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Difference in stretching of sarcomeres between medial gastrocnemius and tibialis anterior by tibial lengthening: an experiment in rabbits

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ABSTRACT

Purpose. To compare the degree of stretching of sarcomeres—in the medial gastrocnemius versus the tibialis anterior—acutely extended by tibial lengthening.
Methods. The right lower legs of 6 New Zealand White rabbits were acutely distracted by 6.3 mm using an external fixator, whereas the left lower legs served as controls. Immediately after distraction, the ankle was dorsiflexed at 50 degrees and the knee flexed at 140 degrees, with respect to the posture of resting on the ground. Both legs were immersed in 10% buffered formalin for fixation, and the medial gastrocnemius and tibialis anterior were removed. Length of the 2 sarcomeres was measured by a laser diffraction technique using isolated muscle fibre bundles.
Results. The mean lengths of sarcomeres in the medial gastrocnemius and tibialis anterior were 3.0 µm and 2.3 µm in the lengthened legs, and 2.4 µm and 2.1 µm in control legs, respectively. The degree of stretching of corresponding sarcomeres was therefore 25% and 10%, this difference being significant ($p<0.01$).
Conclusion. Sarcomeres of the medial gastrocnemius are more stretchable than those of the tibialis anterior following tibial lengthening.

Key words: *bone lengthening; muscle fibers; sarcomeres*

INTRODUCTION

Muscle stiffness and weakness are major problems encountered in limb lengthening.[1–3] Stiffness is mostly caused by increased tension of connective tissue components within a lengthened muscle.[4] Weakness of muscle power is associated with damage to muscle fibres, including degeneration and necrosis (histologically) and overstretching of sarcomeres with disarrangement of sarcomere striation (microscopically).[4,5]

Dysfunction of ankle plantar flexors is frequently

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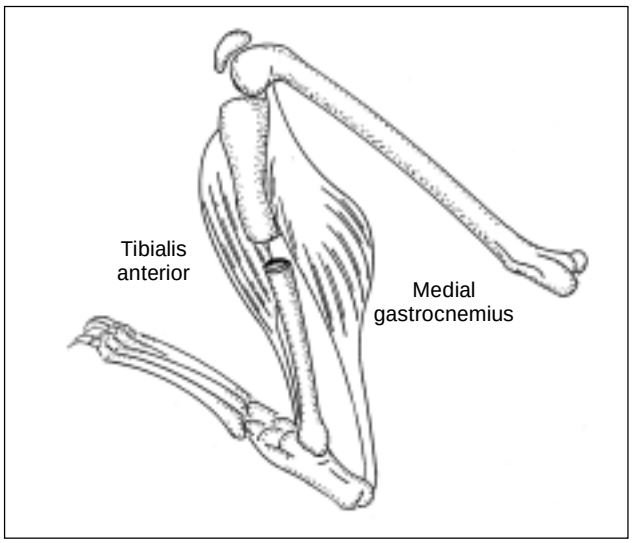

Figure 1 A leg is fixed in a position of 50° ankle dorsiflexion and 140° knee flexion after tibial lengthening.

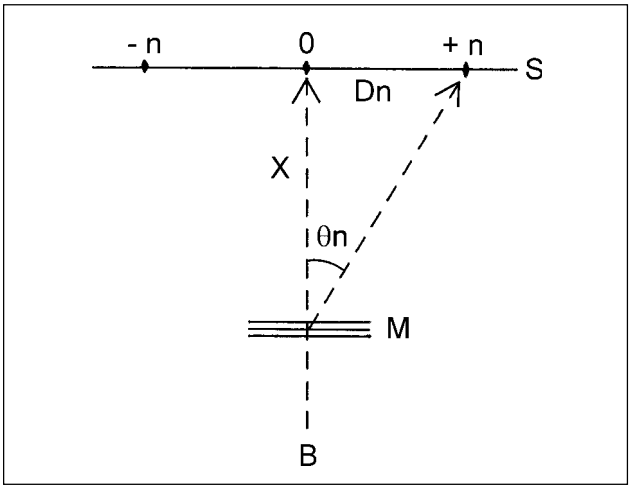

Figure 2 The muscle fibre bundle (M) is illuminated by the laser beam (B) and the diffracted (n) and undiffracted (0) lines are shown on the screen (S). The value of $\sin \theta_n$ was determined from the relationship $\sin \theta_n = D_n / \sqrt{X^2 + D_n^2}$. D_n denotes the distance between the zeroth and n-th order lines, and X the distance between the screen and muscle fibre bundle (10 cm).

experienced in tibial lengthening. Histological changes of muscle fibres have been reported predominantly in ankle plantar flexors but rarely in ankle dorsiflexors.[6] Muscle fibres in ankle plantar flexors are particularly susceptible to damage by lengthening because they have the physiological characteristics of large power and slow contraction velocity and are composed of short muscle fibre bundles with a highly pennated arrangement compared with ankle dorsiflexors.[7–9] It remains uncertain whether the degree of stretching of sarcomeres by tibial lengthening differs between ankle plantar flexors and ankle dorsiflexors. We compared the degree of stretching of sarcomeres between the medial gastrocnemius (MG) and tibialis anterior (TA) in rabbits when both muscles were acutely extended by tibial lengthening.

MATERIALS AND METHODS

Six New Zealand White rabbits weighing 2.3 to 2.7 kg were used. The right lower legs were lengthened and the left lower legs served as controls. Under general anaesthesia with intravenous sodium pentobarbital (35 mg/kg), a longitudinal skin incision was made on the medial aspect of the right lower leg, and the tibia was subperiosteally exposed. Four cortical screws were inserted into the tibia, perpendicularly to the long axis. A unilateral external fixator (Orthofix M-100; Orthofix, Verona, Italy) was tightened on the screws. The tibia and fibula were osteotomised at the mid-shaft between the 2 inner screws. The tibia was lengthened on a single occasion by distraction to 6.3 mm—a length corresponding to the difference between the length of MG in full dorsiflexion and in full plantar flexion of the ankle. Immediately after distraction, animals were killed with an intravenous barbiturate overdose and disarticulated at the hip joint. Under irrigation of Tyrode solution (NaCl 8.0 g, KCl 0.2 g, CaCl 0.2 g, MgCl 0.05 g, NaHCO 1.0 g, NaHPO 0.04 g, and glucose 1.0 g in 1000 ml water), both the distracted and control legs were skinned and mounted on a board with the ankle dorsiflexed at 50° and the knee flexed at 140° to eliminate the effect of joint angle on muscle length (Fig. 1). The leg position was determined with respect to the posture of rabbits resting on the ground.[10] The specimens were immersed in 10% phosphate-buffered formalin (0.4 M phosphate buffer, pH 7.2) for 5 days to fix the muscle, and then rinsed in phosphate buffer (0.4 M, pH 7.2) for 24 hours. The MG and TA were removed afterwards.

The length of a whole muscle was measured as the distance from the origin of the most proximal muscle fibre bundles to the insertion of the most distal muscle fibre bundles. To isolate muscle fibre bundles without disruption, muscles were placed in 20% sulphuric acid for 48 hours to digest the surrounding connective tissue, and then rinsed in phosphate buffer (0.4 M, pH 7.2) for 24 hours. Five muscle fibre bundles were isolated from different regions of the muscle under a dissecting microscope, and their lengths

Table
Comparison between lengthened and control legs

Muscle	Lengthened leg Mean (SD)	Control leg Mean (SD)	Difference Mean (SD)
Medial gastrocnemius			
Whole muscle (mm)	75.3 (2.5)	69.7 (2.2)	5.5 (0.7)
Muscle fibre bundle (mm)	24.7 (1.3)	19.8 (1.1)	4.8 (0.5)
Sarcomere (μm)	3.0 (0.1)	2.4 (0.1)	0.6 (0.1)*
Tibialis anterior			
Whole muscle (mm)	65.6 (2.5)	60.7 (2.0)	4.9 (1.0)
Muscle fibre bundle (mm)	47.1 (3.1)	42.9 (3.2)	4.2 (0.8)
Sarcomere (μm)	2.3 (0.1)	2.1 (0.1)	0.2 (0.1)*

* $p<0.01$, paired *t*-test

measured with a caliper. The 5 measurements were averaged to yield one value per muscle.

Sarcomere length of the isolated muscle fibre bundles was determined by a laser diffraction technique (Fig. 2).[11] The muscle fibre bundle was mounted on a glass slide and illuminated by the beam of a helium-neon laser (wavelength, 0.632 μm). The diffraction pattern was projected onto a screen perpendicular to the beam and 10 cm apart from the muscle fibre bundle on the glass slide. Sarcomere length was calculated as the distance between the zeroth and first order diffraction lines using the grating equation:

$$n\lambda = d \cdot \sin \theta_n$$

where n is the order of the diffraction line assuming the zeroth order bisected the orders on either side, λ is the wavelength of the light, d is the sarcomere length, and θ_n is the n-th order diffraction angle.[12,13] Measurements were obtained from 10 different sites in the middle two thirds on each muscle fibre bundle and averaged to one value per muscle fibre bundle. The mean sarcomeric length of a given muscle was calculated by averaging the sarcomeric lengths of 5 muscle fibre bundles.

RESULTS

The lengths of the whole muscle, muscle fibre bundle, and sarcomere in the lengthened legs and control legs are listed in the Table. The mean length of sarcomeres in MG and TA was 3.0 μm and 2.3 μm in lengthened legs, and 2.4 μm and 2.1 μm in control legs, respectively, with standard deviation of 0.1 μm in all. The degree of stretching of sarcomeres in MG and TA was therefore 25% and 10%, respectively; the difference in these proportions was significant ($p<0.01$, paired *t*-test).

There was no significant difference in the extent of lengthening of the whole muscles or the muscle fibre bundles between MG and TA. The length of whole muscles of MG and TA in the control legs was almost equal. The degree of lengthening of whole muscles of both MG and TA was 8%. The length of muscle fibre bundles of MG was about half that of TA in the control muscles. The degree of lengthening of muscle fibre bundles in MG and TA was 24% and 10%, respectively.

DISCUSSION

A muscle can be lengthened properly without causing degeneration or necrosis through sarcomere lengthening by increasing the serial sarcomere number along the muscle fibres.[4,14] Although the true pathogenesis of irreversible changes to muscle fibres in an excessively lengthened muscle is unclear, a mechanical injury to muscle fibres by stretching of sarcomeres beyond the physiological overlapping of myofilaments is a likely cause.

The results of our study show that the sarcomeres of MG are more stretchable than those of TA. This difference was primarily due to the relative shortness of muscle fibre bundles in MG, because most length changes are translated to the sarcomeres from muscle fibre bundles.[15] Muscles are composed of pennated muscle fibre bundles that run obliquely between 2 facing tendinous planes that extend from its tendons. As the elasticity of the tendinous planes is minimal, a whole muscle is distracted primarily by lengthening muscle fibre bundles.[16] Theoretically, in a muscle having muscle fibre bundles with a large pennation angle, the muscle fibre bundles are less stretched when the muscle is lengthened. This effect is negligible because the pennation angles of the lower leg muscles are small, and the range is less than 30°.[7–9]

In human lower legs, ankle plantar flexors are composed of shorter muscle fibre bundles than ankle dorsiflexors.[8,9] In tibial lengthening, ankle plantar flexors are more likely to sustain mechanical injury of muscle fibre bundles than ankle dorsiflexors caused by overstreching of sarcomeres.

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