Comparison of the Sarcomere Alterations after Muscle Contraction and Tension Loading in the Rat Soleus Muscle.

Natsue Matsuura, Seiichi Kawamata, Junya Ozawa, Satoru Kai,

Harutoshi Sakakima and Sachiko Abiko

Institute of Health Sciences, Faculty of Medicine, Hiroshima University,

Hiroshima, 734-8551 Japan

Running title: Sarcomeres after contraction and tension

Address correspondence to:
Dr. Seiichi Kawamata, Institute of Health Sciences, Faculty of Medicine,
Hiroshima University, Kasumi 1-2-3, Hiroshima 734-8551 Japan
Tel: +81-82-257-5410
Fax: +81-82-257-5344
E-mail: kawamat@hiroshima-u.ac.jp

Abstract

Muscle contraction induced by 30 min of continuous nerve stimulation at 50 Hz resulted in sarcomere changes of the soleus muscle in the rat. To further investigate the cause of sarcomere alterations, the sciatic nerve was electrically stimulated intermittently for 30 min. Nerve stimulation was also conducted after cutting the tendons of the soleus, gastrocnemius and plantaris muscles in order to prevent imposing tension on these muscles due to their own contractions. In addition, the muscles were pulled by weights via their tendons to load high tension for 30 min without nerve stimulation. Sarcomere alterations immediately after these treatments were quantified by electron microscopy. The percentages of aberrant sarcomere areas of the soleus muscle were 25.7±16.4% (mean±SD) in the group of intermittent nerve stimulation with intact tendons and 21.1±35.4% in the group of tenotomy plus continuous nerve stimulation, which were roughly equal to or more severe than the group of continuous nerve stimulation with intact tendons (18.8±15.8%) in our previous study. Sarcomere alterations consisted mainly of hypercontraction in these groups. Almost all sarcomere changes in the tension-loaded (pulled) soleus muscles were scarce myofilaments (1.7±1.0% by 600 g; 4.5±2.9% by 1,200 g), and hypercontraction was not observed. These findings indicate that neither high tension nor a decrease of muscle blood flow during continuous contraction seems to be the primary cause of sarcomere alterations in the present study. There are probably other cause(s) which produce aberrant sarcomeres.

Key words: skeletal muscle, lesion, nerve stimulation, tension, electron microscopy

Introduction

Muscle injury with sarcomere alterations occurs under various conditions such as overexercise. It has been demonstrated that treadmill running (Armstrong et al, 1983; Ogilvie et al, 1988; Komulainen et al, 1994) and reloading on atrophied adductor longus muscles (Riley et al, 1992, 1996; Krippendorf & Riley, 1994) resulted in sarcomere lesions in rats. Electrical stimulation of the muscle with concomitant muscle stretching (eccentric contractions, i.e., the muscles lengthen while they are actively developing tension; Armstrong et al, 1983) produces several types of sarcomere lesions (Thompson et al, 1999). In our previous study, the formation and recovery process during up to 7 days of sarcomere changes of the soleus muscle were clearly shown after 30 min of continuous electrical stimulation of the sciatic nerve with intact tendons (Matsuura et al, 2001). However, the cause of the muscle injury remains to be clarified. One candidate cause is high tension on the muscle due to its own contraction. Other candidates include deficiencies of oxygen and the nutrient supply as well as accumulation of wastes such as lactate in muscles due to a decrease of blood flow during contraction. In addition, there may be other causes which also induce such abnormal sarcomeres. To evaluate the role of tension and a possible decrease of blood flow (which would presumably lead to unfavorable metabolic conditions) in the production of muscle injury, we examined the rat soleus muscle after the following treatments: 1) intermittent nerve stimulation with intact tendons to minimize the deficiency of oxygen and nutrients as well as accumulation of metabolic wastes during contraction, 2) tenotomy plus continuous nerve stimulation to avoid muscle tension, and 3) loading high tension on the soleus muscle by pulling tendons of the soleus, gastrocnemius and plantaris muscles together by weights without nerve stimulation. We compared sarcomere alterations after these treatments with those after continuous nerve stimulation with intact tendons (Matsuura et al, 2001).

Materials and Methods

Eight-week-old female Wistar rats (12 animals; B.W. 181.5±4.9 g) were used in this study. Rats were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg BW) with supplemental ether inhalation when necessary.

In nerve-stimulated rats (9 animals), the skin covering the buttocks was cut on the experimental side, and the sciatic nerve was exposed. The nerve was separated from the surrounding tissues, connected with plus and minus wire electrodes and stimulated by a 1-Hz, monophasic 1-ms square-wave pulse (Nihonkohden SEN-2201, Tokyo, Japan). First we gradually increased the voltage from 0 V until plantar flexion occurred to determine the twitch threshold voltage. Then we doubled the voltage to 3-5 V and stimulated the nerve at 50 Hz. Rats in the intermittent nerve stimulation with intact tendons group (3 rats) were stimulated for 30 min (10 bouts of 3 min each of stimulation separated by 2-min rests). In rats of the tenotomy plus continuous nerve stimulation group (3 rats), the distal tendons of the soleus, gastrocnemius and plantaris muscles were severed prior to nerve stimulation to avoid tension on these muscles during their own contractions, and the nerve was stimulated continuously for 30 min. There were considerable individual differences in aberrant sarcomere areas in this group. Then an additional 3 rats were subjected to the same treatment, which again resulted in some variability from animal to animal. In all rats, the hindlimbs were not restrained, and the sciatic nerve and wound were covered with cotton that had been soaked in saline solution to prevent drying. Data from rats of the continuous nerve stimulation with intact tendons group (3 rats) and their contralateral hindlimbs (the untreated controls) were derived from our previous study (Matsuura et al, 2001).

Rats of the tension-loaded group (3 rats) were loaded with 600 or 1,200 g, which was estimated to be 500 or 1,000 times the muscle wet weight by pulling tendons of the soleus, gastrocnemius and plantaris muscles. (The tonic force and the maximum tetanic tension are 350 times and 1,200 times the muscle wet weight, respectively, in the rat extensor digitorum longus muscle; Pachter and Eberstein, 1989). Anesthetized rats were laid in a prone position. Small incisions were made behind the ankle joints. Tendons of the gastrocnemius, soleus and plantaris muscles of each hindlimb were cut, ligatured to a wire and pulled horizontally by a weight secured at the other end

of the wire via a pulley fixed at the edge of a table. Tendons of one hindlimb were pulled by a weight of 600 g and those of the other hindlimb by 1,200 g. Both sides were pulled simultaneously for 30 min.

All rats were placed on a bag containing water at about 37℃ during treatment to prevent a decrease of body temperature and immediately sacrificed (within 10 min) after treatment.

An incision was made through the ribs to expose the heart and the inferior vena cava was cut. The animal was perfused first with 50 ml of saline and then with 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) through the apex of the heart into the left ventricle. The soleus muscles of the treated and control sides were sampled and the midbelly portions were dissected in rectangular pieces to facilitate orientation during embedding and sectioning. Specimens were immersed in the same fixative for 2 h to 1 day, postfixed in 1% OsO_4 in 0.1 M phosphate buffer (pH 7.4) overnight at 4℃, rinsed 3 times (10 min each) in 10% saccharose and stained en bloc in 3% uranyl acetate for 1 h at room temperature. Then specimens were dehydrated in ethanol and flat embedded in epoxy resin. Ultrathin sections were cut, stained with uranyl acetate and lead citrate and observed using a JEM-1200 electron microscope. Experimental procedures were approved by the Animal Facility Ethical Committee of Hiroshima University.

We analyzed sarcomere alterations quantitatively according to Matsuura et al. (2001). Three randomly selected blocks from each soleus muscle were cut, collected on grids and stained. Three holes of each grid were chosen following a protocol and the centers of these 3 holes of each grid were photographed at a magnification of 2,500 and printed at a final magnification of 5,600. A total of 135 micrographs were inspected by two investigators on a blinded basis, and analyzed using a scanner and a computer, and the percentages of each aberrant sarcomere type were separately calculated using NIH Image software.

Results

Four out of 5 types of sarcomere alterations previously reported (Matsuura et al, 2001) were

found focally in the soleus muscle. Myofilament disorganization was not observed.We briefly profile the features of each type of alteration.

Types of sarcomere alterations

1) Sarcomere hypercontraction

Sarcomeres were segmentally shortened and less than 70% in length compared with surrounding normal-looking sarcomeres. The A and I bands were difficult to distinguish. The Z bands were slightly wavy or curved. This type was a prevalent sarcomere change after nerve stimulation.

2) Sarcomere hyperstretching

Sarcomeres were lengthened to more than 130% the length of surrounding sarcomeres. This type was very rare in the present study.

3) Z band disarrangement

The length of sarcomeres was within the normal range (between 70% and 130%) compared with the surrounding sarcomeres. However, the Z bands were wavy, often blurred and sometimes broken down into fragments. The A and I bands were not distinguishable.

4) Regions of scarce myofilaments

Myofilaments and the Z bands were very scarce in these regions and mitochondria were easily seen.

Findings after treatments

1. The untreated controls

We employed the soleus muscles contralateral to those with the continuous nerve stimulation with intact tendons as controls. These specimens showed a few regions of scarce myofilaments ($0.9 \pm 0.5\%$; Matsuura et al, 2001). Other types of sarcomere changes were not seen.

2. Continuous nerve stimulation with intact tendons

Aberrant sarcomeres were found in 18.8±15.8% of the muscle sectional area (Matsuura et al, 2001). Most of the aberrations were classified as sarcomere hypercontraction (16.8±17.8%). Z band disarrangement (1.3±2.3%) and regions of scarce myofilaments (0.7±1.2%) were also observed.

3. Intermittent nerve stimulation with intact tendons

The soleus muscle showed total 25.7±16.4% of sarcomere alterations, with sarcomere hypercontraction in 21.3±15.7%, Z band disarrangement in 3.6±1.6% and regions with scarce myofilaments in 0.8±1.0% of the muscle area (Fig. 1).

4. Tenotomy plus continuous nerve stimulation

Sarcomere changes were found in 21.1±35.4% of the muscle area. Most of the changes were sarcomere hypercontraction (19.5±36.4%). Sarcomeres were highly contracted and the Z bands were slightly curved (Fig. 2), which was in close accordance with the features observed after continuous nerve stimulation with intact tendons (Matsuura et al, 2001). Regions with scarce myofilaments were also found (1.6±1.9%).

5. Tension loading by weights

Muscles pulled by a weight of 600 g contained sarcomere alterations in 1.7±1.0% of the muscle area, with regions of scarce myofilaments in 1.6±1.0% and Z band disarrangement in 0.1±0.1% of the muscle area (Fig. 3). Muscles pulled by a weight of 1,200 g contained sarcomere alterations in 4.5±2.9% of the muscle area, with regions of scarce myofilaments in 4.1±2.9%, sarcomere hyperstretching in 0.3±0.5% and Z band disarrangement in 0.1±0.1% of the muscle area (Fig. 4).

The relative incidence of sarcomere alterations in soleus muscles after various treatments is summarized in Fig. 5.

Discussion

Sarcomere alterations have been found after various treatments and are generally believed to represent muscle injuries (Fridén et al, 1981; Armstrong et al, 1983; Newham et al, 1983; Ogilvie et al, 1988; Krippendorf & Riley, 1994; Thompson et al, 1999). The sarcomere hypercontraction found in the present study has also been reported after eccentric contractions (Ogilvie et al, 1988; Fridén & Liber, 1998; Thompson et al, 1999), which cause more injury to the muscle than concentric or isometric contractions. It is plausible that sarcomere hypercontraction is induced by elevations in intracellular Ca^{2+} (Duncan, 1987; Duan et al, 1990; Armstrong et al, 1991). However, the trigger of the elevation of Ca^{2+} has not been identified. In our previous study, sarcomere alterations were demonstrated in the soleus muscle after continuous nerve stimulation (Matsuura et al, 2001). However, the cause of aberrant sarcomeres was not clarified. Several factors are suspected to be causes of muscle injury. The high tension which is imposed on muscle fibers during muscle contraction was reported to correlate with muscle injury (Fridén et al, 1981; Newham et al, 1983; Gibala et al, 1995). Fridén and Lieber (1992) and Lieber and Friden (1999) believed that active strain (strain that occurs during active lengthening of an activated muscle; Lieber & Fridén, 1993) plays a key role in producing muscle injury. Those authors also stated that passive strain alone does not induce muscle injury.

In the present study, a small number of abnormal sarcomeres were found after tension loading by weights. Furthermore, the type of sarcomere alterations (regions of scarce myofilaments) obviously differed from that (hypercontraction) after nerve stimulation. Tenotomy presumably eliminates high tension, and consequently also active strain, on muscles. However, tenotomy does not reduce sarcomere hypercontraction. One explanation for these findings is that tension does not correlate with sarcomere hypercontraction. Another possibility is that sufficient tension to give rise to muscle injury was not loaded on muscles even with intact tendons since the hindlimbs were not restrained, suggesting that not tension or strain but some other causative factor(s) are involved in the formation of sarcomere hypercontraction. In either case, high tension and active strain do not seem to play major roles in the production of sarcomere alterations observed in the present study.

When the human peroneal nerve is stimulated at 50 Hz, intramuscular pressure rises to about

300 mmHg and the blood supply stops (Nilsson & Ingvar, 1967). Several minutes later blood reflow starts after a decrease of the intramuscular pressure as the muscle contraction diminishes. Similarly, it seems reasonable to speculate that the muscle blood flow probably decreases and the metabolic environment of muscle fibers deteriorates to some extent during muscle contraction. However, the soleus muscle showed more severe sarcomere hypercontraction after intermittent nerve stimulation than after continuous nerve stimulation. Duncan (1987) reported that changes in pH, loss of ATP and Ca-activated neutral protease were not involved in rapid myofilament damage. Armstrong et al (1991) pointed out that the injury in the muscle fibers is not a result of insufficient ATP production. Those authors also suggested that the degree of injury is proportional to the amount of work done by the muscle. Our findings are in agreement with their conclusions. Some unidentified factor which is related to muscle contraction may play an important role in inducing sarcomere hypercontraction. Recently, evidence has been accumulating that various types of sarcomere alterations occur as a result of a wide range of treatments such as denervation (Sakakima et al, 2000), feeding of a low-protein diet (Oumi et al, 2000) and tenotomy (Abou Salem et al, 2001). The muscles of rats in those studies were not, at least intentionally, overloaded, and some of the sarcomere changes observed may not necessarily have reflected muscle injury. However, the results of those studies support our idea that high tension, active strain and a decrease of blood flow are unlikely to have induced sarcomere alterations in the present study. The causes and mechanisms of sarcomere alterations need to be further elucidated.

Acknowledgements

We greatly appreciate the advice and help of Prof. Kanji Matsukawa, Hiroshima University. This study was supported in part by grants-in-aid for scientific research from the Ministry of Education, Science, Sports and Culture of Japan, and the Tsuchiya Memorial Medical Foundation.

References

Abou Salem EA, Fujimaki N, Ishikawa H, Tashiro T, Komiya Y (2001) Morphological changes and recovery process in the tenotomized soleus muscles of the rat. *Arch Histol Cytol* **64**, 127-137.

Armstrong RB, Ogilvie RW, Schwane JA (1983) Eccentric exercise-induced injury to rat skeletal muscle. *J Appl Physiol* **54**, 80-93.

Armstrong RB, Warren GL, Warren JA (1991) Mechanisms of exercise-induced muscle fibre injury. *Sports Med* **12**, 184-207.

Duan C, Delp MD, Hayes DA, Delp PD, Armstrong RB (1990) Rat skeletal muscle mitochondrial [Ca^{2+}] and injury from downhill walking. *J Appl Physiol* **68**, 1241-1251.

Duncan CJ (1987) Role of calcium in triggering rapid ultrastructural damage in muscle: a study with chemically skinned fibres. *J Cell Sci* **87**, 581-594.

Fridén J, Lieber RL (1992) Structural and mechanical basis of exercise-induced muscle injury. *Med Sci Sports Exerc* **24**, 521-530.

Fridén J, Lieber RL (1998) Segmental muscle fiber lesions after repetitive eccentric contractions. *Cell Tissue Res* **293**, 165-171.

Fridén J, Sjöström M, Ekblom B (1981) A morphological study of delayed muscle soreness. *Experientia* **37**, 506-507.

Gibala MJ, MacDougall JD, Tarnopolsky MA, Stauber WT, Elorriaga A (1995) Changes in humar skeletal muscle ultrastructure and force production after acute resistance exercise. *J Appl Physiol* **78**, 702-708.

Komulainen J, Kytölä J, Vihko V (1994) Running-induced muscle injury and myocellular enzyme release in rats. *J Appl Physiol* **77**, 2299-2304.

Krippendorf BB, Riley DA (1994) Temporal changes in sarcomere lesions of rat adductor longus muscles during hindlimb reloading. *Anat Rec* **238**, 304-310.

Lieber RL, Fridén J (1993) Muscle damage is not a function of muscle force but active muscle strain. *J Appl Physiol* **74**, 520-526.

Lieber RL, Fridén J (1999). Mechanisms of muscle injury after eccentric contraction. *J Sci Med Sport* **2**, 253-265.

Matsuura N, Kawamata S, Ozawa J, Kai S, Sakakima H, Abiko S (2001) Injury and repair of the soleus muscle after electrical stimulation of the sciatic nerve in the rat. *Arch Histol Cytol* **64**, 393-400.

Newham DJ, McPhail G, Mills KR, Edwards RHT (1983) Ultrastructural changes after concentric and eccentric contractions of human muscle. *J Neurol Sci* **61**, 109-122.

Nilsson B, Ingvar DH (1967) Intramuscular pressure and contractile strength related to muscle blood flow in man. *Scand J Clin Lab Invest (Suppl)* **93**, 31-38.

Ogilvie RW, Armstrong RB, Baird KE, Bottoms CL (1988) Lesions in the rat soleus muscle following eccentrically biased exercise. *Am J Anat* **182**, 335-346.

Oumi M, Miyoshi M, Yamamoto T (2000) The ultrastructure of skeletal and smooth muscle in experimental protein malnutrition in rats fed a low protein diet. *Arch Histol Cytol* **63**, 451-457.

Pachter BR, Eberstein A (1989) Passive exercise and reinnervation of the rat denervated extensor digitorum longus muscle after nerve crush. *Am J Phys Med Rehabil* **68**, 179-182.

Riley DA, Ellis S, Giometti CS, Hoh JFY, Ilyina-Kakueva EI, Oganov VS, Slocum GR, Bain JLW, Sedlak FR (1992) Muscle sarcomere lesions and thrombosis after spaceflight and suspension unloading. *J Appl Physiol* **73**, 33S-43S.

Riley DA, Ellis S, Slocum GR, Sedlak FR, Bain JLW, Krippendorf BB, Lehman CT, Macias MY, Thompson JL, Vijayan K, De Bruin JA (1996) In-flight and postflight changes in skeletal muscles of SLS-1 and SLS-2 spaceflown rats. *J Appl Physiol* **81**, 133-144.

Sakakima H, Kawamata S, Kai S, Ozawa J, Matsuura N (2000) Effects of short-term denervation and subsequent reinnervation on motor endplates and the soleus muscle in the rat. *Arch Histol Cytol* **63**, 495-506.

Thompson JL, Balog EM, Fitts RH, Riley DA (1999) Five myofibrillar lesion types in eccentrically challenged, unloaded rat adductor longus muscle—a test model. *Anat Rec* **254**, 39-52.

Explanations of Figures

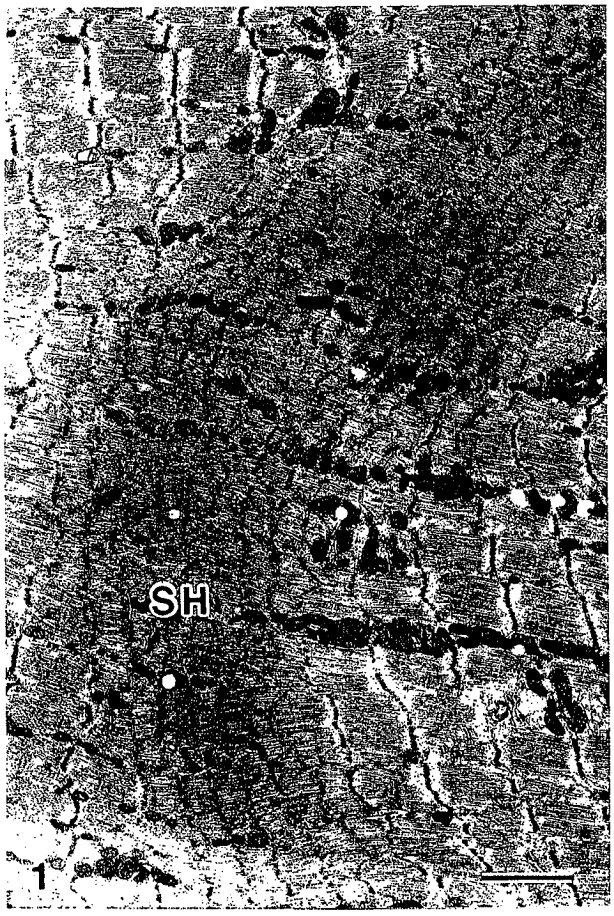
Fig. 1. Sarcomere hypercontraction (SH) after intermittent nerve stimulation with intact tendons. Hypercontracted sarcomeres extend transversely across a muscle fiber, which is in close agreement with the observations after continuous nerve stimulation with intact tendons. Bar = 2μm.

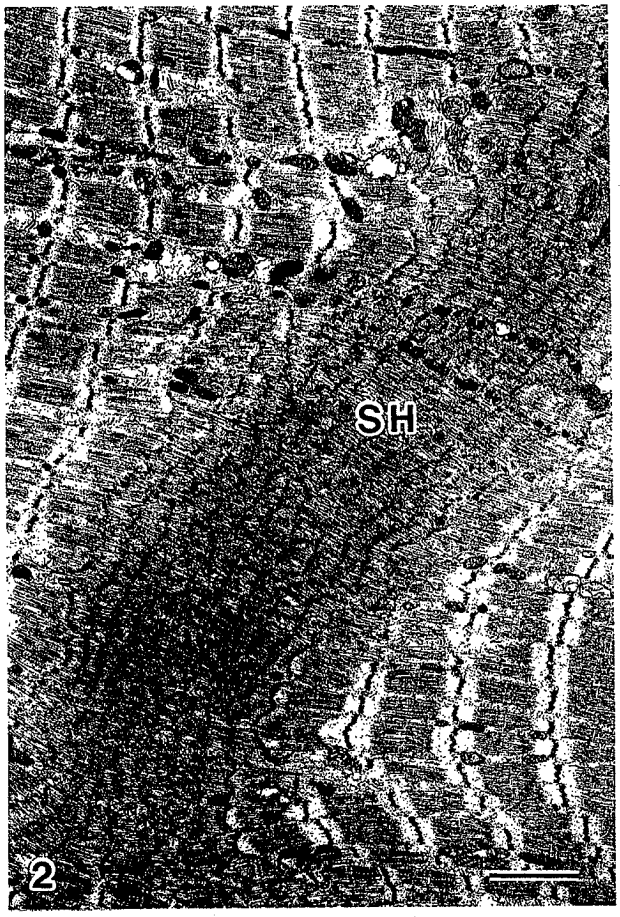
Fig. 2. Sarcomere hypercontraction (SH) after tenotomy plus continuous nerve stimulation. The sarcomere lesions resemble those in Fig 1. Bar = 2μm.

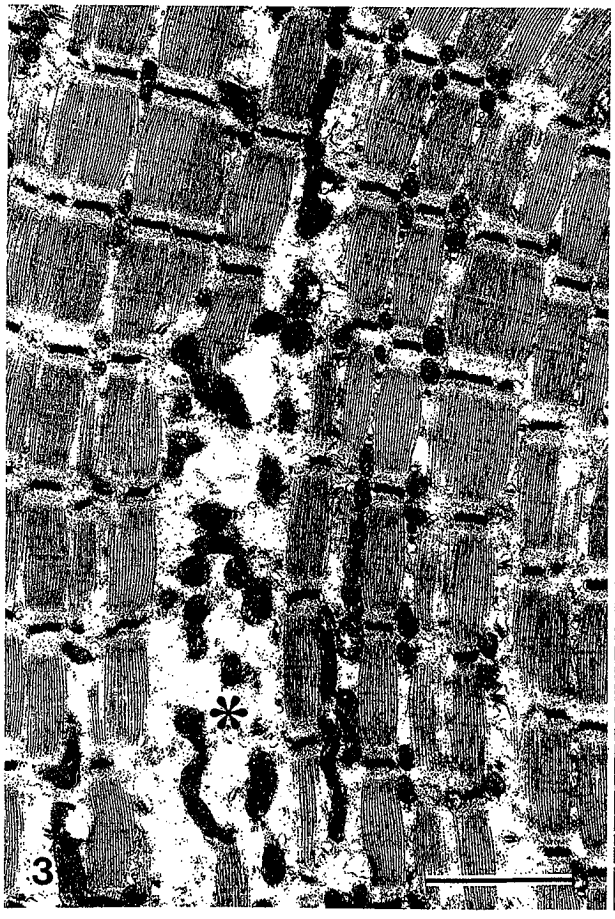
Fig. 3. Regions of scarce myofilaments after tension loading (600 g). Most sarcomeres maintain a normal structure, except for a small region which lacks myofilaments (*). Bar = 2μm.

Fig. 4. Z band disarrangement rarely found after tension loading (1,200 g). Z (Z) bands are wavy. Bar = 2μm.

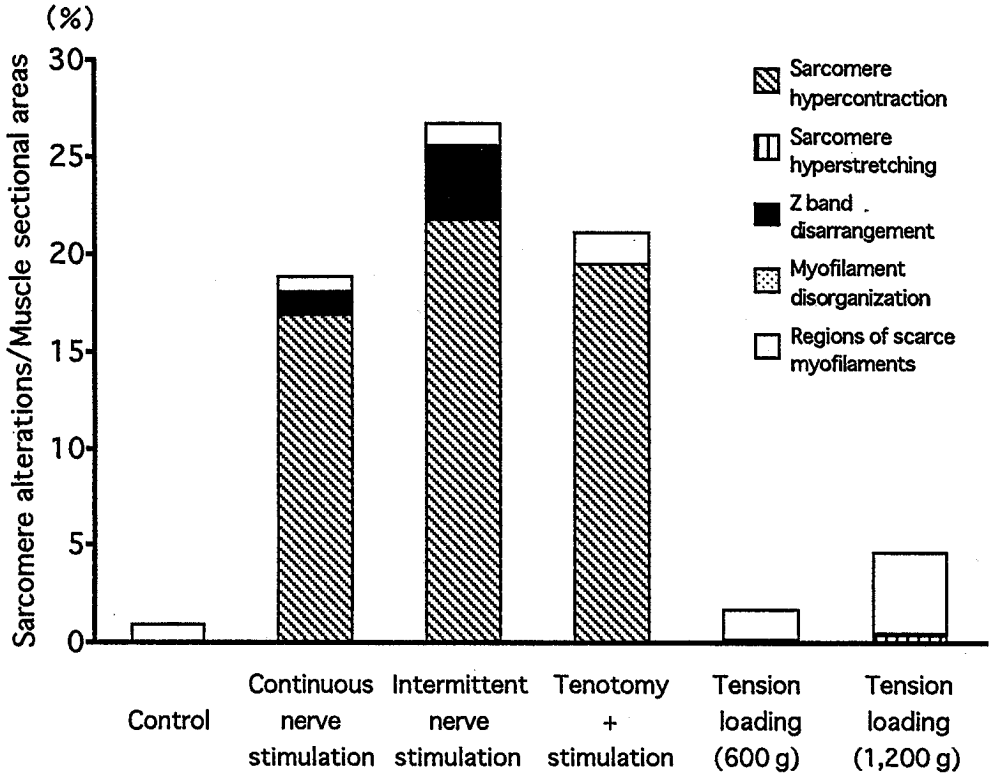
Fig. 5. Areas of aberrant sarcomeres in the soleus muscle after various treatments. Sarcomere alterations were widely found after continuous nerve stimulation, intermittent nerve stimulation and tenotomy plus nerve stimulation, whereas there were far fewer lesions after tension loading.


SH
1


SH
2


*
3


Z
4


(%)
Sarcomere alterations/Muscle sectional areas
30
25
20
15
10
5
0
Sarcomere hypercontraction
Sarcomere hyperstretching
Z band disarrangement
Myofilament disorganization
Regions of scarce myofilaments
Control
Continuous nerve stimulation
Intermittent nerve stimulation
Tenotomy + stimulation
Tension loading (600 g)
Tension loading (1,200 g)

5