

Responses of Vastus Lateralis Muscle Fibers to Cycling Training with Different Loading Parameters in Human

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ABSTRACT

Responses of single muscle fibers of human vastus lateralis muscle to 8-week training with different loading parameters were studied in 22 male subjects, using a specially designed training machine with compulsory rotation of pedals induced by electrical engine. Three types of cycling were compared: 1) forward (concentric) with high velocity and 2) rearward (eccentric) compulsory rotation of pedals with high or 3) low velocity. Pedaling/rotation rate was 70 per min for high velocity groups and 35 rotations per min for low velocity group. Loads for high and low velocity groups were approximately 60% and 90% of the maximal alactic power accordingly. The repetitive exercise consisting of 1-min work periods divided by long (10 min) resting periods was used. Exercise bouts were repeated 5-7 times with 10-min resting periods. The training was performed twice a week for 8 weeks. Biopsy samples were taken from the vastus lateralis muscle before and after the training. Single muscle fibers were mechanically isolated and fiber cross-sectional area (CSA), myonuclear number, myonuclear CSA, and myonuclear domain were analyzed using a confocal microscope. Myonuclear CSA and myonuclear number per mm of fiber length did not change significantly after training in both regimes, although fiber CSA was increased significantly after eccentric training at either low or high intensity. Myonuclear domain size was also increased

significantly by concentric and eccentric exercise. It was suggested that eccentric training was more effective for induction of muscle fiber hypertrophy. However, this phenomenon was not directly related to the increase of number and/or size of myonuclei.

(Received: 3 June, 2007 Accepted: 19 July, 2007)

Key words: concentric or eccentric bicycle exercise, vastus lateralis muscle, fiber size, myonuclei

INTRODUCTION

It is well-known that the patterns of activity and/or mechanical load influence the morphological, metabolic, and contractile properties of skeletal muscles. For example, decreased use in response to gravitational unloading by exposure to actual weightlessness¹²⁾ or its simulation models, such as human bedrest¹⁾ or hindlimb suspension of rats⁸⁾, causes a rapid atrophy in muscles that are composed of slow-twitch fibers predominantly. On the contrary, an increased utilization of muscle by removing the synergists, as well as the strength training, causes a hypertrophy¹⁸⁾. Hypertrophy is also induced by stretching of muscle *in vivo*⁴⁾ and *in vitro*¹⁷⁾. It is reported that the stretching prevents the unloading-related atrophy of muscle fibers^{13,15,16)}. However, it was also reported that exertional muscle injury was induced in response to exhaustive

eccentric contraction, in which the forced active lengthening and/or stretching of muscles are involved, even though such a phenomenon was not observed after concentric contraction¹⁹⁾.

Unloading-related atrophy of muscle fibers is also associated with a decreased number of myonuclei¹⁴⁾. Further, the mean size of myonuclei is generally greater in atrophied muscle fibers than the normal controls, even though the DNA content in each nucleus is constant^{9,20)}. It is suggested that protein synthesis by larger nucleus may be impaired. However, the precise mechanism responsible for the regulation of various properties of skeletal muscles is still unclear. Therefore, the responses of human skeletal muscle fibers to eccentric or concentric exercise training were investigated in the present study.

METHODS

Twenty two healthy male volunteers participated in the current study. The mean age, height, and weight were 21 ± 1 (mean \pm SEM) years old, 177 ± 1 cm, and 78 ± 2 kg, respectively. All subjects were evaluated clinically and were considered to be in good physical condition. They were informed about the possible risks in attending the exercise training and taking muscle biopsies. And a signed informed consent was obtained from each subject. This study was approved by the Human Use Committees at the Institute of Biomedical Problems (Moscow, Russia). All experimental procedures were conducted in accordance with World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects).

The effects of bicycle training using a specially designed bicycle ergometer with different pedaling parameters were investigated. Characteristic property of the ergometer is compulsory rotation of pedals induced by electrical engine. The difference of ergometer from the classic one is that the seat is not fixed firmly, but slides along an inclined beam. As a result lower extremities carry the load which is a part of the subject's weight and depends on the angle of beam inclination. To proportion the pressure on the pedal, the subjects were asked to maintain the position of the seat at the constant level.

Three types of compulsory rotation were performed. Eight subjects performed pedaling with forward compulsory rotation as the classical bicycling at the velocity of 70 per min. Eight subjects performed pedaling with rearward compulsory rotation at the same velocity. And the remaining 6 subjects performed rearward rotation with the velocity of 35 per min. It was assumed that the concentric and eccentric loads are applied to the knee and hip extensors during the forward and rearward compulsory rotation, respectively. Thus, the groups were classified as 1) high velocity-concentric, 2) high velocity-eccentric, and 3) low velocity-eccentric training group, respectively. Training loads were chosen to fatigue muscles after approximately 60 seconds of rotations. For high and low velocity groups, the loads were approximately 60 and 90% of the maximal alactic power. The mean mechanical power per one exercise day was the same in all three groups. The repetitive exercise consisting of 5-7 working periods (1 min) divided by long resting periods (10 min) was used as daily training session. Two training sessions per week were performed and the total duration of training was 8 weeks.

Biopsy samples were taken from the vastus lateralis muscle of one leg before and from the other leg after the training. The samples were immediately frozen in liquid nitrogen and stored at -80°C until analyses. Each sample was gradually thawed to room temperature in a low-calcium relaxing solution, as describe previously²⁾. Single muscle fibers were mechanically isolated in relaxing solution (at least $n=20$ in each muscle). Each muscle sample was put into a tube filled with $500 \mu\text{l}$ relaxing solution and $500 \mu\text{l}$ of 100% glycerol. Relaxing solution contained 1.4 mM ethylene glycol bis (β -aminoethyl ether)-N, N, N', N'-tetraacetic acid (EGTA) and 8.6 mM MgCl_2 , 70 mM KCl, 80 mM imidazol, 4 mM adenosine-5'-triphosphate (ATP) disodium salt, and 11.3 mM phosphocreatine disodium salt in distilled water (7.4 pH adjusted with propionic acid). These tubes were then stored in a freezer (-20°C) over night. Next day, the muscle sample was placed on a glass plate, coated with silicon, and fixed using pins, and was immersed in the relaxing solution. Under microscope, a single fiber was separated using two pairs of

tweezers and was placed on a gelatin-coated glass slide and air-dried. Then, the slides were stored in a freezer (-5°C) until analysed. Mechanical isolation of single fibers has been shown to strip off the basal lamina of muscle fiber including satellite cells²⁾.

Immunohistochemistry and nuclear labeling

The single muscle fiber segments were thawed and dried at room temperature. The single fibers ($n=20$ for each muscle) was stained with 4.1×10^{-5} M acridine orange, diluted in phosphate-buffered saline (PBS), for 5 min to label the cytoplasm. And the myonuclei were stained with 1.5×10^{-7} M propidium iodide, diluted in PBS, for 4 min. Immediately before the analysis, the fibers were mounted in 50% glycerol with coverslips with "struts" of hardened nail polish on the corners to minimize the fiber compression.

Confocal microscopy

A FV-300 confocal microscope with an argon laser (488 nm of peak wavelength, Olympus) was used to analyze the fiber cross-sectional area (CSA), and the number and CSA of the myonuclei. First, a maximum-intensity projection rotated orthogonally to the long axis of the fiber was produced, and the fiber CSA was measured at three non-overlapping regions, randomly chosen along the fiber length. The number of myonuclei in the single fiber

segment was counted. The length of the fiber segment was also measured. Myonuclear domain^{3,6)} was calculated by multiplying the fiber CSA and length of the fiber segment and dividing by myonuclear number. The fiber CSA and the length of fiber segment were normalized at a $2.5 \mu\text{m}$ sarcomere length. The myonuclear number per mm of fiber length was calculated. The myonuclear size was also measured in each myonucleus by Nomarski optic scan. To measure the myonuclear size, the outline of the myonucleus was enclosed in the scanned image and the area was measured. At least 30 myonuclei were checked for each fiber.

Statistical analyses

All values were expressed as means \pm SEM. Significant differences were examined by repeated measures of ANOVA followed by Scheffé's post hoc test. Differences were considered significant at the 0.05 level of confidence.

RESULTS AND DISCUSSION

The absolute fiber CSA was significantly increased after eccentric, but not concentric, training at both high and low intensities (Fig. 1A, $p < 0.05$). Since the subject-specific fluctuations were noted in the levels obtained before the initiation of exercise training, the percent change in each subject was also calculated (Fig. 1B). The mean

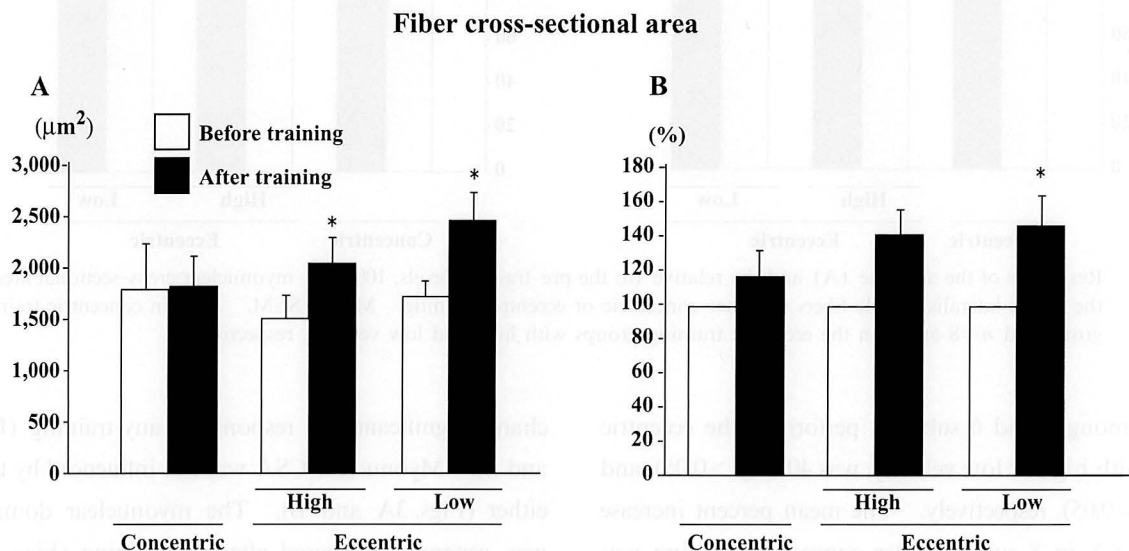


Fig. 1. Responses of the absolute (A) and the relative (to the pre-training levels, 100%, B) fiber cross-sectional area of the vastus lateralis muscle fibers to either concentric or eccentric training. Mean \pm SEM. $n=8$ in concentric training group and $n=8$ and 6 in the eccentric training groups with high and low velocity, respectively. *: $p < 0.05$ vs. before training.

Myonuclear number/mm

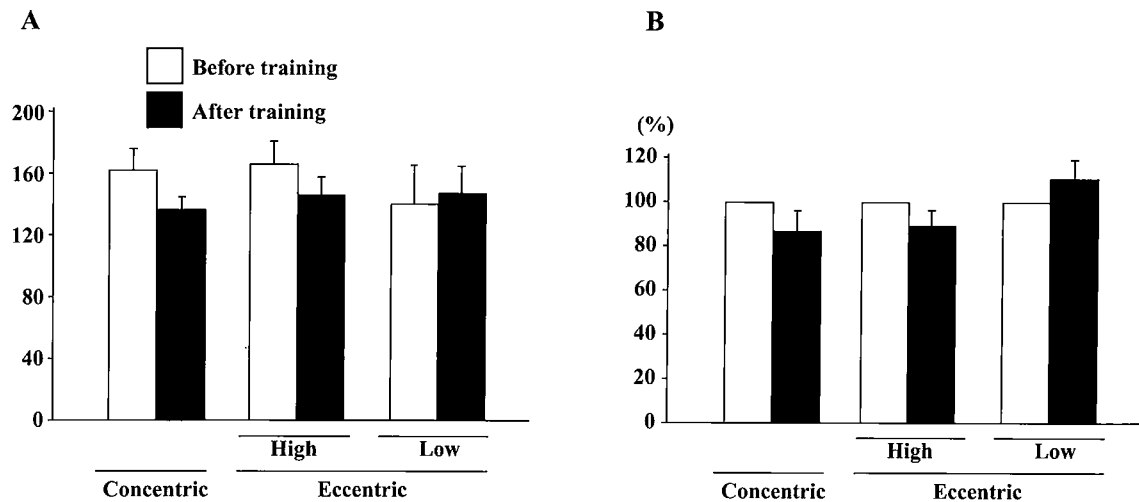


Fig. 2. Responses of the absolute (A) and the relative (to the pre-training levels, 100%, B) myonuclear number per mm of fiber length of the vastus lateralis muscle fibers to either concentric or eccentric training. Mean \pm SEM. $n=8$ in concentric training group and $n=8$ and 6 in the eccentric training groups with high and low velocity, respectively.

Myonuclear cross-sectional area

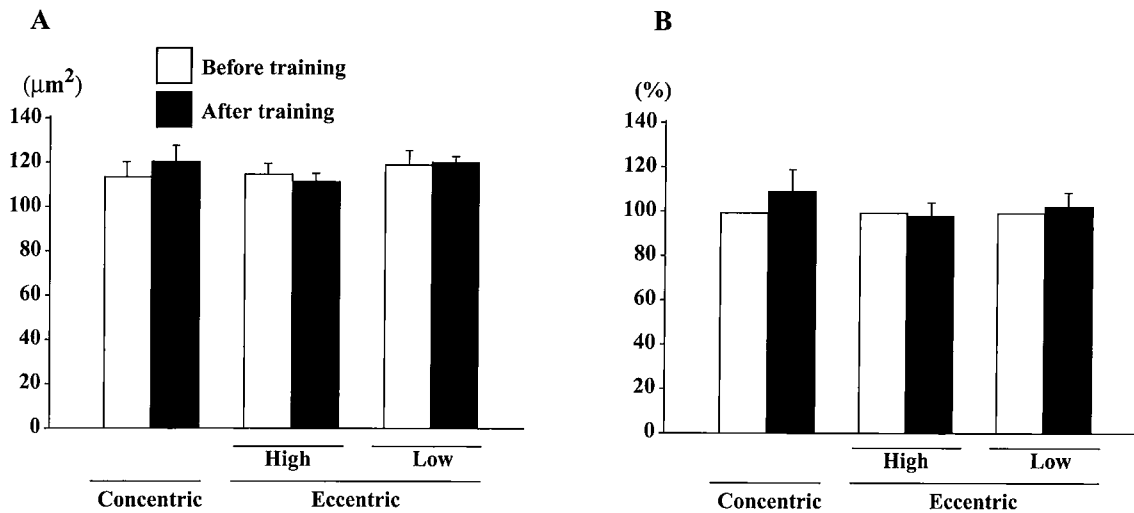


Fig. 3. Responses of the absolute (A) and the relative (to the pre-training levels, 100%, B) myonuclear cross-sectional area of the vastus lateralis muscle fibers to either concentric or eccentric training. Mean \pm SEM. $n=8$ in concentric training group and $n=8$ and 6 in the eccentric training groups with high and low velocity, respectively.

increase among 8 and 6 subjects, performed the eccentric training with high or low velocity, was 40.0 ($p > 0.05$) and 46.7% ($p < 0.05$), respectively. The mean percent increase of fiber CSA in 8 subjects after concentric training was 16.6% ($p > 0.05$).

Myonuclear number per mm of fiber length did not

change significantly in response to any training (Figs. 2A and B). Myonuclear CSA was not influenced by training, either (Figs. 3A and B). The myonuclear domain size was, generally, increased after the training (Figs. 4A and B). The absolute values of subjects, performed the eccentric training with high velocity, significantly increased ($p <$

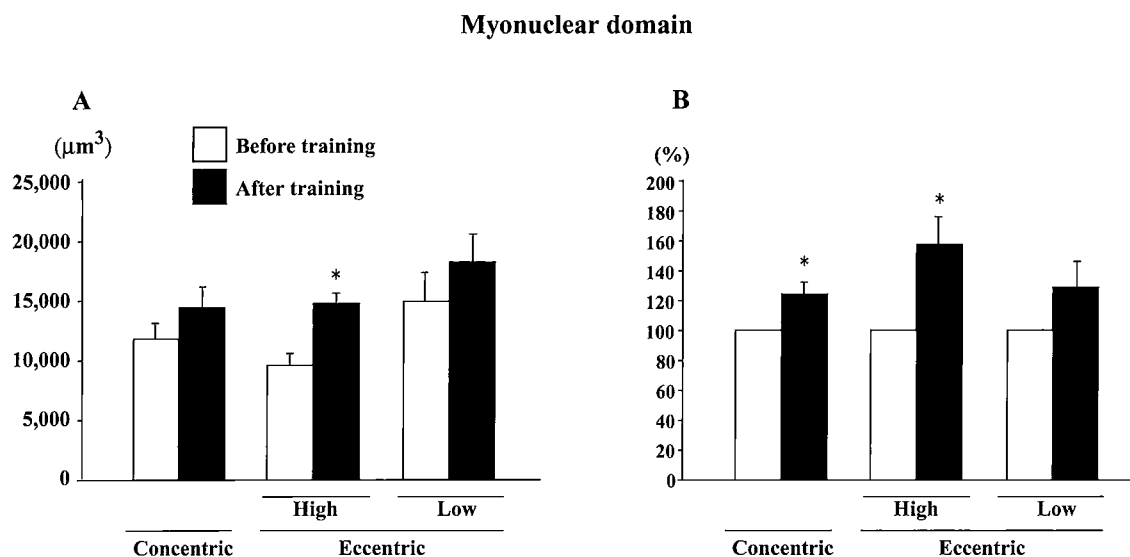


Fig. 4. Responses of the absolute (A) and the relative (to the pre-training levels, 100%, B) myonuclear domain of the vastus lateralis muscle fibers to either concentric or eccentric training. Mean \pm SEM. $n=8$ in concentric training group and $n=8$ and 6 in the eccentric training groups with high and low velocity, respectively. *: $p<0.05$ vs. before training.

0.05). The mean relative increase after concentric or eccentric training with high or low velocity was 25.9% ($p<0.05$), 59.3% ($p<0.05$), and 29.6% ($p>0.05$), respectively.

LaStayo *et al.*¹⁰⁾ and Higbie *et al.*⁷⁾ reported that fiber CSA was increased after eccentric training more than after concentric training. It was further reported that eccentric contractions caused more profound changes in some aspects of muscle function than concentric contractions¹¹⁾. The fiber CSA was also increased significantly by eccentric exercise but not by concentric exercise in the present study.

The degree of increase in myonuclear domain size tended to be greater after high-velocity eccentric exercise than concentric exercise in the present study. Chen *et al.*⁵⁾ also reported that the expressions of cardiac ankyrin-repeated protein, chemokine ligand 2, CCAAT/enhancer binding protein delta, IL-1 receptor, tenascin C, and cysteine-rich angiogenic inducer 61 in vastus lateralis muscle of human subjects, analyzed by quantitative real-time polymerase chain reaction, were increased following the eccentric training. These results may suggest that the function of myonuclei might be improved by eccentric exercise. Furthermore, myonuclear CSA tended to increase slightly following the concentric, but not eccentric,

training ($p>0.05$). We previously reported that the smaller myonuclei may have the greater function than the myonuclei with larger size^{9,20)}. Thus, it is also suggested that the protein synthesis by myonuclei might be more stimulated by eccentric than concentric training.

Significant atrophy of soleus muscle fibers was induced in response to 4-month bedrest in male subjects, although the myonuclear number was stable¹⁵⁾. Therefore, the myonuclear domain size was decreased after bedrest, suggesting that the function of each myonucleus might be inhibited or that the breakdown of cytoplasmic proteins, which may be unrelated to myonuclei, could be stimulated. The fiber atrophy was prevented by passive stretching of soleus due to dorsiflexion of ankle joints. These results suggest that the plasticity of morphological properties of muscle fibers could be modulated by some mechanism (s), which is not necessarily directly related to the function of myonuclei.

In conclusion, the eccentric training was more effective for induction of muscle fiber hypertrophy compared with the concentric training. However, this phenomenon was not directly related to the changes of the number and/or size of myonuclei. Beneficial effect of eccentric training is suggested, although it is also possible to cause muscle injury if the work intensity is too severe¹⁹⁾.

ACKNOWLEDGEMENTS

This investigation was sponsored by the Russian Ministry of Education and Sciences, Governmental Contract No. 02.522.11.2004. This study was also carried out as a part of Grant-in-Aid for Scientific Research (S, 19100009, Y.O.) from Japan Society for the Promotion of Science, Grant-in Aid for Exploratory Research (18650188, Y.O.) and for Young Scientists (B, 15700417, F.K.) from Ministry of Education, Culture, Sports, Science and Technology, and Grant-in-Aid for the Academic Frontier Project (Waseda University: 2005-2010) of the Ministry of Education, Culture, Sports, Science and Technology (K.I.).

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