Effect of tibial lengthening on the gastrocnemius muscle

A histopathologic and morphometric study in rabbits

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We observed the changes of the gastrocnemius muscle in relation to the percentage of lengthening of the rabbit's tibia by callotasis. 75 rabbits were separated into 3 lengthening groups, 10, 20, and 30 percent lengthening, respectively. Histopathologic observations, based on the fiber size variation, internalization of the nuclei, degeneration, regeneration, and endomysial fibrosis of muscle fibers, revealed that substantial changes occurred in the latter groups. Histomorphometrically, the decrease in the mean size of Types I and II muscle fibers was observed in all lengthening groups, but there was no significant change in the proportion of the muscle fiber types in any of the lengthening groups.

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Gradual distraction techniques according to Ilizarov (1989a,b) and de Bastiani et al. (1987) have recently gained popularity around the world. However, permanent tissue damage and joint contracture or subluxation can occur if the bone is lengthened beyond a certain safe limit—"the most significant unsolved problem in limb lengthening today is the muscle rather than the bone" (Paley 1990).

We report histopathologic and histomorphometric changes in the gastrocnemius muscle in rabbits, in relation to the amount of lengthening of the tibia.

Animals and methods

75 New Zealand white rabbits of both sexes, weighing 1.5–1.6 kg, had the left tibia lengthened 10 (n 23), 20 (n 23), 30 (n 29) percent. Under general anesthesia, the left hindleg was shaved and prepared for operation. The tibia and fibula were osteotomized at the proximal metaphysiodiaphyseal junction between 2 middle transfixing pins, and then all 4 transfixing pins were fixed to a pair of our modification of the dynamic fixator (Lee et al. 1992). Distraction was started at the rate of 0.25 mm twice a day from the 5th postoperative day. Preoperative and postoperative radiograms of both legs were taken, using a scanogram technique. The length of the tibia was measured from the lateral condyle of the proximal tibia to the lateral portion of the articular surface of the distal tibia. Percentage of lengthening was measured from the radiograms, after cessation of lengthening, by the change in the distance between the 2 central pins of the left tibia relative to the length of the right tibia which served as control. The rabbits were killed when the desired amount of lengthening was reached.

With the rabbits in deep anesthesia before killing, biopsy specimens were obtained from the medial gastrocnemius muscles of both hindlegs The specimens from the distal half of the medial gastrocnemius were fixed with formalin solution, and transverse sections were prepared and stained with HE, PAS, Masson trichrome. The specimen from the proximal half was frozen by liquid nitrogen, and transverse sections were stained with NADH-TR, ATPase pH 9.4, pH 4.6 and pH 4.3 (Dubowitz 1985).

In order to permit semi-quantitative analysis of the histopathologic study, a scoring system was devised, based on 5 parameters consisting of size variation of muscle fibers, internalization of the nuclei of muscle fibers, degeneration of muscle fibers, regeneration of muscle fibers, and endomysial fibrosis of muscle fibers. Points from 0 to 3 were given to each parameter.

Muscle fiber-size variation (200 ×): 0, normal; 1, atrophied muscle fibers less than 2/3 the size of the

normal contralateral side in less than 20 percent of the field; 2, atrophied fibers less than 2/3 the size of the control side in between 20–40 percent of the field; 3, atrophied fibers less than 2/3 the normal size in more than 40 percent of the field.

Internalization of the nuclei of muscle fiber (400 ×): 0, normal; 1, 4–5 muscle fibers with central nuclei in 10 fields; 2, muscle fibers with central nuclei between scores 1 and 3; 3, more than 5 muscle fibers with central nuclei in 1 field.

Degeneration of muscle fiber (400 ×): 0, normal; 1, 1–2 degenerating muscle fibers in 10 fields; 2, degenerating muscle fibers between scores 1 and 3; 3, more than 10 degenerating muscle fibers in 10 fields. Degenerated muscle fiber has pale-staining liquefied or hyalinized acidophilic cytoplasm losing striation with HE stain. This represents necrosis, and such a fiber frequently becomes filled with phagocytoses by macrophages (Dubowitz 1985; Figure 1).

Regeneration of muscle fiber (400 ×): 0, normal; 1, 1–2 regenerating muscle fibers in 10 fields; 2, regenerating fibers between scores 1 and 3; 3, more than 10 regenerating muscle fibers in 10 fields. Regenerating muscle fiber has a relatively large nucleus with prominent nucleoli and basophilic cytoplasm with HE stain (Dubowitz 1985; Figure 2).

Endomysial fibrosis of muscle fibers (400 ×): 0, normal; 1, mild focal fibrosis; 2, fibrosis between scores 1 and 3; 3, severe multifocal endomysial fibrosis. Endomysial fibrosis, if present, stained blue on Masson trichrome staining in the spaces among the muscle fibers (Figure 3).

The PC-SAS system was used for the statistical procedure (SAS Institute 1987). The statistical test of histopathologic scores, based on the ordinal scale, among the lengthening groups was done by the Kruskal-Wallis test, followed by the Wilcoxon rank-sum test for individual comparisons among the lengthening groups and within each lengthening group between the lengthened side and the control side. The severity trend of the histopathologic score was verified by the Mantel-Haenszel Chi- square test.

For the histomorphometric study, all tissue slides of the NADH-TR staining were photographed with Kodak film using 100 × and developed in 12 × 9-cm color photographs. The boundary of each muscle fiber was traced with a digitizer excluding incomplete fibers, and the mean size of muscle fibers was calculated using an image analyzer (VIDAS Image processing system). NADH-TR histochemical staining was useful in evaluating changes in the size and proportions of muscle fiber types in relation to the percentage of lengthening, because Type I fibers stained dark, and

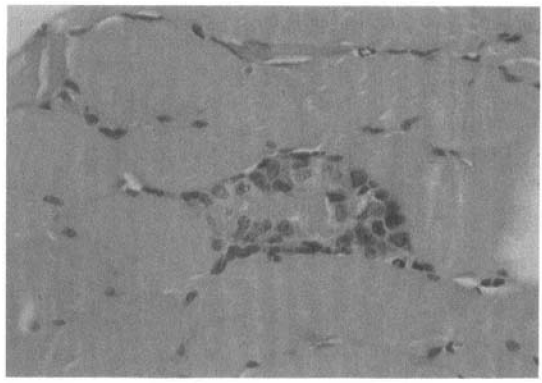

Figure 1. Degenerating muscle fiber has homogeneous hyalinized acidophilic cytoplasm losing striation on HE staining. Degenerated muscle fiber becomes filled with phagocytosis by macrophages, HE, ×400.

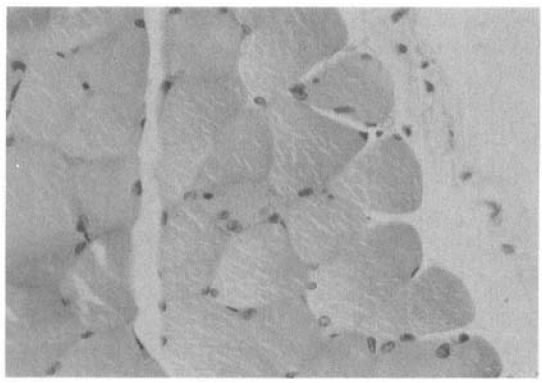

Figure 2. Regenerating muscle fiber (arrow) has relatively large nucleus with prominent nucleolus and basophilic cytoplasm, HE, ×400.

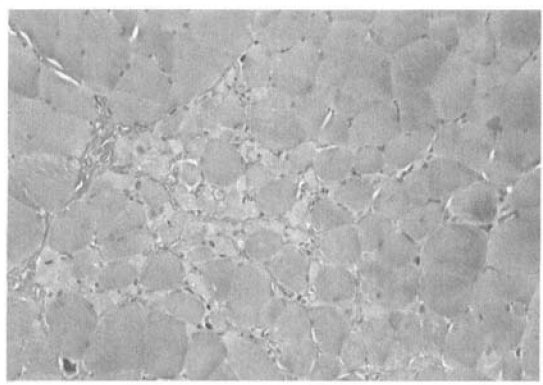

Figure 3. Severe multifocal endomysial fibrosis (arrow) is seen in the spaces among muscle fibers. Muscle fiber size variation is also conspicuous along with internalization of the nucleus (arrowhead), Masson-trichrome, ×200.

Table 1. Histopathologic scores

	Lengthening side							Control side					
Lengthening	S	N	D	R	F	Σ	Rabbit	S	N	D	R	F	Σ
10 percent	1	0	0	0	0	1	1	0	0	0	0	0	0
(Rabbit 1–23)	0	0	0	0	0	0	2	0	0	0	0	0	0
	0	0	0	0	1	1	3	0	0	0	0	0	0
	0	0	0	0	0	0	4	0	0	0	0	0	0
	1	0	0	0	0	1	5	0	0	0	0	0	0
	1	0	0	0	0	1	6	0	0	0	0	0	0
	2	1	0	0	0	3	7	0	0	0	0	0	0
	1	0	0	0	0	1	8	0	0	0	0	0	0
	1	1	0	1	0	3	9	0	0	0	0	0	0
	1	0	0	0	0	1	10	0	0	0	0	0	0
	0	0	0	0	0	0	11	0	0	0	0	0	0
	1	0	0	0	0	1	12	0	0	0	0	0	0
	0	0	0	0	0	0	13	0	0	0	0	0	0
	0	0	0	0	1	1	14	0	0	0	0	0	0
	0	0	0	0	1	1	15	0	0	0	0	0	0
	1	0	0	1	0	2	16	0	0	0	1	0	1
	1	0	0	1	0	2	17	0	0	0	1	0	1
	0	0	0	0	0	0	18	0	0	0	0	0	0
	0	0	0	0	0	0	19	0	0	0	0	0	0
	0	0	0	0	0	0	20	0	0	0	0	0	0
	0	0	0	0	0	0	21	0	0	0	0	0	0
	0	0	0	0	0	0	22	0	0	0	0	0	0
	1	0	0	1	0	2	23	0	0	0	0	0	0
20 percent	1	0	0	1	1	3	24	0	0	1	1	0	2
(Rabbit 24–46)	2	1	2	1	1	7	25	1	0	1	1	0	3
	2	0	0	0	2	4	26	0	0	0	0	0	0
	3	0	0	0	0	3	27	0	0	0	0	0	0
	3	0	0	0	0	3	28	0	0	0	0	0	0
	1	1	0	0	0	2	29	0	0	0	0	0	0
	2	0	0	0	1	3	30	0	0	0	0	0	0
	1	1	0	0	0	2	31	0	0	0	0	0	0
	1	1	0	0	0	2	32	0	0	0	0	0	0
	1	0	1	0	0	2	33	1	0	0	0	0	1
	1	1	1	1	1	5	34	0	0	0	0	0	0
	2	1	0	1	1	3	35	0	0	0	0	0	0
	1	1	0	0	0	2	36	1	0	1	0	1	3
	2	0	0	0	0	2	37	1	0	0	0	0	1
	1	0	0	0	0	1	38	0	0	0	0	0	0
	1	1	0	0	0	2	39	0	0	0	0	0	0
	2	0	0	1	0	3	40	1	0	0	0	0	1
	2	1	0	0	2	5	41	1	0	0	0	0	1
	1	3	1	0	0	5	42	0	1	0	0	0	1
	2	3	2	3	1	11	43	0	1	1	2	0	4
	1	0	0	0	0	1	44	0	0	0	0	0	0
	0	0	0	0	0	0	45	0	0	0	1	0	1
	2	1	3	3	2	11	46	0	0	0	0	0	0
30 percent	1	1	1	3	1	7	47	1	0	0	0	1	2
(Rabbit 47–75)	3	1	3	3	2	12	48	0	0	0	0	0	0
	1	2	0	2	2	7	49	0	1	2	1	1	5
	2	0	0	0	1	3	50	0	0	0	0	0	0
	1	1	1	0	1	4	51	1	0	1	0	0	2
	2	0	0	0	2	4	52	0	0	0	0	0	0
	2	0	0	0	1	3	53	0	0	0	0	0	0
	2	2	2	2	3	11	54	0	0	0	0	0	0
	2	0	2	2	3	9	55	0	0	0	0	0	0
	3	0	2	1	2	8	56	0	0	0	0	0	0
	2	1	0	0	0	3	57	1	0	1	0	0	2
	3	1	0	0	1	5	58	1	1	0	0	0	2
	1	1	0	0	1	3	59	0	0	1	0	0	1
	2	1	0	0	0	3	60	2	1	1	2	0	6
	2	2	0	0	0	4	61	2	1	0	0	0	3
	2	0	0	0	0	2	62	1	1	1	0	0	3
	3	0	0	0	0	3	63	2	0	1	0	0	3
	1	1	1	0	0	3	64	0	0	0	0	0	0
	2	0	0	0	0	2	65	1	0	0	0	0	1
	3	0	0	0	0	3	66	1	0	0	0	0	1
	2	1	0	0	1	4	67	1	0	0	0	0	1
	3	1	1	3	0	8	68	1	0	2	1	0	4
	1	1	0	0	0	2	69	1	0	0	0	0	1
	1	0	0	0	0	1	70	0	0	1	2	0	3
	1	0	0	0	1	2	71	1	0	0	0	0	1
	2	3	3	3	2	13	72	1	2	2	3	0	8
	2	3	0	1	1	7	73	0	0	0	1	0	1
	1	0	0	0	1	2	74	0	0	1	1	0	2
	2	2	0	0	1	5	75	0	0	0	0	0	0

S muscle fiber size variation, N internalization of nucleus,
D degeneration of muscle fibers, R regeneration of muscle fibers
F endomysial fibrosis.

Type II fibers stained light. Subgrouping of Type II fibers was not tried because of inconsistency in the quality of staining.

The mean sizes of Types I and II fibers on the lengthening side were compared with those of the control side by the Wilcoxon rank-sum test. The relative proportion of each fiber type was calculated in percentage, after counting the number of each fiber type, and compared statistically among the lengthening groups. The statistical significance among the lengthening groups was analyzed by the Kruskal-Wallis test, followed by the Wilcoxon rank-sum test for individual comparisons among the lengthening groups and within each lengthening group between the lengthened side and the control side.

Results

The individual histopathologic scores are listed in Table 1. As compared with the control side, the lengthened side had substantial differences in fiber size variation in all 3 lengthening groups ($P < 0.001$). However, significant differences in internalization of nucleus and endomysial fibrosis (Figure 3), which may imply irreversible damage, were observed only after 20 and 30 percent lengthening ($P < 0.01$), and there were no differences in degeneration or regeneration among the lengthening groups. Fiber size variation was mainly due to an increase in the proportion of atrophied fibers instead of hypertrophied fibers, which were found only occasionally. When the histopathologic scores of the lengthened side were compared among the lengthening groups, there were differences in all parameters, except regeneration. As the lengthening percentage increased, the histopathologic scores of each parameter of the lengthened side showed a linearly increasing trend which reflects an increasing severity of the histopathologic changes ($P < 0.05$). The relative proportion of the rabbits that had higher histopathologic scores—either 2 or 3 in each parameter—increased. The mean scores of each parameter and the mean of the cumulated score of all 5 parameters of the lengthened side increased, as the lengthening percentage increased ($P < 0.05$).

The mean size of Types I and II fibers decreased in all 3 lengthening groups ($P < 0.05$). However, the decrease was not significant among the 3 lengthening groups (Table 2). The proportions of the 2 fiber types did not change in any of the 3 lengthening groups, as compared with their control side, nor among the 3 lengthening groups.

Table 2. Size of muscle fibers (1,000 μm²), mean *SD*

Lengthening (%)	Side	Type I	Type II
10	Lengthened	2.08 *0.74*	4.05 *1.30*
	Control	3.08 *1.07*	5.29 *1.83*
20	Lengthened	2.82 *1.01*	5.40 *1.35*
	Control	3.79 *1.27*	7.18 *1.98*
30	Lengthened	2.92 *1.01*	6.18 *1.63*
	Control	4.05 *1.25*	7.78 *2.80*

Discussion

Kawamura et al. (1968) conducted histochemical and electromyographic studies on dogs and patients and concluded that there was no damage in soft tissues if initial lengthening was not more than 3 percent and gradual lengthening was up to 10 percent of the initial bone length. Carroll et al. (1981) reported that tibial lengthening by more than 11 percent of the initial length consistently produced irreversible changes in the gastrocnemius and the flexor digitorum profundus muscles in sheep, including loss of myofibrils, empty sarcolemmal sheaths, central migration of nuclei, hyaline staining character of sarcoplasm, and irregular sizes and shapes of myofibrils. He suggested that such changes were probably myogenic by distraction itself and not neurogenic. It should be noted, however, that these studies were based on relatively rapid distraction, compared to the current use of callotasis.

Dyachkova and Utenkin (1980) reported on a model using callotasis—lengthening up to 10 percent—and found that the lengthening was accommodated by a tighter packing and sliding effect between muscle fibers. After 10 percent, myogenesis and fibrogenesis were found to account for the production of new muscle, tendon, and fascia. The level of new tissue production was evenly distributed along the length of a muscle and fascia until 20 percent lengthening was achieved. After 20 percent lengthening, both muscle and fascia tended to lengthen more at the level of the osteotomy site than throughout the entire muscle or fascia. Yasui et al. (1991) also reported that when the tibia of a growing rabbit was lengthened by callotasis to 20 percent of its initial length, elongation of the muscle occurred throughout the muscle substance and not only at the site of the osteotomy.

Our experiment, simulating the current use of callotasis, revealed that histopathologic changes, such as endomysial fibrosis and internalization of nuclei which reflect abnormalities of the contractile elements of the muscle, did not occur when the tibia was not lengthened more than 10 percent. These changes, however, increased when the tibia was lengthened 20 percent or more, and were more conspicuous when the tibia was lengthened to 30 percent. These results may suggest that irreversible damage to muscle can occur when the lengthening is more than 20 percent with the current use of callotasis. Our results may support the observations by Shen and Aronson (1993) who demonstrated in a rat callotasis that tibial lengthening of more than 20 percent caused acute stiffness of the gastrocnemius muscle, presumably due to an increase in endomysial and perimysial fibrosis. We therefore think that our results would support in part the hypothesis of Calandriello (1968) who postulated that rupture of the muscle fibers occurs beyond a certain point of distraction, and muscle cells proliferate for regeneration of muscle fibers and connective tissue repair by fibrosis occurs simultaneously.

Muscle fibers of animals consist of Types I and II, the former being slow twitching and the latter fast twitching (Kakulas and Adams 1985). There are few reports about the effect of distraction on histomorphometric changes and the proportion of muscle fiber types, and these reports differ in results. Holley et al. (1980) reported muscle hypertrophy and hyperplasia by distraction. Ilizarov (1989a) observed the changes in muscle during bone lengthening under the electron microscope and reported that by the influence of tension-stress effect, muscle fibers become enlarged with multiple mitochondrial cristae and with hypertrophy of their nucleoli, and that elongation of the muscle occurs not only by myofibrillogenesis in preexisting muscle fibers but also by the formation of new muscle tissue characterized by an increased number of muscle satellite cells, the appearance of muscle myoblasts, and their fusion into myotubes.

On the other hand, Lindboe et al. (1985) reported that, during 10–20 percent bone lengthening, the Type II fibers decreased in size, but the Type I fibers did not change in size. They suggested that atrophy of the Type II fibers was mainly caused by muscular inactivity during the postoperative period, but an additional effect of continuous stretching of the muscle cannot be excluded. Their observations are open to some doubt, however, because they compared only the muscle of the lengthened side preoperatively and postoperatively. By contrast, our study showed that the mean size of both Types I and II fibers decreased in all 3 lengthening groups, compared to their normal control side. Our histopathologic study also revealed that fiber size variation was mainly due to an increase in the proportion of atrophied fibers rather than to hypertrophied fibers, which were found only occasionally. We think that these changes in fiber size may be due to the combined effects of muscle-stretching and atrophy.

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