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Effect of Resting Interval for Muscle Regeneration in Mice

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Abstract

Background: Muscle tissue has an exceptional ability to regenerate, however, unresting damage to the muscles by intense and frequent exercises occasionally causes prolonged muscle fatigue, soreness, and underperformance in sports. Taking rest is generally considered to be crucial for regular training to avoid the accumulation of muscle damage. We hypothesized that differences in the resting intervals between two periods of exercise may result in histological differences in muscle regeneration.

Method: An eccentric contraction model of mouse gastrocnemius muscle was made using percutaneus electrical stimulation. The mice received eccentric exercises twice with resting intervals of 0, 12, 24 hours, 2, and 3 days. The authors investigated the ratio of myofibers with central nuclei to whole myofibers histologically (the centronuclear cell ratio; CNCR) at 14 days after the second exercise as an index of the muscle regeneration.

Results: The CNCR of the group that exercised one-time was 29.5%. In the groups exercised twice, it increased from 31.8% with an interval of 0 hours to a peak of 43.9% with 24 hours, then decreased to 32.8% with an interval of 3 days. The ratios of the groups with intervals of 12 and 24 hours were higher than those with one-time exercise and those with the intervals of 0 hours, 2 days, and 3 days.

Conclusions: The resting interval between two periods of eccentric exercises affected the histology of muscle regeneration. The amount of muscle damage and/or the recovery process of damaged muscles should vary depending on the length of resting interval between strenuous exercises. An appropriate interval for rest must be necessary in order to avoid further muscle damage.

Introduction

Basically, skeletal muscles undergo three kinds of exercise: isometric, isotonic, and isokinetic. Isotonic exercise consists of two different types: concentric, and eccentric. Various combinations of such exercises are employed for training [8,13,18]. Intense and frequent exercises, particularly the eccentric type, causes histological muscle damage [11,15]. Muscle tissue generally has an excellent ability to regenerate and consequently develop muscular strength and mass (the supercompensation effect). Nevertheless, unresting damage to the muscles occasionally leads to prolonged muscle fatigue, soreness, and underperformance in sports [3,20]. The appropriate balance of exercise and rest, therefore, should be crucial to avoid the accumulation of muscle damage. So far, little has been reported on the effects of resting based on

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histological evaluations of muscle regeneration. We hypothesized that differences in the resting intervals between two periods of exercise may result in histological differences in muscle regeneration. T

Materials and methods

Eccentric contraction animal model

The experiment was carried out according to the guidelines of the Ethics Committee of Tohoku University. Thirty 10-week-old male C57BL mice were used . The mice were anesthetized by an intraperitoneal injection of pentobarbital sodium at 50 mg/kg body mass. The hair of the right lower leg was removed with vanishing cream. The right hind limb was fixed at the knee, and the right foot was strapped with adhesive tape to a metal plate attached to the servomotor (RU-72, Kimura Seisakujo, Japan). Contractions of the gastrocnemius muscle were induced by percutaneus electrical stimulation, with a pair of electrodes applied to the skin of the right lower leg and an electrical stimulator (DPS-07, Daiya Medical System, Japan) with 0.5 millisecond pulses at 150 Hz and 15V for 0.6 seconds. During the 0.6-second activation period, the ankle was passively dorsiflexed by the motor by 90° from the position of maximum plantar flexion, which resulted in an eccentric contraction of the gastrocunemius muscle. This action was repeated every 4 seconds for 180 repetitions with a 3-minute rest in the middle [23].

Intervals between the two periods of exercises

Each group received exercise twice with a resting interval of either 0 hour (continuously), 12 hours, 24 hours, 2 days, and 3 days, and were designated as the 0-hour, 12-hour, 24-hour, 2-day, and 3-day groups, respectively. The mice that underwent just one-time exercise comprised the single-exercise group. Untreated mice were used as a control. Those mice were allowed free cage activity between or after exercises.

Muscle sampling and histological procedure

Five mice of each group were euthanized 14 days after the last exercise. The gastrocnemius muscles of the right leg were removed for this study. The midportion of the muscle was cross-sectioned 5 mm thick, embedded in paraffin, and cut into slices 2-mm thick. Sections from all mice were stained with hematoxylin and eosin (H&E) for histological investigation [23].

Quantification of muscle regeneration

The central nucleus was used as an indicator of muscle regeneration. A higher number of central nuclei can be interpreted as reflecting greater damage and/or a slower

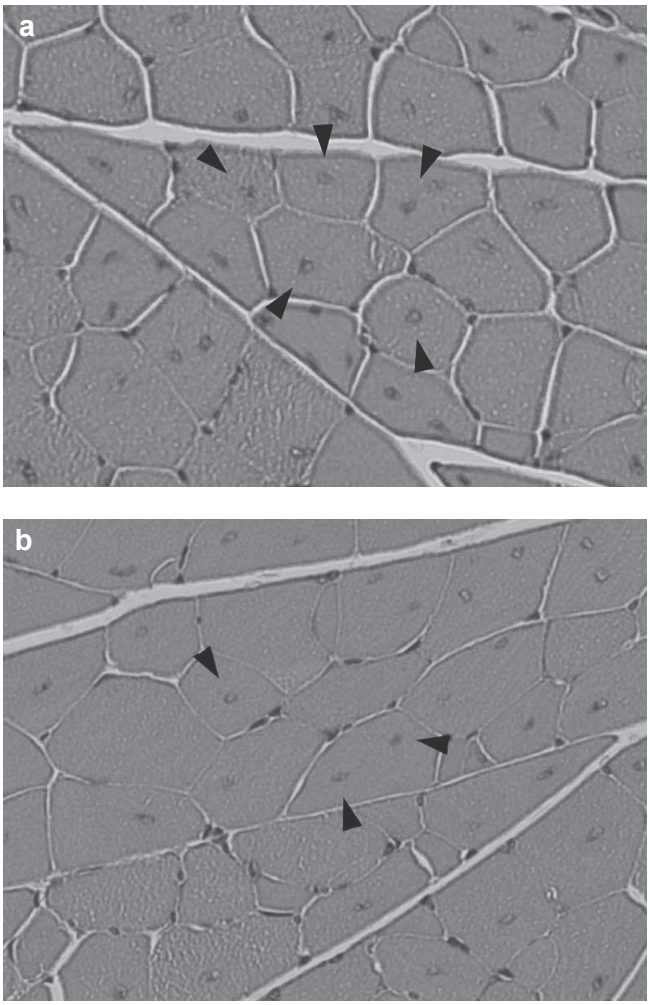

Figure 1. Light microscopy of muscle regeneration after eccentric exercises. a) 24-hour group. b) 3-day group. Myofibers with central nuclei (arrows) were detected. The central nuclei were more frequently found in the 24-hour group. Original magnification is x 200.

recovery of the muscle [2,3,17,22]. We evaluated the centronucleated cell ratio (CNCR), defined by the percentage of central nucleated myofibers divided by the total number of myofibers. Five fields containing the largest number of central nucleated myofibers were selected at a magnification of x200. Myofibers with central nuclei and those with nuclei peripherally shifted were counted three times, each. Then, the CNCR was calculated using the mean numbers of those data.

Statistical analysis

Post hoc analysis was performed using the Tukey-Kramer test to determine differences in the CNCR among all exercise groups. The level of significance was set at $P<0.05$. All statistical analyses were performed with commercially available software, JMP (SAS Institute Inc.,Cary, NC, USA).

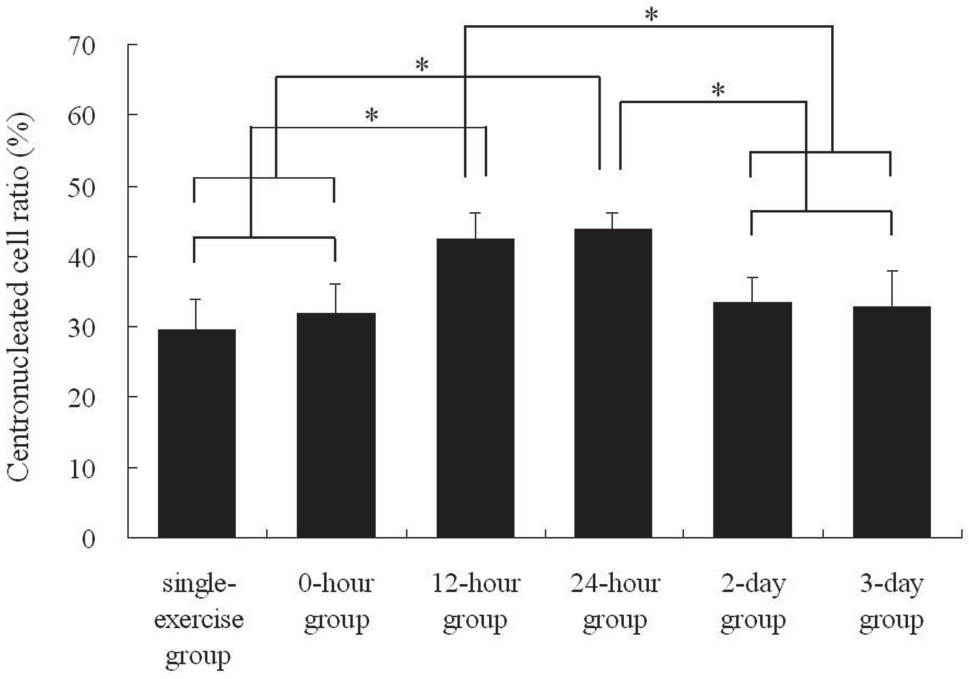

Figure 2. Centronuclear cell ratios. The 12- and 24-hour groups showed higher ratios than the other groups. Values are means ± SD; n =5. Statistical significance at $P < 0.05$ is denoted by *.

Results

Myofibers in each fascicle fit closely together with little variation in size and shape in all groups. In the untreated control group, the nuclei of myofibers were relatively inconspicuous and situated peripherally. No myofibers with central nuclei, which indicates regenerating ones, were found. On the other hand, in every exercise group myofibers with central nuclei were detected in all sections and could be distinguished easily from those whose nuclei were shifted peripherally. Neither disrupted myofibers nor inflammatory cells were found. Myofibers with central nuclei were more frequently found in the 12- and 24-hour groups (Figure 1).

The CNCR of the single-exercise group was 29.5%. In the twice exercised groups, it increased from 31.8% in the 0-hour group to a peak of 43.9% in the 24-hour group (Figure 1a), then decreased to 32.8% in the 3-day group (Figure 1b). The ratios of the 12- and 24-hour groups were statistically higher than those of the single-exercise, 0-hour, 2-day, and 3-day groups (Figure 2).

Discussion

The recovery process of myofibers begins with satellite cells, the primary stem cells in skeletal muscle [14]. Simultaneously with the occurrence of muscle dam-

age, satellite cells are activated, caused to proliferate and become differentiated, and finally fuse with other satellite cells to form myofibers with central nuclei. Then, their nuclei are shifted peripherally with the production of muscle-specific proteins and the regeneration process is completed. Thus, central nuclei are used as an indicator of the regenerating muscle, and the large number of those can be interpreted as a greater muscle damage and/or a slower recovery of the damaged muscle [2,3,17,22].

Exercise-induced muscle damage is primarily caused mechanically by direct muscular strain during the lengthening of contracting muscle [5,21], and secondarily by free radicals, an increase in the permeability of the membranes of myocytes, the inflammatory response, and phagocytosis of neutrophils and macrophages [4,19]. In our study, the CNCRs showed no significant differences among the single-exercise, the 0-hour, the 2-day, and the 3-day group mice. It should be explained that repetitive eccentric exercises reduced the muscular strain by the mechanism such as the overstretched sarcomere to fail to reinterdigitate [1] and the intramuscular exhaustion of glycogen and adenosine tri-phosphate [9,10]. Thus, the damage of myofibers in both the single-exercise and the 0-hour group should have reached a plateau in the middle of the exercises by the repetitive electrical stimulation. In the interval from 2 to 3 days after the eccentric exercises, the structural damage of myofibers was most prominent. The subsequent exercises in those groups, therefore, might have produced less muscular strain and no significant accumulation of disrupted myofibers.

The CNCRs in the groups exercised twice with resting intervals from 12 to 24 hours were higher than those of the other groups. The muscular strain in those phases might recover temporarily and yield greater muscle damage according to the improvement of the metabolic supply. Another possibility is that the muscle regenerative capacity might have been disturbed by the subsequent exercises in those groups. The regulatory factors of muscle regeneration are reported to be locally produced cytokines and growth factors, the local and systemic immune system, endocrine system [12,16], blood flow [7], and innervation [2,5]. Particularly, several cytokines and growth factors are well known as substances secreted from muscle tissue locally after intense exercise and are expressed in a time-dependent manner with specific roles in the action of satellite cells [6,23]. After the subsequent exercise in the 12- and 24-hour group, those substances might not be produced and/or activated in the optimal manner, leading to a delay the process of muscle regeneration.

Muscle has an excellent ability for regeneration. However, our results demonstrate that the resting interval between two periods of eccentric exercises affects the histology of muscle regeneration. This implies that an appropriate interval for rest must be necessary in order to avoid the accumulation of muscle damage in mice. A limitation of our study is that we cannot extrapolate from our data in mice on the amounts of exercise and rest needed to achieve much more safe and effective training in human beings.

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References

1. Allen DG (2001) Eccentric muscle damage: mechanisms of early reduction of force. Acta Physiol Scand Mar 171: 311–319.
2. Anderson JE (1991) Dystrophic changes in mdx muscle regenerating from denervation and devascularization. Muscle Nerve 14: 268–279.
3. Angeli A, Minetto M, Dovio A, Paccotti P (2004) The overtraining syndrome in athletes: a stress-related disorder. J Endocrinol Invest 27: 603–612.
4. Armstrong RB, Warren GL, Warren JA (1991) Mechanisms of exercise-induced muscle fibre injury. Sports Med 12: 184–207.
5. Atha J (1981) Strengthening muscle. Exerc Sport Sci Rev 9: 1–73.
6. Bischoff R (1990) Cell cycle commitment of rat muscle satellite cells. J Cell Biol 111: 201–207.
7. Delp MD, Laughlin MH (1998) Regulation of skeletal muscle perfusion during exercise. Acta Physiol Scand 162: 411–419.
8. Faulkner JA, Opiteck JA, Brooks SV (1992) Injury to skeletal muscle during altitude training: induction and prevention. Int J Sports Med 13: 160–162.
9. Hesselink MK, Kuipers H, Geurten P, Van Straaten H (1996) Structural muscle damage and muscle strength after incremental number of isometric and forced lengthening contractions. J Muscle Res Cell Motil 17: 335–341.
10. Hesselink MK, Kuipers H, Keizer HA, Drost MR, van der Vusse GJ (1998) Acute and sustained effects of isometric and lengthening muscle contractions on high-energy phosphates and glycogen metabolism in rat tibialis anterior muscle. J Muscle Res Cell Motil 19: 373–380.
11. Hough (1902) Ergographic studies in muscular soreness. Am J Physiol 7: 76–92.
12. Kraemer WJ, Fleck SJ, Dziados JE, Hahrrman EA, Marchitelli LJ, Gordon SE, Mello R, Frykman PN, Koziris LP, Triplett NT (1993) Changes in hormonal concentrations after different heavy-resistance exercise protocols in women. J Appl Physiol 75: 594–604.
13. LaStayo PC, Woolf JM, Lewek MD, Snyder-Mackler L, Reich T, Lindstedt SL (2003) Eccentric muscle contractions: their contribution to injury, prevention, rehabilitation, and sport. J Orthop Sports Phys Ther 33: 557–71.
14. Mauro A (1961) Satellite cell of skeletal muscle fibers. J Biophys Biochem Cytol 9: 493–495.
15. McCully KK, Faulkner JA (1985) Injury to skeletal muscle fibers of mice following lengthening contractions. J Appl Physiol 59: 119–126.
16. Naveri H, Kuoppasalmi K, Harkonen M (1985) Metabolic and hormonal changes in moderate and intense long-term running exercises. Int J Sports Med 6: 276–281.
17. Pizza FX, Peterson JM, Baas JH, Koh TJ (2005) Neutrophils contribute to muscle injury and impair its resolution after lengthening contractions in mice. J Physiol 1: 899–913.
18. Rhea MR, Alderman BL (2004) A meta-analysis of periodized versus nonperiodized strength and power training programs. Res Q Exerc Sport 75: 413–422.
19. Smith LL (1991) Acute inflammation: the underlying mechanism in delayed onset muscle soreness? Med Sci Sports Exerc 23: 542–551.
20. Smith LL (2004) Tissue trauma: the underlying cause of overtraining syndrome? J Strength Cond Res 18: 185–193.
21. Teague BN, Schwane JA (1995) Effect of intermittent eccentric contractions on symptoms of muscle microinjury. Med Sci Sports Exerc 27: 1378–1384.
22. Thaloor D, Miller KJ, Gephart J, Mitchell PO, Pavlath GK (1999) Systemic administration of the NF-kappaB inhibitor curcumin stimulates muscle regeneration after traumatic injury. Am J Physiol 277: C320–329.
23. Tomiya A, Aizawa T, Nagatomi R, Sensui H, Kokubun S (2004) Myofibers express IL-6 after eccentric exercise. Am J Sports Med 32: 503–508.

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