Distraction frequency and the gastrocnemius muscle in tibial lengthening

Studies in rabbits

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We compared the effect of various distraction frequencies on the gastrocnemius muscle by evaluating the histological findings, intramuscular enzyme contents, and DNA contents. In 15 rabbits, both tibiae were distracted 1 mm per day. The distraction frequency was 2 steps (0.5 mm/12 hour) by hand on the right side and 120 steps (0.0083 mm/12 minutes) by an auto-distractor on the left. The rabbits were divided into 3 subgroups based on length gain: 10%, 20%, and 30%. Histologically, there were no signs of fibrosis or edema and no differences in the number of necrotic cells, and intramuscular enzyme contents between the 2- and 120-step groups. The DNA content, however, was higher in the 120-step group at 30% lengthening in the middle of the muscle belly, and at 20% and 30% lengthening in the musculotendinous junction. Our findings suggest that an increase in the distraction frequency may promote DNA synthesis in the muscle, thus providing better muscle accommodation during bone lengthening.

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Several authors have reported the effects of the lengthening rate and the distraction speed on muscle (Lee et al.1993, Simpson et al.1995). However, it is little known how different distraction frequencies affect adjacent muscle during bone lengthening. We investigated the effects of various distraction frequencies on muscle, using histological findings, intramuscular enzyme contents, and DNA contents in muscle with tibial lengthening in rabbits.

Animals and methods

15 Japanese white rabbits weighing 3.0–3.5 kg underwent general anesthesia with sodium pentobarbital (30 mg/kg). A longitudinal skin incision was made on the medial aspect of the tibia, and the periosteum was incised and retracted. 4 threaded 2.0-mm pins were inserted at right angles to the diaphysis after drilling with a drill-guide. A transverse osteotomy was performed using a hand-saw just below the tibiofibular junction between the second and third pins. The pins were then clamped to a unilateral external fixator. Both tibiae were operated on in the same manner.

Distractions were started from the following day at a rate of 1 mm per day. The frequency of distraction was 2 steps per day (0.5 mm/12 hours) by hand on the right tibia and 120 steps per day (0.0083 mm/12 minutes) by an auto-distractor on the left (Nakamura et al. 1993). The 15 animals were divided into 3 subgroups of 5 each based on length gain. The first subgroup had a 10% increase in length, the second 20%, and the third 30%.

When the desired length was achieved, the animal was anesthetized with sodium pentobarbital (30 mg/kg). Muscle sections were taken from the middle of the muscle belly and from the distal musculotendinous junction (MTJ) of the gastrocnemius muscle. They were then quickly frozen in isopentane precooled by liquid nitrogen.

Histology. Muscles were cut into sections 6 μm thick. After H.E. staining, we looked for whorled fibers, edema between muscle fibers, fibrosis, degenerative or necrotic muscle fibers, and infiltrating cells in the necrotic area. We counted the number of necrotic muscle fibers and the cells infiltrating them per 10 fields in 5 serial sections.

Intramuscular enzymes. Aldolase and creatinine phosphokinase (CPK) in the gastrocnemius muscle were determined as an index of muscle damage. Frozen tissues were thawed, homogenized, and centrifuged, and the contents of aldolase and CPK were determined according to their activity in the supernatant. Since the amount of tissue from the MTJ was insufficient, measurements were made only in tissues from the middle of the muscle belly.

Table 1. Number of necrotic fibers

A	B		C	
	2-step	120-step	2-step	120-step
10	0.4	0.2	0.4	0.4
20	0.6	0.4	0.4	0.4
30	0.6	0.4	0.6	0.6

A percent increase in length.
B number per 10 fields from the middle of the muscle belly.
C number per 10 fields from the distal musculotendinous junction.

Table 2. Number of infiltrating cells in necrotic fibers

A	B		C	
	2-step	120-step	2-step	120-step
10	8.2	8.6	9.0	9.8
20	11.8	10.2	10.7	10.6
30	11.0	12.2	11.6	10.4

A percent increase in length.
B number per 10 fields from the middle of the muscle belly.
C number per 10 fields from the distal musculotendinous junction.

Table 3. Contents of intramuscular enzymes related to increase in length

A	B		C	
	2-step	120-step	2-step	120-step
10	44 (37–51)	46 (38–54)	3449 (2103–4419)	3981 (2602–5792)
20	73 (66–77) [b]	82 (79–86) [b]	4507 (3249–5712)	5006 (3281–6860)
30	84 (70–97) [b]	91 (77–124) [a]	5049 (3659–5666) [a]	5266 (3784–6162)

A percent increase in length.
B contents of aldolase; median (range) in IU/gram wet weight.
C contents of CPK; median (range) in IU/gram wet weight.
Significant difference from value for 10% increase in length: [a] $p < 0.05$, [b] $p < 0.01$.

DNA content. From each tissue, 5 sections 6 µm thick were cut serially, dried for 60 minutes, fixed in 70% ethyl alcohol at 4 °C, and washed in distilled water and in PBS (pH 7.4) for at least 60 minutes. The sections were incubated in 0.1% RNase at 37 °C for 60 minutes, then washed in distilled water and in PBS (pH 7.4) for 20 minutes. These specimens were stained with propidium iodide (0.025 mg/mL) shielding the light at 4 °C for 20 minutes (Kusuzaki et al.1984).

The intensity of fluorescence per unit area in 10 fields of 5 serial specimens, which is proportional to the DNA contents, was measured by NICON Microphoto-FXA with the excitation light at 510–560 nm and an emission wavelength of 590 nm or longer.

Statistics

ANOVA was used to evaluate the influence of the length gain and the daily frequency of distraction. The nonparametric Kruskall-Wallis test was used to compare the 3 subgroups in the same step. When a significant difference was found, the Mann-Whitney U-test was used. It was also employed to evaluate the difference between the 2- and 120-step groups in the same elongation. $P < 0.05$ was considered significant.

Results

Histology. We found no whorled fibers, no edema between muscle fibers and no fibrosis in any specimens either from the middle of the muscle belly or from the MTJ. The numbers of necrotic fibers and infiltrating cells in the necrotic fibers at the middle of the muscle belly and from the MTJ were very small (Tables 1 and 2). There was no significant difference between the 2-step and 120-step groups at 10%, 20%, and 30% length gain and no significant increase was found from 10% to 30% lengthening.

Intramuscular enzymes. There was no difference in the aldolase or CPK contents at any lengthening rate between the 2-step and 120-step groups (Table 3). Significant increases in aldolase were found from 10% to 30% length gain in both groups. As regards CPK, only the 30%-value on the 2-step side increased significantly.

DNA content. In the middle of the muscle belly, the DNA contents increased with elongation in both groups. The 120-step group showed 1.0, 1.1, and 1.2-fold increases at 10%, 20%, and 30% lengthening, compared to the 2-step group. The increase was significant at 30% (Table 4).

At the distal musculotendinous junction, the DNA contents increased in both groups as the lengthening proceeded. The values in the 120-step group showed

Table 4. Fluorescent intensity as DNA contents related to increase in length

A	B		C	
	2-step	120-step	2-step	120-step
10	341 (264–397)	356 (297–454)	358 (293–385)	390 (343–449)
20	396 (316–461)	438 (367–509)	418 (382–472) [b]	465 (417–492) [ab]
30	461 (388–534) [b]	535 (467–612) [ac]	481 (434–556) [c]	563 (502–635) [ac]

A percent increase in length.
B middle of the muscle belly; median (range) in arbitrary unit..
C distal musculotendinous junction; median (range) in arbitrary unit.
Significant difference from value for 2-step side: [a] $p < 0.05$, and from value for 10% increase in length: [b] $p < 0.05$, [c] $p < 0.01$.

1.1, 1.1, 1.2-fold increase at 10%, 20%, and 30% compared to the 2-step group. The increase was significant at 20% and 30%.

There was no difference between the middle of the muscle belly and the MTJ in the 2 groups at 10%, 20%, and 30%.

Discussion

Muscle adaptation during bone lengthening is controversial. Calandriello (1975) hypothesized that after tears occur in the muscle fibers, muscular regeneration proceeds with connective tissue repair at various levels. Lee et al. (1993) partly supported this hypothesis with histological findings of both necrosis and fibrosis in a study on rabbits. On the other hand, Ilizarov (1989) suggested that muscle growth under the influence of tension-stress occurred without tears in the muscle fibers, not only by myofibrillogenesis in preexisting muscle fibers but also by new muscle formation. Schumacher et al. (1994) reported an increase in muscle weight and in the number of dividing cells during tibial lengthening in rabbits, and Simpson et al. (1995) observed, in rabbits, an increase in the number of sarcomeres with the creation of new muscle tissue. These recent reports indicate that muscle accommodation during bone lengthening takes place mainly by forming new muscle.

P.I. staining, useful in evaluating DNA synthesis (Ellwart et al. 1982), reflects all the DNA in the tissue. Differences in the DNA contents between the 2-step and 120-step groups in our study may have been due to the following: 1) satellite cells, which activate, divide, and proliferate to become new muscle when certain changes occur in the muscle (Chou and Nonaka 1977), 2) newly formed muscle, and 3) infiltrating cells in necrotic fibers. We found no differences in the number of necrotic fibers, infiltrating cells, or intramuscular enzyme contents between the 2- and 120-step groups. Thus, differences in the groups' DNA contents could be explained mainly by the proliferated satellite cells and the newly formed muscle.

Ohnishi et al. (1992) clinically measured the resistance to distraction on the fixator, which may be due mainly to the tension of the adjacent muscles (Paley 1990), by comparing the daily distraction frequency between 2 steps by hand and 1440 steps by an autodistractor in femoral lengthening. They found very little change in the baseline of this resistance time-course in the 1440-step group, but a large one in the 2-step group. On the basis of this biomechanical report and our results, we suggest that an increase in the distraction frequency may promote new muscle formation in the muscle, thus providing better muscle accommodation during limb lengthening.

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