983; Rubin *et al.* 1984). They are a significant health problem for both young physically active people (McBryde, 1976; James *et al.* 1978; Clement *et al.* 1981; Rosen *et al.* 1982) and for older osteopenic individuals (Blickenstaff and Morris, 1966; Todd *et al.* 1972; Freeman *et al.* 1974). Bone fatigue and stress fractures are more common when bones are repetitively loaded above a certain stress or strain threshold (Carter *et al.* 1981*b*,*c*; Carter and Caler, 1983, 1985; Caler and Carter, 1989; Nunamaker *et al.* 1987, 1990).

We hypothesize that this threshold is exceeded when the neuromuscular system is impaired, for example following muscular fatigue. Muscles and other soft tissues can potentially dissipate dynamic forces in the skeleton by eccentric contraction (Paul *et al.* 1978; Pauwels, 1980; Hill, 1962; McMahon, 1984; Bergmann *et al.* 1991), but whether they do has never been experimentally verified. Muscle contraction on the tensile side of a bone may limit tensile strains generated by bone bending during mechanical loading (Radin *et al.* 1979). Bone is weakest in tension and in greatest danger of fracturing from high tensile stress (Yamada, 1970; Evans, 1973). When a bone is bent, one surface is subject to compression and the opposite surface to tension. Theoretically, when muscles that span the tensile surface of a bone contract appropriately, they limit the magnitude of tension by adding compression to this surface (Radin *et al.* 1979). Gait is altered when muscles become fatigued, and it is possible that this also produces a change in strain magnitude or distribution. Gait alterations may cause regions of bone that are usually under low strain to experience momentary applications of very high strain. If either eccentric or concentric muscle contraction can reduce tensile strain in bone during movement, stress fractures may be prevented as long as the capacity of muscles to protect bones from overloading is not exceeded.

To determine whether neuromuscular response could be a factor in preventing stress fractures, we tested the hypothesis that muscle fatigue causes a significant increase in peak principal and shear strains in bone.

Materials and methods

The design of this experiment was to subject animals to fatiguing exercise, while simultaneously measuring myoelectrical activity of the quadriceps and hamstrings as well as bone strain in the distal one-third of the tibial diaphysis. The myoelectrical recordings (EMGs) were used to assess muscular fatigue. The hamstrings and quadriceps were chosen because these muscles control acceleration and deceleration of the limb, as well as knee joint angles, and these have been shown in humans to increase ground reaction loading rate (Jefferson *et al.* 1990; Radin *et al.* 1991).

Ten skeletally mature 1- to 2-year-old foxhounds were trained to walk bipedally on their hindlimbs on a treadmill. Their forelimbs were held up by a padded support located under the axilla to prevent weightbearing and to prevent the dog from moving backwards on the treadmill (Fig. 1). Hindlimb walking was utilized to minimize the time to onset of

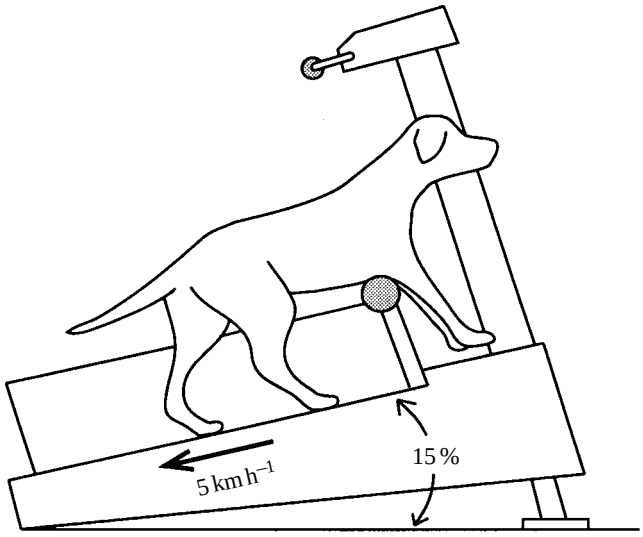

Fig. 1. Diagram showing the configuration of the treadmill used to exercise dogs to the point of muscular fatigue. The dog's forelimbs were placed over a bar so that they bore no weight. The treadmill was inclined by 15 % (8.5 °) while the dog trotted at 5 km h^{-1} for 20 min.

fatigue. This is an unnatural mode of walking for the dogs but, because all measurements were paired before and after the fatiguing exercise, it serves as a reasonable model to test the hypothesis that muscle fatigue is correlated to increased bone strain. The dogs were conditioned to walk on their hindlimbs for 20 min day^{-1} at 5 km h^{-1} with the treadmill inclined by 15 % (8.5 °). A minimum of 2 days was spent for training prior to entering the animal into the experiment.

The animals were housed in cages approximately 2 m×1.5 m and received a standard diet and water *ad libitum*. Upon arrival, skeletal maturity was verified radiographically by assessment of tibial and femoral growth plate closure.

Force plate recording and analysis

An initial force plate measurement was made on each dog upon arrival. Another was made 1 day prior to the surgical application of strain gauges, and a third was made 1 day before bone strain measurement to determine how the vertical component (F_z) of the ground reaction force (GRF) was altered by the surgical procedures and also to screen for gait abnormalities prior to entering the animal into the experiment. F_z was measured as the dogs trotted briskly (9 km h^{-1}) in one direction along a specially constructed runway, and also when they were allowed to walk using only their hindlimbs to simulate bipedal walking on the treadmill. Hindlimb walking was accomplished by placing their forelimbs over a transverse bar attached to wheels that ran along parallel tracks fixed 50 cm above the floor of the walkway (Fig. 2).

GRF was measured using an AMTI (Advanced Mechanical Technology Inc., Newton, MA) strain-gauge force plate flush with the surface of the runway. The signals were

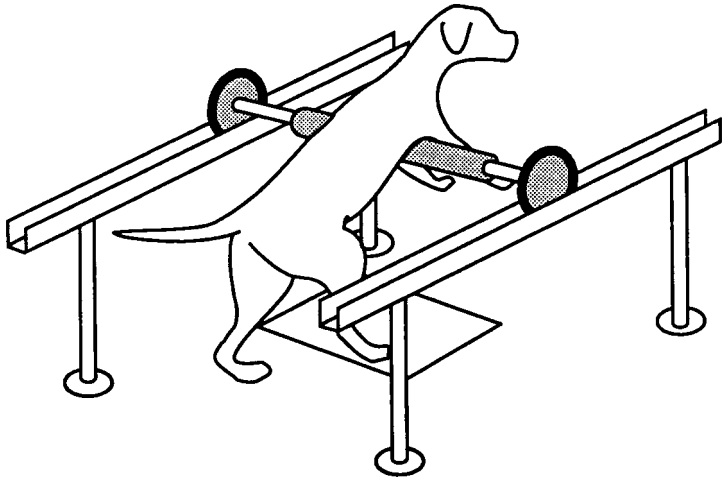

Fig. 2. Ground reaction forces on the operated and non-operated hindlimb were measured before and after surgical implantation of strain gauges. Forces were measured while the dog walked bipedally across the force plate with its forelegs placed over a transverse bar with rollers that ran along parallel tracks 50 cm above the floor of the walkway.

processed using AMTI signal conditioners, digitized at 200 Hz and transmitted to a Gateway 386-20 microcomputer, *via* a Scientific Solutions A/D board, for storage and subsequent analysis. Data acquisition and analysis was accomplished using Asystant+ scientific software (Scientific Solutions Software, Rochester, NY). For each animal, a minimum of eight successful trials was collected. Each trial was videotaped for archival purposes and to ensure that only paw strikes that were completely on the force plate were included in the evaluation. A successful trial was defined as one in which the waveforms of the mean peak vertical forces at paw strike are represented by two well-defined peaks representing one forelimb and one hindlimb strike. When the dog was walking on two limbs, a successful trial required only one well-defined peak. The means and standard deviations of F_z were determined for each hindlimb of each dog walking bipedally for the eight trials. The means were normalized to the animal's body mass for each limb. The normalized means of the presurgery and postsurgery F_z were compared using a paired *t*-test.

Strain gauge application

Fine conducting wires (38 gauge, Phoenix Inc.) were soldered to the copper-coated tabs of planar rectangular rosette strain gauges (Micromeasurements EA-06-015-RJ-120), and the surface of the rosette was coated with M-Coat A (Micromeasurements, Raleigh, NC). After 24 h, M-Coat C (Micromeasurements) was applied and cured for 24 h. The rosettes were trimmed and packed for gas sterilization.

Each dog was tranquilized with Inovar (0.07 ml kg^{-1}) followed by a general anesthesia with halothane. A tourniquet was applied to the thigh, and a 7 cm incision was made through the skin along the anterior crest of the tibia. The tibialis anterior was carefully retracted along with the anterior tibial artery and deep peroneal nerve. At the tibial

midshaft, the periosteum was stripped and hemostasis was obtained using a bipolar coagulator. The bone surface was roughened with 400 grit silicon carbide sandpaper, cleaned and dried with absolute alcohol.

Five rosettes were arranged at the same cross-sectional level halfway between the tibial midshaft and the distal tibio-fibular junction. The midshaft was chosen because theoretically it is likely to be the region of maximum bending strain. Rosettes were applied to the lateral, antero-lateral, anterior, antero-medial and medial cortices. These cortices were chosen because they were easily accessible with little disruption to muscles and muscle attachments. Gauges could not be applied posteriorly without significant damage to muscle insertions that could have adversely affected the dog's gait. Each rosette was aligned with its long axis parallel to the long axis of the tibia and attached to the bone using a cyanoacrylate adhesive (M-Bond 200, Micromeasurements). 3 min was allowed for bonding. After verification of firm attachment, a silicone coating (Petrarch Systemes, Inc.) was applied to the rosette and marginal bone and cured with ultraviolet light. The wires from the rosette strain gauges were sutured around the bone at a distance of 5 mm from the proximal side of the rosettes to provide strain relief on the wires and soldered connections.

After releasing the tourniquet, two plastic extension tubes from an intravenous line were led subcutaneously from the surgical site to the dorsal aspect of the thorax. A 1 cm incision was made and the tubing brought out on the back. The strain gauge wires were drawn through the plastic tubing, and the tubing was sutured to the skin on the back. The incisions were closed and the dog was fitted with a mesh jacket. The ends of the exposed wires were soldered to connectors in an electrical pack, and the connector box was carried in a pocket of the jacket.

After the surgery, the position and orientation of the strain gauges was verified radiographically. The dogs were kept in the recovery room for observation and to protect the connectors from moisture and the dog's physical activity until measurements were collected 5 days after surgery. All dogs received an antibiotic (Cefazolin, 1 g day^{-1}) for 3 days following surgery.

Application of electromyographic (EMG) electrodes

Electromyographic measurements were made to determine whether the quadriceps and hamstring muscles became fatigued during the 20 min exercise period. Muscle fatigue was defined as a significant spectral shift to lower frequencies in the myoelectric signal (Lindstrom *et al.* 1977; Petrofsky *et al.* 1982; Nagata *et al.* 1990). The skin overlying the vastus lateralis and biceps femoris muscles was shaved. The skin was washed and lightly abraded with gauze wetted in a detergent solution, and collodian paste was applied to the skin to improve adherence of the electrodes. Two Sensormedic silver/silver chloride surface electrodes measuring 1.5 mm in diameter were applied to biceps femoris and the vastus lateralis midway between the hip and knee joints at an inter-electrode distance of 2.5 cm. This was generally the thickest part of the muscle belly. A 2 cm ground electrode was applied to the skin overlying the greater trochanter. All electrodes were affixed with Dermicel adhesive tape (Johnson and Johnson, Inc. New Brunswick, NJ) and Coban self-adherent wrap (3M Inc., St Paul, MN).

Strain and EMG data collection

Electrodes were attached to Grass 7DA amplifiers (Grass Instruments, Quincy, MA) using coaxial cables. The amplifiers were calibrated before each experiment and the low-frequency analog filter was set at 500 Hz. Amplifiers were interfaced with a Keithley 575 measurement system (Keithley Instruments, Inc. Cleveland, OH) and computer. High-frequency filters were set at 2 kHz on the Keithley RTMDS data-collection program. The electrical activity of each muscle was sampled at 1 kHz (sampling frequency should be more than twofold faster than the highest frequency of the analog signal) for 5 s and stored on hard disk.

The rosette strain gauges were connected to a Minerva data-acquisition system through a Wheatstone bridge. The Wheatstone bridge circuit was balanced while the animal was lifted off the floor. Strain data collected for a different study (C. H. Turner, T. Yoshikawa, M. R. Forwood, T. C. Sun and D.B. Burr, in preparation) show that strains when the dog is lifted off the ground are less than 25 microstrain. Nevertheless, to ensure that muscle activity that could pre-load the tibia was absent, EMG recordings were made during the zeroing process. Twelve channels of strain data from four of the strain gauge rosettes were acquired at 100 Hz for 4 s.

All animals were made to walk bipedally on the inclined treadmill at 5 km h^{-1} (80 steps min^{-1}) for 20 min as they had been trained to do. Strain and EMG recordings were taken for 4 s and 5 s, respectively, with the dogs standing at rest and after 0 and 20 min of walking.

Strain analysis procedures

Raw voltage data from each gauge element were converted into microstrain based on calibration factors pre-determined on an instrumented aluminum bar. Principal compressive and tensile strains, material (anatomical) axis strains and shear strain were calculated using standard formulae (Carter, 1978), incorporating the rosette deviation angle into the calculation.

The mean peak strain at 0 min was compared with that at 20 min for each dog using a paired *t*-test to investigate whether there was a significant increase in bone strain following 20 min of fatiguing exercise.

The strain distribution was calculated from the material axis strains of three rosettes for one dog using methods presented by Carter *et al.* (1981*a*,*d*) and Caler *et al.* (1982). The relative coordinates of the rosette gauges were determined from the cross sections of the tibiae obtained after the animal had been killed.

EMG analysis procedures

Five bursts of muscle activity were collected for each 5 s recording. A segment consisting of 128 points was cut from each stationary burst and subjected to a power spectrum analysis. PC-DSP software was used for signal processing using an autoregression (AR, all pole) approach with a model order of approximately 12. This model order was determined by the method of parsimonious modeling by analyzing several spectra using model orders from 1 to 50. An AR estimator of order p cannot

generate more than p peaks. Accordingly, a low model order generates a smoothed estimate, whereas a higher model order generates a more uneven curve. We used the smallest AR model order at which little change in the number of major peaks could be detected. The power spectral density was computed using Burg's method (Marple, 1987). The median frequency root-mean-square amplitude and the percentage of energy in frequency bands 4–60 Hz, 60–100 Hz, 100–200 Hz and 200–500 Hz were calculated for each burst. When more than one 'stationary' burst was available, the means of the above parameters were calculated and used for comparison of the EMG power density change before and after the 20 min treadmill walking.

The median frequencies of the EMG signal at baseline and after 20 min of exercise were compared using one-tailed nonparametric Wilcoxon signed-ranks tests. The median frequency extracted from an EMG signal is considered to be ordinal level data and cannot be assumed to be normally distributed. Therefore, we chose to use a nonparametric test rather than a paired t-test for this analysis.

Treatment of the dogs

Dogs were killed by intravenous injection of Uthol D (0.22 ml kg^{-1}; Butler, Co, Columbus, OH). Death was ensured by inducing pneumothorax. National Institutes of Health guidelines for the care and use of laboratory animals were observed in all phases of the research. All phases of the protocol were approved by the Indiana University School of Medicine Animal Care and Use Committee (protocol MD 1209).

Results

Force plate measurements

Dogs routinely bear approximately 60 % of their weight through their forelimbs and 40 % through their hindlimbs (Kimura *et al.* 1979; O'Connor *et al.* 1989). The average mass transmitted through the the forelimbs in the 10 dogs used for our study was 62.07 % of body mass: not significantly different from expected values (O'Connor *et al.* 1989). No limping or other signs of abnormal gait were visible.

Nine out of ten dogs gave both pre- and post-surgery force plate measurements while walking only on their hindlimbs. One dog was unable to give a post-surgery measurement because it had a severe limp after surgery, and it was eliminated from the study. No significant differences were found between pre-surgery and post-surgery values for the normalized axial force (F_z) on either the operated limb or the non-operated limb (Fig. 3). We interpreted this to mean that the effects of surgery on gait were negligible and could be disregarded as a significant factor in this experiment.

Muscle fatigue following exercise

EMG recordings of quadriceps activity at 5 min intervals for the entire 20 min exercise period were obtained from six dogs, and recordings of hamstring activity for the exercise period were obtained from five dogs. Owing to non-stationarity of the EMG signal, other EMG recordings could not be used. Non-stationarity prevents the calculation of statistics describing the data because the signals change over time. Because the EMG signal can be

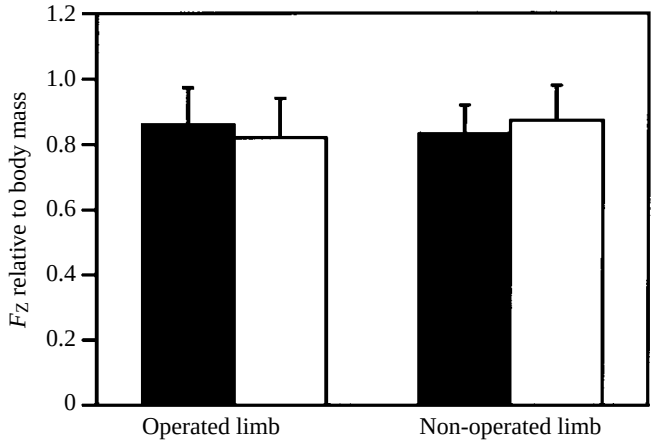

Fig. 3. No significant differences were found in normalized axial ground reaction force (F_z) on either the operated or non-operated limb during bipedal walking before (filled columns) and after (open columns) surgery. Values are mean + S.D., N=9.

assumed to be stationary for only short periods, we were forced to select data from bursts in which the data were stationary. Additional non-stationarity was introduced into the data by movement artifacts. These were superimposed on real changes in the electrical signal caused by muscle contraction and changed the frequency components in the EMG signal. We felt it would be best to be highly selective in the bursts that were selected for analysis and to eliminate any burst in which stationarity was questionable.

No significant shift in the median frequency could be detected for the hamstrings. However, there was a significant decrease (from 138.6±17.6 to 124.5±6.5 Hz; P=0.05) in median frequency for the quadriceps (Table 1). Moreover, there was a significant increase (P<0.05) in the energy in the 4–60 Hz (from 13.5±2.1 to 18.8±5.0 Hz) and 60–100 Hz (from 10.3±2.9 to 14.0±4.0 Hz) ranges for the quadriceps, and a significant decline in the 100–200 Hz range (from 56.3±6.4 to 51.2±4.6 Hz; P=0.05; Table 1). These changes indicate a shift of energy in the EMG signal to lower frequencies (Petrofsky *et al.* 1982; Nagata *et al.* 1990). We concluded that fatigue had occurred in the quadriceps muscle group.

Strain change following exercise

One dog was not used because it was limping. Strain data were collected for nine dogs. The means of the peak principal strains and shear strain on compressive (medial, antero-medial) and tensile (lateral, anterior) cortices of the tibia were calculated, and 0 min data and 20 min data were compared using Wilcoxon signed-ranks tests (Fig. 4). The tibia was placed in bending by the exercise. Peak principal strains on both compressive and tensile cortices of the tibia increased non-significantly. Maximum shear strain increased significantly (P=0.02) on the tensile (antero-lateral) cortex of the tibia. The largest changes were along the anterior and antero-lateral surfaces of the tibia, where the strain increased by an average of 26–35 % following muscular fatigue (Fig. 5).

Table 1. *Change in EMG frequency bands following exercise*

Dog	Muscle group	Exercise period (min)	Median frequency (Hz)	Frequency distribution (% energy)			
				4–60 Hz	60–100 Hz	100–200 Hz	200–500 Hz
D	Hamstrings	0	146.8	11.5	3.3	48.3	29.3
D	Hamstrings	20	150.0	10.8	12.6	56.9	18.9
E	Hamstrings	0	180.4	9.0	5.0	43.2	41.0
E	Hamstrings	20	134.1	16.0	12.7	47.1	20.8
F	Hamstrings	0	109.7	29.7	12.2	35.5	19.5
F	Hamstrings	20	90.4	35.5	15.9	37.0	7.3
H	Hamstrings	0	112.5	43.2	3.4	36.8	14.6
H	Hamstrings	20	236.9	8.2	4.1	26.1	57.7
K	Hamstrings	0	108.9	15.6	22.8	50.2	10.4
K	Hamstrings	20	59.7	46.9	7.1	31.3	11.9
Mean (±S.D.)		0	131.6±28.2	21.8±12.9	9.3±7.5	42.8±5.9	23.0±11.0
		20	134.2±60.4	23.5±15.1	10.5±4.3	39.7±11.1	23.3±17.9
D	Quadriceps	0	143.3	12.2	8.6	59.8	18.7
D	Quadriceps	20	120.9	20.0	15.9	49.0	14.0
E	Quadriceps	0	170.2	9.4	6.0	46.4	37.2
E	Quadriceps	20	137.7	16.3	10.7	44.2	28.1
F	Quadriceps	0	123.4	15.6	13.9	48.6	20.7
F	Quadriceps	20	117.4	18.4	15.2	52.6	11.4
H	Quadriceps	0	130.6	15.2	10.7	59.8	13.2
H	Quadriceps	20	123.0	28.8	7.4	53.0	9.8
I	Quadriceps	0	117.1	14.4	13.9	58.9	11.3
I	Quadriceps	20	124.4	13.2	19.8	49.5	16.5
K	Quadriceps	0	147.2	14.1	8.9	64.0	12.2
K	Quadriceps	20	120.5	15.8	15.1	59.1	9.0
Mean (±S.D.)		0	138.6±17.6	13.5±2.1	10.3±2.9	56.3±6.4	18.9±8.9
		20	124.5±6.5	18.8±5.0	14.0±4.0	51.2±4.6	14.8±6.5

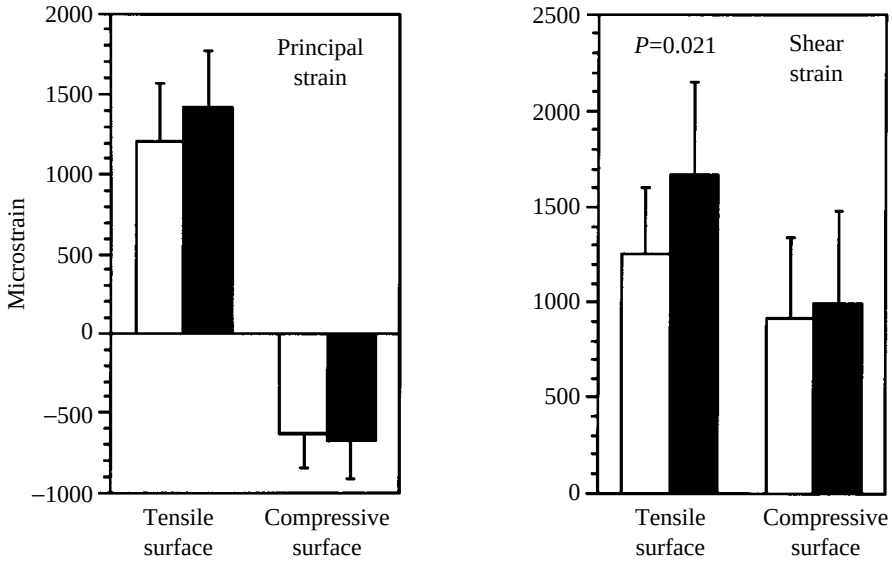

Fig. 4. The change in peak principal and shear strains on compressive and tensile tibial cortices at the beginning of each exercise period (open columns) and following 20 min of fatiguing exercise (filled columns). Error bars represent standard errors. The *P* value was calculated using the Wilcoxon signed-ranks test.

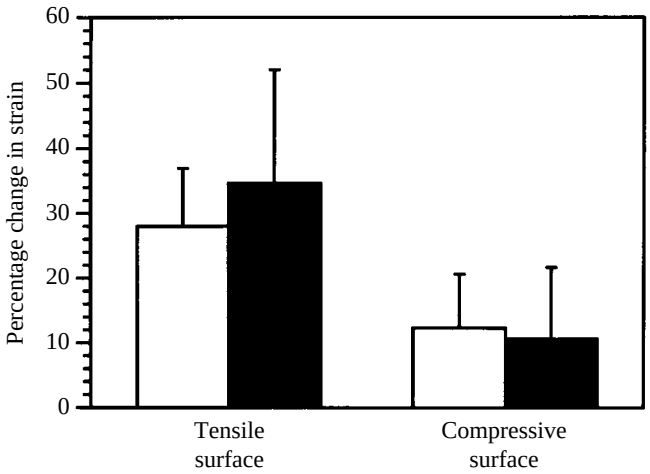

Fig. 5. The percentage change in peak principal strain (open columns) and shear strain (filled columns) on the compressive and tensile cortices of the dog tibia following 20 min of fatiguing exercise. Changes were greatest on the tensile cortex, averaging 26–35 %. Error bars represent standard errors.

Material axis strains calculated from rosettes on the antero-lateral, antero-medial and medial surfaces of the tibia were used to predict strains measured by the gauge on the anterior surface over the entire duration of loading. Predicted strains were compared with

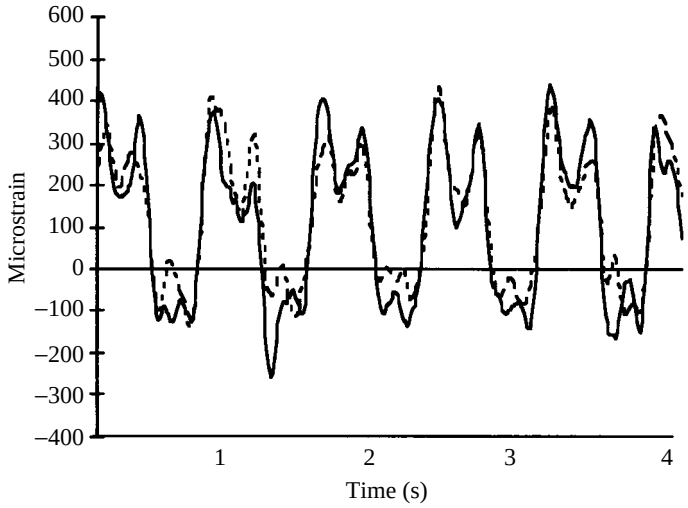

Fig. 6. Comparison of measured strain (solid line) and strain predicted by strain distribution calculations (dashed line), showing that strains from three rosettes can be used to predict strains accurately at any point on the periosteal surface of the bone. Material axis strains calculated from rosettes on the antero-lateral, antero-medial and medial surfaces of the tibia were used to predict strain measured by the gauge on the anterior tibial surface over the entire duration of strain collection. Differences between predicted and actual strains were less than 15 % at every point.

actual strains measured by the anterior rosette gauge (Fig. 6) (Carter *et al.* 1981*a*). The predicted strains were nearly identical to measured strains over the 4 s duration of the loading. Peak values of predicted and actual strain differ by less than 15 % at every point.

Using material axis strains, the cross-sectional strain distribution was calculated at the gauge site for data collected at baseline (0 min) and following 20 min of fatiguing exercise for one dog. These data show that the strain distribution across the tibial cortex changes with fatiguing exercise (Fig. 7). Prior to muscle fatigue, the orientation of the neutral axis at the moment of peak strain is nearly parallel to the anatomical axes (i.e. antero-posterior and medio-lateral axes) of the bone. Following exercise, the neutral axis at peak strain rotates clockwise by approximately 25 °.

The relationship between strain change and muscle fatigue

Significant muscle fatigue could not be demonstrated for every dog, and not all dogs demonstrated an increase in bone strain following fatiguing exercise. Therefore, we used correlation analysis to examine the relationship between the shift in median frequency of the EMG signal and the change in bone strain. Four sets of data were available to compare the EMG and strain changes on the anterior and antero-lateral cortex of the tibia after 20 min of walking (Table 2). Spearman nonparametric rank correlations were used to assess the relationship between percentage strain change and a shift in median frequency. We also employed Pearson product moment correlations to assess the relationship between median frequency and the absolute magnitude (in microstrain) of strain change.

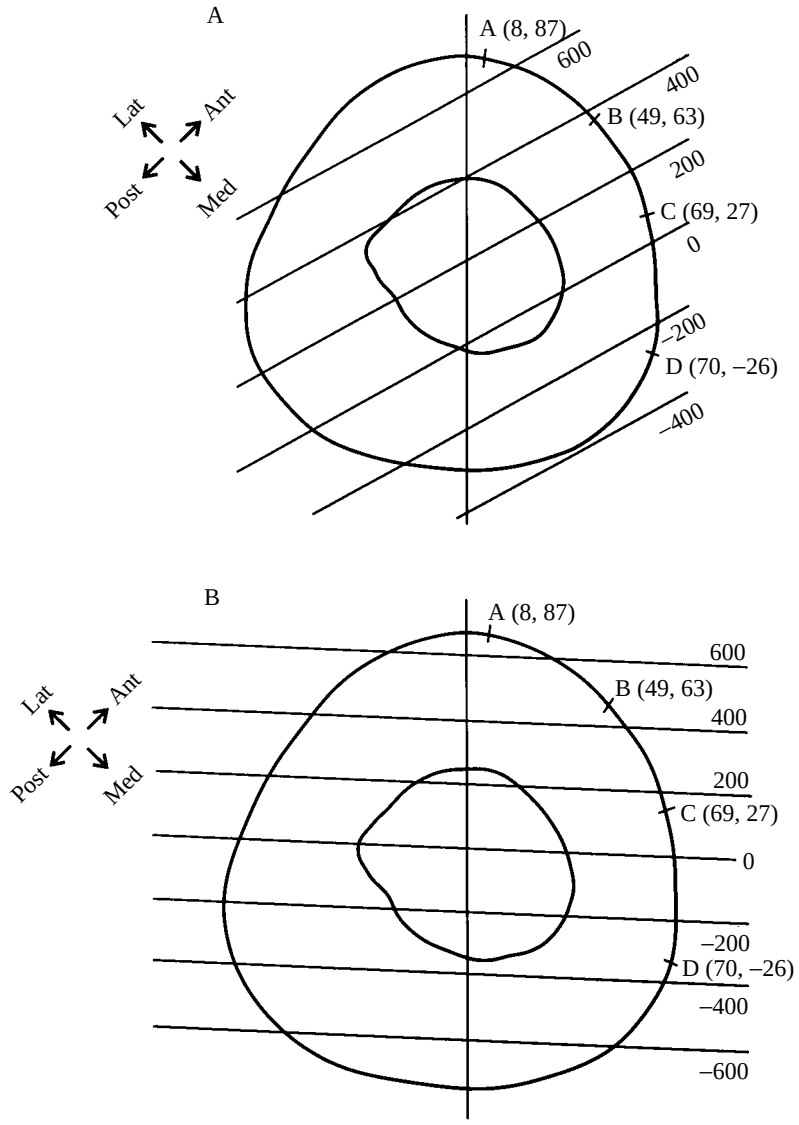

Fig. 7. The change in strain distribution on the dog tibia following 20 min of fatiguing exercise (B) is primarily due to a 25 ˚ clockwise rotation of the neutral axis. This exposed some regions of the tibia to much higher strains than were usual during normal non-fatigued locomotion (A). The strain isobars are given in units of microstrain. The letters followed by numbers in parentheses [e.g. A (8,87)] identify the strain gauge and its coordinate location on the bone surface.

Pearson and Spearman correlation tests demonstrated strong inverse correlations between median EMG frequency and bone strain after 20 min of walking (Spearman r^2=1.0, P=0.05; Pearson r^2=0.82; P=0.03, one-tailed test).

Table 2. *Changes in median EMG frequency with strain change*

Dog (limb)	Median frequency	Strain change (microstrain)	Strain change (%)
E (L)	–32.5	347.2	84.2
I (R)	7.3	–380.6	–10.4
F (R)	–6.0	136.4	21.5
H (R)	–7.6	36.4	39.0

Correlations between strain values and median frequency were r^2=1.0 (Spearman, $P = 0.05$) and r^2=0.82 (Pearson, P=0.03, one-tailed test).

Discussion

Bone fractures after very few loading cycles when tensile strains are hyperphysiological. Using reversed uniaxial fatigue loading on machined specimens from human femora, Carter *et al.* (1981*b*,*c*) showed that bone will fail within 1000–10 000 loading cycles when strain ranges are between 5000 and 10 000 microstrain. A strain range of 6000 microstrain (−3000 to +3000 microstrain) caused failure in 2147 cycles. This translates into approximately 3 km of running. Loading in uniaxial tension at 3000 microstrain, slightly higher than normal peak strains in tension *in vivo*, caused bone to fail within 100 000 loading cycles (Carter and Caler, 1983, 1985).

Carter's estimates show that bone fails quickly at strains above those normally encountered in daily living. By contrast, peak physiological strains measured *in vivo* are approximately 1500–2500 microstrain in tension (Rubin and Lanyon, 1982). *Ex vivo* mechanical tests using strains within this physiological range indicate that the fatigue life of bone under these conditions is much longer. Long-term fatigue tests of bovine primary and Haversian bone using strains and strain rates within the physiological range (1200–1500 microstrain in tension at 0.01–0.03 s^{-1}) (Lanyon and Baggott, 1976; Rubin and Lanyon, 1982) demonstrate that healthy bone does not fail in tension by fatigue (defined as a 30 % stiffness loss) even after 30×10^6 loading cycles (Schaffler *et al.* 1990). This demonstrates that the fatigue properties of bone at physiological strains are very good.

Because stress fractures do occur in life, there must be circumstances (e.g. muscular fatigue, loss of bone mass, changes in normal movement patterns or loss of coordination) under which strain ranges are greater than those measured in healthy non-fatigued individuals. Clinical observations indicate that stress fractures (Koplan *et al.* 1982; Dressendorfer *et al.* 1991) and other overuse injuries (Roy *et al.* 1989, 1990) occur most often following muscular fatigue, when the capacity of muscles to relieve strain and to protect bone from excessive overloads is compromised. Roy *et al.* (1990) showed that a shift in the median frequency of the EMG signal, indicating muscular fatigue, discriminated 100 % of those rowers with low back pain. It is known that muscles can attenuate peak dynamic forces in joints (Radin *et al.* 1991) and that neuromuscular disease is associated with higher than normal joint forces (Bergmann *et al.* 1991). Grimston *et al.* (1991) showed that subjects who had suffered a previous stress fracture produced significantly greater vertical ground reaction and propulsive forces on impact

than subjects who had not previously had a stress fracture. This suggests an association between the magnitude of impact forces on the leg and the risk of stress fracture. Whether the higher ground reaction forces were the cause of the stress fracture is uncertain. These data suggest that the way forces on the limb are controlled is important to preventing injury, but whether muscles can control bone strain has never been systematically tested in a well-controlled experiment.

The solution to this paradox (that bone does not fail at physiological strains to which it would normally be exposed, yet stress fractures are observed in overloaded bone *in vivo*) is that bone strains may periodically exceed the 'peak physiological thresholds' defined by the experiments of Rubin and Lanyon (1982) and Biewener and Taylor (1986). Nunamaker *et al.* (1990) measured strains ranging between 3317 and 5216 microstrain in running Thoroughbred racehorses and discovered that stress fractures are more common in animals subject to these high strains. At these strain magnitudes, horses presented with fatigue fractures of the third metacarpal after about 55 000 cycles of loading during 5 months of training. This is consistent with *ex vivo* estimates made by Carter *et al.* (1981*b*,*c*). The high strains in these animals may either be caused by muscular fatigue or be a consequence of the low mean tissue age and low stiffness of young bone. Age has been shown to be a risk factor for stress fractures (Nunamaker *et al.* 1987; Grimston *et al.* 1991; C. Milgrom, personal communication). These competing hypotheses cannot be discriminated without data showing what happens to bone strain when muscle becomes fatigued.

The data presented here are the first to demonstrate an association between muscle fatigue and increased bone strain magnitude or altered strain distribution. Bone strain increased when muscles became fatigued and did not increase when muscles failed to become fatigued. Fatigue of the quadriceps muscles, as indicated by a significant shift to lower frequencies in the spectral density profile, was associated with an average strain increase of as much as 35 % on the lateral surface of the tibia. However, because the distribution of strain also changed, actual local increases in bone strain were even greater in some cases. The greatest percentage increase in strain occurred near the neutral axis along the posterior cortex, where strains prior to exercise were low.

It is particularly significant that shear strains increased at a greater rate following muscle fatigue than did either principal tensile or principal compressive strains. Rubin *et al.* (1989) found that shear produced primarily by bending forces was a significant component of the strain induced by normal loading events. Because of the varying loading conditions and the complex geometry of bones, shear stresses produced by bending will not be uniform, but will concentrate in specific regions. Beam bending theory predicts the highest shear stresses close to the neutral axis. This may explain the observation of Rubin and Nunamaker (1989) that stress fractures in the cannon bone of racehorses occur near the momentary neutral axis at the instant of peak strain. It is significant that muscular fatigue caused a shift in this axis which, in addition to increasing the magnitude of the shear strains, would alter the location of the maximum shear strain. Because bone is weak in shear (Reilly and Burstein, 1974; Cowin, 1989), significant increases in shear strain near the neutral axis as a consequence of muscle fatigue could cause bone to fatigue relatively rapidly in shear.

We conclude that muscle fatigue is associated with a modest increase in strain and, perhaps more importantly, can alter the distribution of strain within the bone. This may contribute to the development of stress fractures in those cases in which fracture occurs subsequent to fatiguing exercise.

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