INTRODUCTION

It has been found that muscles become harder under several conditions including spasms, cramps, oedema, (Fisher; 1987) muscle shortening and swelling (Murayama et al.; 2000). Physiotherapists often examine the condition of muscles by palpation. Several methods for quantitative palpation in humans have been developed (Fisher; 1987, Horikawa et al.; 1993). Muscle hardness has some potential for use as a general index of physiological condition; however, the physiological mechanism of the hardness change in fatigued or damaged muscle has been unclear.

Muscle fiber that has suffered damage by eccentric exercise shows a remarkable increment of muscle hardness (Murayama et al.; 2000). Damaged muscle accompanying muscle shortening shows a decrement in its range of motion. Nevertheless, muscle thickness and circumference, which relate to circulation change, also increase after eccentric exercise. Therefore, it is necessary to understand whether or not the physiological reason for this increment involves changes in the muscle fiber itself.

This study was carried out to investigate the mechanism of fatigued muscle hardness on isolated frog muscles. We made a comparison between fatigued muscles stimulated by two types of tetanic pulse trains, one having a saw-toothed pattern and the other a trapezoid pattern. We also varied the loading weight on the muscle.

METHODS

Preparation and mounting
Experiments were carried out according to procedures approved by the Animal Ethics Committee of the Juntendo University. The gastrocnemius muscle and sciatic nerve were dissected from frogs (Rana catesbeiana) and submerged in Ringer’s solution. At first, the muscle was mounted horizontally and a force transducer was attached to the Achilles tendon to measure the maximum tetanic force (MTF). The sciatic nerve was placed on platinum electrodes mounted horizontally and a force transducer was attached to the Achilles tendon to be intercepted by the stopper. (Figure 1). The muscle was fatigued by lifting a connected to the Achilles tendon to be intercepted by the stopper. (Figure 1). The muscle was fatigued by lifting a bead was put through the end of the string connected to the Achilles tendon to be intercepted by the stopper. (Figure 1). The muscle was fatigued by lifting a weight using two types of modulated tetanic pulse stimulation (Figure 2). For a saw-toothed pattern pulses, the voltage was increased gradually for 1sec, held for 0.5sec and ended abruptly. The trapezoid pattern pulse had same rising and holding phase, but decreased gradually over 2sec. Later pulse trains can be expected to induce damage to a part of the muscle fiber. The patterned pulses were applied intermittently for 6-8 min until the muscle could not lift a weight. The fatigue task was finished when contraction height decayed to 5% of the initial value, according to a fatigue curve recorded during the stimulation (Figure 3).

Muscle hardness measurement
To evaluate muscle hardness, a mechanical pressing method was used, in which the force required to deform the muscle belly was recorded. A linear motor vibrator system (DPM-270: Dia Medical Systems Co. Ltd., Japan) which had an electrical feedback servomechanism was used to deform the muscle belly (Figure1). A 3.5 mm vertical pressure was applied to the muscle belly, and the muscle hardness value was determined from the peak response force (Figure4).
Muscle hardness was measured before the fatigue task and during the 12 min recovery after the fatigue task.

(a) Responding force

(b) Displacement by pushing

(c) Changes of contraction height (fatigue curve)

(d) Stimulation

![Figure 3: Example of recording data and stimulation in fatigue task. (a) Responding force (muscle hardness) (b) Displacement of pushing (c) Change of contraction height (fatigue curve) (d) Stimulation (saw-toothed pattern)](image)

**RESULTS**

Figure 5 shows the time courses of muscle hardness changes before and after the fatigue task using saw-toothed stimulation pattern. All time courses showed a slight decrement. There was also decrement after the fatigue task at 40% MTF loading.

On the other hand, for the trapezoid stimulation pattern at 40% MTF loading, almost all the time courses of muscle hardness showed an increment within 12 minutes after the fatigue (Figure 6). Those at 20% MTF loading were similar to the time courses for the saw-toothed pattern stimulation.

![Figure 4: Relationship between the displacement of the muscle deformation and responding force. The peak value was determined as muscle hardness value.](image)

![Figure 6: Percent changes of muscle hardness before and after fatigue task using trapezoid stimulation pattern. Upper; 20% MTF loading. Lower; 40% MTF loading.](image)
DISCUSSION

For the saw-toothed pattern and for 20% MTF loading in the trapezoid pattern, muscle hardness did not increase; it did increase, however, after fatigue at 40% MTF loading in the trapezoid pattern. Since the stiffness change would affect the muscle hardness change detected by the vertical pressure method (Murayama et al.; 2001), this result suggested that the hardness change is caused at least in part by some essential change in the muscle cell. During the falling phase in the trapezoid pattern stimulation some muscle fibers should be damaged because they are exposed to extension tension before the derecruitment. However, it is clear that 20% MTF loading was not critical that could damage the contracting muscle fiber.

On the contrary, muscle hardness rather tended to decrease from the pre-fatigue level in the saw-toothed pattern. This could be because the resting tension of the mounted muscle must decrease due to the slack of epimysium or connective tissue in the muscle or tendon during the fatigue task.

In conclusion, this study showed that muscle hardness of muscle itself was increased in the fatigued frog muscle having contraction with gradual falling phase.

REFERENCES

Murayama et al. (2001). IUPS '01 Abstract CD-ROM, ID NO. 1798

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