Muscle contribution to tibial fracture strength in rats

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We studied the contribution of anesthetized and dead muscle to the loading capacity of the tibia in ventral three-point cantilever bending directly by a newly developed method in rats. Ketamine anesthesia, known to give poor muscle relaxation, did not increase the loading capacity more than Hypnorm®/ Dormicum® anesthesia and dead muscle. The ultimate bending moment increased by about 40 percent and the ultimate absorbed energy by about 85 percent when the tibia was tested in situ compared to testing in the dissected state; the tibia could withstand approximately 50 percent more deflection when loaded intact with soft tissues. No differences were observed between the tibia of anesthetized and newly-killed rats. The results indicate that the soft tissues are quantitatively important for the strength of long bones, and for the understanding and prevention of fractures.

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There are numerous reports on mechanical testing of bone stripped of all soft tissues, but the total strength of an intact long bone with muscles has, however, not been studied in the laboratory. We report results of a method developed to determine the ultimate strength of the lower leg in anesthetized rats.

Material and methods

28 male Wistar/Han/Mol SPF rats (Møllegård, Copenhagen), median weight 285 g (Table 1), were randomly divided into 3 groups. The experiments conformed to the Norwegian Council of Animal Research Code for the Care and Use of Animals for Experimental Purposes. One group (DEAD, n 10) was killed by neck elongation in ketamine (260 mg/kg body weight; Ketalar; Parke, Davis & Co. Ltd., Pontypool, Gwent, U.K.) anesthesia, and the lower legs were tested after 30 minutes to evaluate any effects of dead muscles. The second group (HYP/DORM, n 10) was tested in situ in fluanisone (4 mg/kg)-fentanyl (0.08 mg/kg; Hypnorm; Jansen Pharmaceutica BV, Beerse, Belgium)/midazolam (1.9 mg/kg; Dormicum; Hoffmann La Roche, Basel, Switzerland) anesthesia. The third group (HYP/KET, n 8) was tested in situ in fluanisone (4 mg/kg)-fentanyl (0.08 mg/kg)/ketamine (40 mg/kg) anesthesia.

The animals were suspended in a "coat" (Figure 1). The distal right lower leg was fixed in a clamp and mounted in a modular test apparatus, originally developed for the rat femur (Engesæter et al. 1978). The tibia with intact soft-tissues was loaded in ventral three-point cantilever bending to failure at a deflection rate of 0.04 radians (2.5 degrees) per second. Both tibiae were resected, and the length of the distal frag-

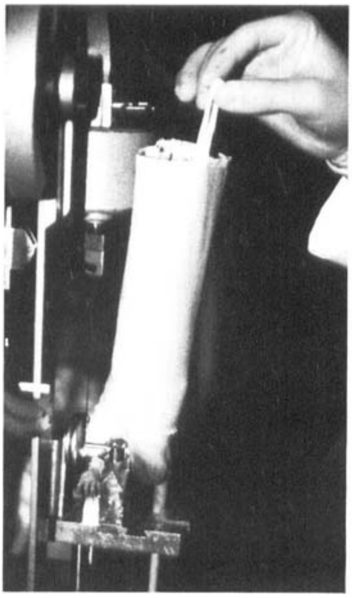

Figure 1. Rat suspended in the "coat", and attached to the modular test apparatus.

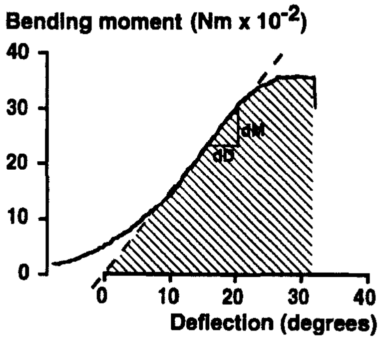

Figure 2. Load deflection curve of the lower leg tested in three-point ventral cantilever bending with intact soft-tissues. Hatched area illustrates ultimate energy absorbtion. dM/dD = bending stiffness.

Table 1. Weight of rats, and length of the distal and proximal tibial fragments after three-point ventral bending until fracture with intact soft tissues in rats anesthetized with Hypnorm/ketamine (HYP/KET), Hypnorm/Dormicum (HYP/DOR) and in animals tested 30 minutes after being killed (DEAD), median (25–75 percentiles)

Group	n	Weight (g)	Distal fragment (mm)	Proximal fragment (mm)
HYP/KET	8	280 (263–285)	18 (18–18)	20.5 (20–21)
HYP/DOR	10	285 (280–290)	17 (17–18)	21 (21–22)
DEAD	9	288 (285–294)	18 (18–18)	21 (21–21)

Table 2. Coefficient of variance for mechanical variables in tibiae tested with and without intact soft tissues

Tibia	n	Bending moment	Energy absorption	Bending stiffness	Deflection
Intact	27	0.06	0.29	0.13	0.21
Dissected	27	0.14	0.20	0.21	0.18

ment of the fractured bone was measured with sliding calipers (accuracy of ± 0.01 mm). The fracture distance from the malleolar plane was marked on the left tibia, which was tested with the fulcrum of the apparatus positioned correspondingly to place the fracture exactly at the same site in both lower legs. 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. During loading of the dissected tibia with fibula, the cam first engaged the fibula and fractured it. Thus the fibula gave negligible contribution to the strength of the tibia (pilot study). During in situ testing the fibula probably fractured during the initial part of the loading. The fibula was therefore removed before testing of the dissected tibia. The maximum difference in fracture position on the right and left sides in one test animal was allowed to be up to 1.5 mm apart before exclusion from the study.

The load-deflection curves were transferred to a chart recorder, and the ultimate bending moment, the ultimate energy absorption, the bending stiffness, and the ultimate deflection angle were calculated (Figure 2; Ekeland et al. 1981). Deflection in the in situ leg was calculated as the distance on the x-axis from the interception of the linear-elastic part of the curve to the point of failure.

Results are presented as medians with 25 and 75 percentiles. Wilcoxon's signed rank test for paired data was used for evaluation of test/control differences. Group differences were evaluated by the Kruskal-Wallis test, followed by the Mann-Whitney U test if significant differences were detected. $P < 0.05$ was considered significant. The coefficient of variance (V) was calculated as

$$V = \frac{(75\text{ percentiles}-25\text{ percentiles})}{\text{median}} \times 0.74$$

(Diem and Lentner 1975).

Results

The fractures were transverse or slightly oblique, and located distally in the middle third of the tibia (Table 1). One animal in the DEAD group was excluded because the fracture position was more than 1.5 mm apart in the two tibiae. The precision of the fracture localization was high, with a coefficient of variance of 0.03. The coefficients of variance for the mechanical parameters obtained after testing the tibiae with and without musculature varied from 0.06 for the ultimate bending moment to 0.29 for ultimate energy absorption (Table 2).

The ultimate bending moment increased by a median of 36, 37, and 45 percent in the HYP/KET, HYP/DOR and DEAD animals, respectively, when the tibia tested in situ was compared to the dissected tibia (Table 3). The ultimate (absorbed) energy increased by 106, 83 and 88 percent in the HYP/KET, HYP/DOR and DEAD animals, respectively. The bending stiffness decreased by 31, 22, and 11 percent, and the ultimate deflection angle (deflection) increased by 62, 51

Table 3. Ultimate bending moment (Nm x 10^{-2}), energy absorption (J), bending stiffness (Nm/° x 10^{-2}), and deflection (°) for the test tibia (T) fractured by three-point ventral bending with intact soft tissues, and for the contralateral control tibia (C) fractured in the dissected state. Groups of rats were either anesthetized with Hypnorm/ketamine (HYP/KET), Hypnorm/Dormicum (HYP/DOR) or tested 30 minutes after being killed (DEAD), median (25–75 percentiles)

		Bending moment		Energy absorption		Bending stiffness		Deflection	
	n	T	C	T	C	T	C	T	C
HYP/KET	8	40	30	9.8^a	4.7	1.8^a	2.7	36^a	21
		39–42	29–33	6.6–12	3.8–5.8	1.7–2.0	2.4–3.9	30–40	19–23
HYP/DOR	10	41^b	33	12^b	5.9	1.9^b	2.6	38^b	23
		35–45	27–33	7.2–13	4.7–6.3	1.7–2.2	2.1–2.9	29–42	23–2
DEAD	9	42^b	29	11^b	5.6	2.0	2.4	35^a	24
		41–43	27–30	8.4–12	4.7–6.0	1.8–2.2	1.8–2.6	30–40	22–28

Intergroup test-control difference ^{a} $P < 0.05$, ^{b} $P < 0.01$ (Wilcoxon's signed rank test for paired differences).

and 49 percent in the HYP/KET, HYP/DOR and DEAD animals, respectively. All in situ/dissected differences were significant, except for the bending stiffness in the dead animals. No significant differences were observed when the three groups of rats were mutually compared (Table 3).

Discussion

To our knowledge, this is the first article reporting a direct measurement of the muscle contribution to the loading capacity of a long bone. The ultimate bending moment increased by approximately 40 percent when the tibia was tested with intact soft-tissues compared with the dissected contralateral tibia. The increase in energy absorption was 85–90 percent, and the tibia could withstand about 50 percent more deflection before fracture.

The presented method for strength-testing of the lower leg in situ gave a precise localization of the fracture. For the mechanical results the coefficient of variance was 0.06 for the ultimate bending moment of the intact leg, but 0.29 for the energy absorption. The coefficient of variance for the testing of dissected femora in the same machine has previously been reported to be 0.08 (Ekeland et al. 1981). The variance in mechanical parameters is mainly due to biological variation of the test specimens including variation in muscle rigidity, as the precision of the test apparatus is high, with a coefficient of variance of only 0.01 for testing of steel rods (Engesæter et al. 1978). Strömberg and Dalén (1976) reported a precision of 0.03 for their test apparatus.

The initial part of the load-deflection curves for the lower leg tested with intact soft-tissues (Figure 2) displayed some similarities to those obtained when testing skin specimens (Ekeland et al. 1983), probably due to the longer loading time of the soft tissues. The increase in ultimate bending moment due to the soft tissues in the present study is lower than the 100 percent reported by Kuo et al. (1983) in telemetric recordings of uninjured alpine skiers. Their tibial bending moments were, however, calculated from recorded forces between the ski and the sole of the boot. The present results are direct measurements, and thus have a higher degree of validity. Furthermore, the skiers probably contracted their triceps surae muscles, in contrast to the anesthetized animals who lost most of the muscle tone. The increase in energy-absorbing capacity was twice as high as the increase in bending moment in the present study. Rosson et al. (1991) states that "given the 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". Deflection was about 50 percent higher in the in situ tibia compared to the resected tibia. Local deformation at the three points of loading due to the presence of soft tissues could have led to an overestimate of the true deflection of the tibia (Lotz et al. 1991). However, deflection was taken from the interception of the linear part of the load/deflection curve with the x-axis, thereby eliminating the effect of local deformation of the soft tissues.

The loading of the lower leg was applied at a quasi-static rate in this study. Ketamine anesthesia is assumed by some authors not to influence muscle reflexes in rats (van den Berge et al. 1990), and a more dynamic loading might have triggered spinal reflexes resulting in increased muscular tone and thus higher ultimate bending moments. The load was transferred from the cam of the test machine to the soft tissues dorsal to the tibial condyles and thus compressed the soft tissues. This could affect the ability of the muscles to maintain their tone, or the possibility of nerve impulses reaching the muscle.

No differences were observed between the three groups of rats. This is probably related to the anesthetics used. Surprisingly, dead musculature increased the ultimate bending moment (Table 2). This may partly be due to rigor mortis in the dead animals. The explanation for the increase in mechanical values in the tibiae tested in situ is probably that the elongational elasticity of the soft calf tissues dorsally transforms tension forces on the dorsal side of the tibia to compression forces. Since bone is from 45 to 65 percent stronger in compression than in tension (Burstein et al. 1972, Reilly and Burstein 1975), the bending strength will increase when soft tissue is present dorsally. If the lower leg had been deflected dorsally or in another direction, the increase in structural capacity would probably have been smaller, since most of the muscles in the rat lower leg lie dorsally to the tibia. Ketamine was used, since this drug has a relatively small influence on the muscle tone in humans (Collins 1976) and in lower doses in rats (Wixson et al. 1987). This did not, however, increase the bending moments in the ketamine group with doses that induced sufficient analgesia. Wixson et al. (1987) reported poor muscle relaxation in rats for a combination of 40 mg/kg ketamine and 5 mg/kg diazepam. Their evaluation of muscle tone was only clinical. In a pilot study (Nordsletten and Ekeland, unpublished data) where anesthesia was induced with ketamine alone, 140 mg/kg was needed for surgical anaesthesia, and all muscle tone was lost. The combination of a low dose of ketamine and fluanisone was used since this clinically seemed to retain some muscle tone. Future experiments should employ anesthetics with less effect on the muscle tone, or electric stimulation of the calf muscles.

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