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# Muscle Contraction Increases the Structural Capacity of the Lower Leg: An In Vivo Study in the Rat

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**Summary:** A model to study the contribution of muscle contraction to the structural capacity of the rat tibia was developed. The right lower leg was tested to failure in three-point ventral bending during electrically stimulated muscle contraction. The left lower leg was tested without stimulation, as a control. The mean ultimate bending moment for the stimulated legs was 0.603 Nm, compared with 0.492 Nm for the unstimulated legs ( $p < 0.001$ ). The ultimate energy absorption was 0.313 and 0.188 J in the stimulated and unstimulated legs, respectively ( $p < 0.01$ ). Fracture strength has been studied nearly exclusively in dissected bone stripped of all soft tissues. The present investigation suggests that studies of dissected bone are incomplete compared with the in vivo situation, as contraction of the muscles substantially increased the fracture strength of the lower leg in rats.

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The loading capacity of whole bones may be considerably higher in vivo than has been detected in laboratory testing of dissected specimens, due to contraction of muscles surrounding the loaded bone (18). This protective effect of the muscles, however, has been studied only by indirect methods. Using finite elemental modeling, Holthe found an increased loading capacity of the femur in single-leg stance during contraction of the muscles attached to the iliotibial tract (12). Kuo et al. calculated bending moments on the tibia exceeding the estimated fracture strength by 100% in telemetric force measurements of uninjured test skiers (14). Furthermore, the available energy in a fall from standing height is about 18 times higher (10) than the energy absorbing capacity of the proximal resected femur in individuals of the same age (15). Since only a small percentage of all falls

results in a fracture (17), the loading capacity in vivo must be higher than has been found by testing of resected bone. The strength of or ability to contract muscles surrounding the loaded bone may be one factor that increases the in vivo structural capacity compared with testing of dissected specimens (18). Although there have been numerous reports on laboratory testing of dissected bone stripped of all soft tissues (2-5,9,15,19,22,23), the structural capacity of an intact leg during muscle contraction has not been reported. In an earlier study in anesthetized and dead rats, the contribution of the unstimulated muscle to the loading capacity of the intact lower leg compared with the contralateral dissected tibia was investigated (7). The anesthetized and dead animals had similar increases in ultimate bending moment of about 40% and in ultimate energy absorption of about 85% compared with the dissected tibia, probably due to relaxation of the muscles induced by the anesthetics. Consequently, the model was not representative of the muscle contribution to fracture strength in vivo.

The aim of the present investigation was to extend

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this method to study whether contraction of muscles increases the *in vivo* structural capacity of the lower leg compared to testing with relaxed muscles.

### MATERIALS AND METHODS

Ten male Wistar/Han/Mol SPF rats (Møllegaard, Copenhagen, Denmark), with a mean weight of 320 g (range 308-337 g), were used. The animals were anesthetized with a combination of Hypnorm (fluanisone, 5mg/ml, and fentanyl citrate, 0.1575 mg/ml) (Janssen Pharmaceutica BV, Beerse, Belgium) and Dormicum (midazolam, 2.5 mg/ml) (Hoffmann La Roche, Basel, Switzerland). The dose was 0.2 ml/100 g body weight, given subcutaneously. The experiment conformed to the Norwegian Council of Animal Research Code for the Care and Use of Animals for Experimental Purposes.

Prior to creation of the stimulated fracture, the right hind limb was shaved and a longitudinal incision was made over the lateral aspect of the right femur. The intermuscular septum between the biceps femoris and quadriceps muscles was divided, and the ischiatic nerve was dissected from its surrounding layer of connective tissue for approximately 1.5 cm, just proximal to the main division of the nerve (tibial and peroneal branches). A bipolar electrode was

connected to the nerve, with the cathode placed distally to induce antegrade depolarization. The electrode consisted of two silicone-coated wires (AS 632 biomed wire; Cooner Wire, Chatsworth, CA, U.S.A.), mounted 7 mm apart in an open silicone tube. The tube was carefully placed around the nerve and ligated to prevent displacement during testing. Proximal to the electrode, the nerve was crushed between forceps to prevent retrograde depolarization. The electrode was connected to a nerve stimulator (Pulsar 6i; Frederick Haer, Brunswick, ME, U.S.A.).

The skin incision was closed, and the rat was suspended in a "coat," leaving the right lower leg free. A clamp was fixed to the lower leg with suspension under the foot, and the rat was placed in a modular test apparatus (Fig. 1). The test machine originally was developed for the rat femur (8), but it also is suitable for testing of the intact lower leg of rats (7). In the present experiment, the lower leg was deflected ventrally in a three-point bending test. The load was transferred to the leg by a cam mounted on the rotating disc of the test apparatus, thus giving a moment-arm equal to the radius of the disc. The cam engaged the soft tissues dorsally to the tibial condyle. A fulcrum positioned anterior to the leg was the third point of force application.

The ischiatic nerve was supramaximally stimu-

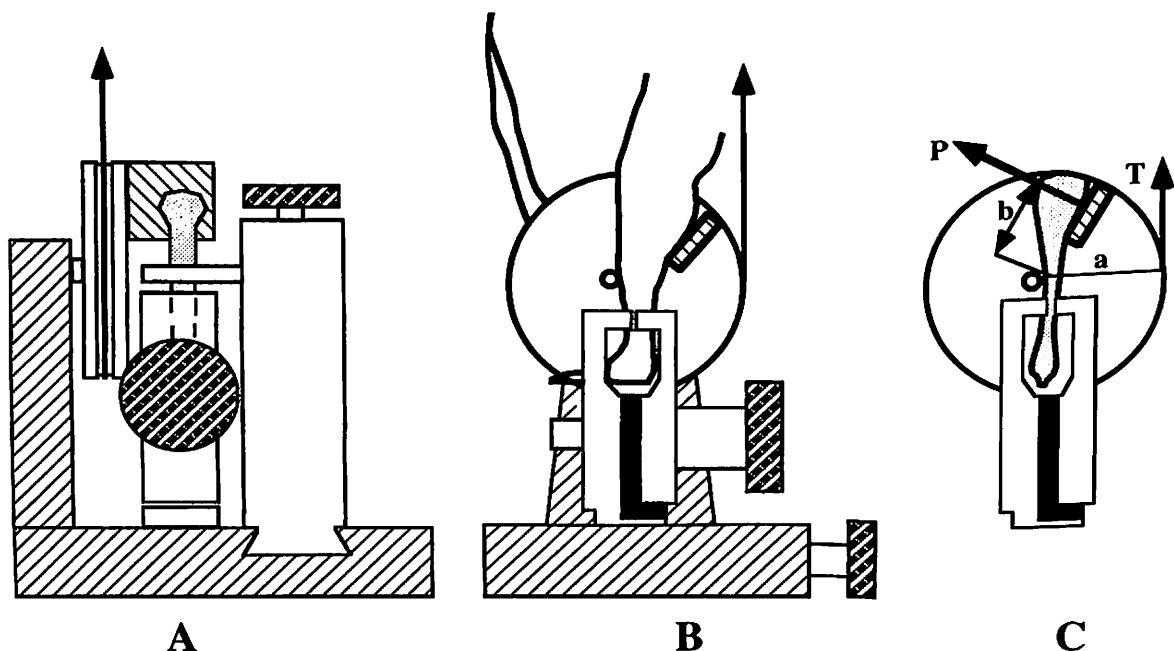


FIG. 1. Test apparatus for bending. Frontal view (A) with dissected specimen. Lateral view (B) with the rat placed for *in vivo* testing. Lateral view (C) shows that the ultimate bending moment ( $T \times a$ ) equaled the moment needed to bend the proximal part of the test specimen (here dissected tibia) ventrally ( $P \times b$ ). The radius ( $a$ ) of the rotating wheel is 21.11 mm.  $T$  = ultimate load,  $a$  = moment-arm,  $P$  = force (in newtons) at one given time during testing, and  $b$  = moment-arm at one given time during testing.

lated with 0.5 ms square pulses of 80 Hz, with an amplitude of 6 V. The right lower leg was deflected ventrally to fracture during contraction of all muscles of the lower leg. The load was applied at a quasi-static rate of 0.095 r/s (5.43°/s). In pilot studies, the force of the tetanic contraction of the triceps surae had shown a slow decline to about 66% of initial force after 6 s. The loading time to failure was 7-8 s from the initial "setting." Stimulation was therefore started shortly after the initiation of loading (Fig. 2), so that the force of contraction was as high as possible at the time of fracture.

The load in the test apparatus was measured with a load cell, which was connected to a microcomputer via an amplifier. The load-deflection curve was recorded online in WorkBenchMac software (Strawberry Tree, Sunnyvale, CA, U.S.A.). Ultimate bending moment, ultimate energy absorption, bending stiffness, and deflection were read directly or were calculated from the computer recordings. Ultimate bending moment was taken as the product of the ultimate load and the moment-arm (Fig. 1). Ultimate energy absorption (work to failure) was the area under the load-deflection curve (Fig. 2). Bending stiffness was defined as the slope of the linear elastic part of the curve and was read directly from the computer. Deflection was taken as the distance on the X-axis from the point of intersection of the linear portion of the load-deflection curve to the point of failure.

For the unstimulated control fracture, the ischiatic nerve on the left side was isolated and sectioned, to ensure that the muscles were totally relaxed during testing. The left lower leg was fixed to the clamp, and the animal was positioned in the test apparatus.

Thereafter, the lower leg was loaded to failure as already described. The maximum difference in fracture position on the right and left side in a test animal was allowed to be as much as 2.0 mm before it was excluded from the study.

The Student paired *t* test (two-tailed) was used for statistical evaluation of differences between findings in the stimulated and the unstimulated legs.  $P < 0.05$  was considered significant.

## RESULTS

Both the tibia and the fibula were fractured during the *in vivo* test procedure. Fractures in the tibias of the stimulated legs were transverse or oblique in the posterior-proximal to anterior-distal direction, whereas tibial fractures in the unstimulated legs were transverse or oblique in the posterior-distal to anterior-proximal direction. The tibial fractures were positioned at a mean distance of 16.4 mm (range, 15.5-17.6 mm) from the malleolar plane in the right stimulated legs and a mean of 17.1 mm (range, 16.1-17.9 mm) from the malleolar plane in the left (unstimulated) legs. One animal was excluded due to a difference in fracture position of more than 2.0 mm.

The initial nonlinear part of the load-deflection curves for the stimulated and unstimulated legs represents compression of the soft tissues, which have a much lower stiffness than the bone (Fig. 2). The vertical part of the stimulated curve (starting from the tip of the arrow) represents the effect of expansion of the calf muscles during contraction and possibly a change in the structural stiffness of the entire limb.

The ratio for the stimulated to the unstimulated

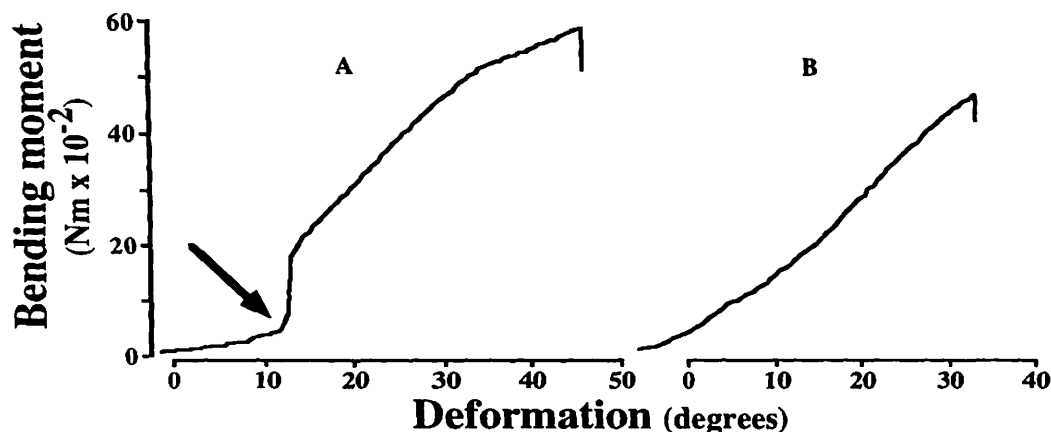


FIG. 2. Representative load-deflection curves for three-point ventral bending of the lower leg during muscle contraction (A), and for the contralateral leg tested with relaxed muscles (B). The arrow indicates the start of muscle contraction.

**TABLE 1.** Biomechanical data (mean  $\pm$  SD) for right lower leg tested during tetanic muscle contraction (stimulated) and left lower leg tested with relaxed muscles (unstimulated)

	Ultimate bending moment (Nm $\times 10^{-2}$ )	Ultimate energy absorption (J $\times 10^{-2}$ )	Bending stiffness (Nm/ $^{\circ}$ $\times 10^{-3}$ )	Deflection ( $^{\circ}$ )
Stimulated leg	60.3 $\pm$ 9.0 <sup>a</sup>	31.3 $\pm$ 8.1 <sup>b</sup>	14.47 $\pm$ 3.1	51.9 $\pm$ 8.3 <sup>b</sup>
Unstimulated leg	49.2 $\pm$ 6.1	18.8 $\pm$ 5.4	15.2 $\pm$ 2.6	35.7 $\pm$ 5.7
Ratio of stimulated to unstimulated values in each animal	1.22 $\pm$ 0.09	1.73 $\pm$ 0.49	0.96 $\pm$ 0.19	1.50 $\pm$ 0.37

<sup>a</sup> P < 0.001.<sup>b</sup> P < 0.01.

leg in each animal gave an average increase of 22.4% in ultimate bending moment due to muscle contraction ( $p < 0.005$ ) (Table 1). The average ultimate energy absorption increased by 73.3% ( $p < 0.005$ ), and the mean deflection increased by 49.7% ( $p < 0.005$ ). The average bending stiffness decreased by 4% in the stimulated legs compared with the unstimulated legs (not significant).

## DISCUSSION

The aim of this study was to develop a model to study how muscle contraction increases the structural capacity of the lower legs in rats. Muscle contraction resulted in a substantial and significant increase in ultimate bending moment, energy absorption to failure, and ultimate deflection.

Testing of an intact limb during muscle contraction is much more complicated than testing of resected whole bone. However, the method gave reproducible results, with a coefficient of variation for the fracture position of 4.1 and 3.5% for the stimulated and unstimulated tibias, respectively. The corresponding coefficients of variation for the ultimate bending moments were 14.9 and 12.3%. The magnitude of these coefficients is probably due to biological variation in both bone (5) and contraction of muscles, as the test apparatus has a coefficient of variation for testing of steel rods to 45° deflection of only 1% (8).

In rats, the fibula is incorporated distally into the tibial shaft. The synostosis is positioned one-third of the tibial length from the malleolar plane, and the fibula lies as a string dorsally and laterally to the tibia. If the fibula remained intact until the tibia fractured, this would diminish the effect of the muscle contraction, since the structural effect of an intact fibula on the tibial strength would be similar to the effect of muscle contraction. However, in a pilot study, during loading of the tibiofibular bone dissected free of all soft tissues, the fibula fractured

during the initial part of the loading, and this was probably also the case during in vivo loading.

The animals used in this study were about 12 weeks old, which is close to skeletal maturity for this strain of rats (6). The lower legs were loaded through the soft-tissue bulk, dorsal to the tibial condyle. This loading probably affected the force of the tetanic contraction in the stimulated legs as muscles, vessels, and nerves were compressed between the cam and the bone. Nevertheless, contraction of the muscles had a significant effect. The ischiatic nerve was stimulated proximal to the division into the tibial and peroneal branches. As a result, there was contraction of all muscles of the lower leg during testing. The contracting muscles anteriorly in the stimulated legs may have lessened the stress concentration in the tibia due to the fulcrum compared with the unstimulated leg. Muscles with a line of action anterior to the neutral axis of bending probably also have a negative effect during ventral deflection, as they produce extra compressive stress anteriorly. In rats, as in humans (13), the strongest muscles have a moment-arm posterior to the tibia. The load-deflection curves for the stimulated legs showed decreasing stiffness just prior to fracture (Fig. 2). This change in structural stiffness could represent plastic deformation of the tibia, but as the decrease in stiffness was less in the unstimulated legs (Fig. 2), it might have been the result of a declining force of muscle contraction.

Muscle contraction increased the ultimate bending moment of the intact limb by 22%. In clinical situations, this protection may be enough to avoid fracture during external loading that just exceeds the fracture limit of the limb. The explanation for the increase in ultimate bending moment and ultimate energy absorption is probably that contraction of the muscles dorsally to the neutral axis of bending produced compressive stress that neutralized the tensile stress (18). Since compact bone can tolerate from 45% (20) to 65% (3) more stress when tested in

compression than in tension, the fracture strength can be expected to increase.

The tibia could be deflected 50% more before fracture during contraction of muscles. This increase in deflection probably was the effect of a posterior shift of the neutral bending axis due to the addition of compressive loading from the contracting muscles (11). Local deformation at the three points of loading may lead to an overestimation of the true deflection (15). However, deflection was calculated from the extrapolation to the X-axis of the slope of the linear-elastic part of the load-deflection curve, thereby eliminating the effects of local deformation.

To our knowledge, this is the first study reporting the direct measurement of the effect of muscle contraction on the structural capacity of an intact limb. Kuo et al. (14) calculated the tibial bending moments in alpine skiers from force measurements between the boot and the ski; they found bending moments exceeding the laboratory strength of dissected tibias by 100% in uninjured skiers. Holthe (12) used finite element modeling of the femur and the iliotibial tract and studied the effects of contraction of the muscles attached to the tract. With a force in the tract equal to the reduced body weight (the weight of the stance leg subtracted from the body weight), tensile stress on the lateral side was reduced by 37% and compressive stress on the medial side, by 26% during loading representing one-legged stance. The increase in ultimate bending moment due to contraction of muscles in the present study is of the same magnitude as the stress changes found by Holthe (12).

The 73% increase in absorbed energy to fracture by muscle contraction was more than three times higher than the increase in ultimate bending moment (22%). Rosson et al. (21) noted that "given that static strength [ultimate bending moment] is sufficient to maintain posture, it would seem that energy absorbing capacity is far more relevant to resistance to fracture in the dynamic activities of everyday life." Lotz and Hayes (15) found the energy absorbing capacity of the dissected proximal femur to be 26.5 J in mechanical testing simulating a fall. The energy available during a fall from standing height in individuals of corresponding age was found by Hayes et al. (10) to be 495 J in those in whom the hip fractured and 447 J in those in whom it did not. In a previous study in rats tested with relaxed muscles, we found the increase in energy absorbing capacity by the soft tissues to be about 85% compared with the dissected tibia (7). An additional increase of 73% by muscle

contraction gives a more than twofold difference between dissected and stimulated in vivo energy absorbing capacity of the lower leg. The soft tissues and muscle contraction therefore may help to explain a part of the discrepancy in energy absorbing capacity, found by Lotz and Hayes (15), and in available energy, reported by Hayes et al. (10). Whether or not a hip fracture is sustained during a fall could also be explained by the degree or ability of muscle contraction, since the available energy in the two groups investigated by Hayes et al. (10) was between 16.7 and 18.7 times higher than the dissected upper femur could sustain.

The method we have presented for in vivo testing of intact lower legs proved to be a reliable laboratory model for study of the increase in structural capacity due to contraction of muscles. Although the present investigation was carried out in the lower legs of rats, we propose that the increase in structural capacity due to muscle contraction is a general principle of the musculoskeletal system. In studies on dissected bone, the structural strength and the material qualities of bone can be evaluated. In research on ski injuries, attempts have been made to combine results from material testing of bone with the contribution of muscle contraction to bone strength (1). Our data imply that the contribution of contracting muscles should be considered before the results of testing of resected bone can be applied to in vivo situations (for instance, the assessment of fracture risk), as fracture strength is significantly increased during contraction of muscles in the lower legs of rats.

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