# Applications of vascular occlusion diminish disuse atrophy of knee extensor muscles

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**ABSTRACT**

TAKARADA, Y., H. TAKAZAWA, and N. ISHII. Applications of vascular occlusion diminish disuse atrophy of knee extensor muscles. *Med. Sci. Sports Exerc.*, Vol. 32, No. 12, 2000, pp. 2035–2039. **Purpose:** We have previously shown that the combination of low-intensity resistive exercise and moderate vascular occlusion induces in humans a marked increase in growth hormone secretion and muscular hypertrophy. The present study investigated the effects of vascular occlusion on the size of thigh muscles in patients who underwent an operation for the reconstruction of the anterior cruciate ligament to see whether it attenuates the disuse muscular atrophy without any exercise combined. **Methods:** Two sessions of occlusive stimulus, each consisting of five repetitions of vascular occlusion (mean maximal pressure, 238 mm Hg) for 5 min and the release of occlusion for 3 min, were applied daily to the proximal end of the thigh from 3rd to 14th days after the operation. Changes in the cross-sectional area (CSA) of thigh muscles were analyzed with magnetic resonance images taken on the 3rd and 14th day after the operation. **Results:** Without occlusive stimulus (control), the CSAs of knee extensors and flexors decreased by 20.7 $\pm$ 2.2% and 11.3 $\pm$ 2.6% (mean $\pm$ SEM, $N = 8$), whereas with the occlusive stimulus, they decreased by 9.4 $\pm$ 1.6% and 9.2 $\pm$ 2.6% ($N = 8$), respectively. The relative decrease in CSA of knee extensors was significantly ($P < 0.05$) larger in the control group than in the experimental group. **Conclusion:** The results indicate that the occlusive stimulus effectively diminishes the postoperation disuse atrophy of knee extensors. **Key Words:** HYPOXIA, ISCHEMIA, REPERFUSION, DISUSE ATROPHY

Skeletal muscles are capable of adapting themselves rapidly to the mechanical environment. They respond with a hypertrophy to the strong mechanical stress such as resistive exercise, whereas they respond with a disuse atrophy to both the unloading such as spaceflight and cast-immobilization. It has been thought that the major cause of the disuse atrophy is an elevation of protein degradation rate relative to the protein synthesis rate (1). In the particular field of knee surgery, suppressing the disuse atrophy of thigh muscles has been regarded as important, because the rehabilitation usually takes a prolonged period of time to regain the original muscular strength. In addition, the age-related atrophy of knee extensor muscles gives rise to serious problems such as inability to stand up among aged population.

We have previously shown that a low-intensity resistance exercise combined with vascular occlusion induced muscular hypertrophy and concomitant increase in strength in elbow flexor muscles of older women, even if the intensity of exercise was much lower than expected to induce the muscular hypertrophy (17). The mechanisms underlying such an effect of externally applied occlusive stimulus have been interpreted as follows: 1) additional recruitment of fast-twitch fibers in a hypoxic condition (18); 2) moderate production of reactive oxygen species (ROS) promoting the tissue growth (13,15,16); and 3) stimulated secretion of catecholamines and growth hormone (17). All of these processes are also thought to be associated with conventional, heavy-resistance exercises, because strong muscular contractions produce a large amount of metabolic products and cause a transient intramuscular ischemia (19). If the processes 2) and 3) play roles in muscular hypertrophy, the occlusive stimulus *per se* is expected to have an effect in either promoting hypertrophy or attenuating atrophy.

In the present study, we investigated the effect of occlusive stimulus on thigh muscles of patients subjected to the surgical reconstruction of the anterior cruciate ligament (ACL) to see whether it has an effect in diminishing the postoperation muscular atrophy without any exercise stimulus combined. The occlusive stimulus applied periodically from the 3rd day after the operation for ACL reconstruction significantly diminished the atrophy of cast-immobilized knee extensor muscles. The results were discussed with special reference to the possible mechanisms underlying such an effect of occlusive stimulus on the disuse atrophy.

## METHODS

**Subjects.** Sixteen patients (8 male and 8 female) after the ACL reconstruction volunteered for the study. Eight

0195-9131/00/3212-2035/$3.00/0
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Received for publication October 1999.
Accepted for publication April 2000.

TABLE 1. Physical characteristics of subjects.

| | Experimental Group ($N = 8$) | | Control Group ($N = 8$) | |
|---|---|---|---|---|
| | Male ($N = 4$) | Female ($N = 4$) | Male ($N = 4$) | Female ($N = 4$) |
| Age (yr) | 22.8 ± 0.9 | 22.0 ± 0.9 | 23.3 ± 1.3 | 22.8 ± 1.0 |
| Height (cm) | 169.8 ± 0.7 | 157.8 ± 1.3 | 173.4 ± 1.0 | 158.5 ± 1.1 |
| Weight (kg) | 67.8 ± 2.0 | 49.8 ± 0.7 | 71.0 ± 5.8 | 53.4 ± 1.6 |

Values are means ± SD.
No significant difference was observed between variables of control group and those of experimental group in the same gender.

patients (4 male and 4 female) participated in an experimental group (aged 22.4 ± 2.1 yr, mean ± SD) and the other eight patients (4 male and 4 female) participated in a control group (aged 23.0 ± 2.5 yr). Physical characteristics of the subjects in experimental group were similar to those of the subjects in the control group (Table 1). All of the subjects were previously informed well about the experimental procedure to be utilized as well as the purpose of the study, and their written informed consent was obtained. The study was approved by the Ethical Committee for Human Experiments, University of Tokyo.

**Experimental procedure.** The experimental period was for 2 wk, including the day of operation. The subjects in both experimental and control groups followed the usual program for recovery in the hospital with their injured knee kept immobilized by means of a knee brace. Magnetic resonance imaging (MRI) was performed to obtain cross-sectional images of the lower extremities of the injured side on the 3rd and 14th days after the operation (Fig. 1A). In the experimental group, occlusive stimuli were given by compressing the proximal end of the thigh (100 mm below the hip joint) with a pneumatic occlusion cuff (width, 90 mm; length, 700 mm) for the period between the 3rd and 14th days after the operation (Fig. 1B). The subjects kept their upper body inclined at about 45° during the occlusive stimulus. A set of occlusive stimulus consisted of occlusion for 5 min and a removal of occlusive pressure for 3 min, and five sets were given to the subjects twice a day, at 9 a.m. and 2 p.m. (Fig. 1C). The occlusive pressure was kept constant during the two sessions of the same day. It was initially set at 180 mm Hg and then was gradually elevated at a 10-mm Hg step depending on the degree of postoperation recovery of each subject. The maximal pressure was 238 ± 8 mm Hg (mean ± SEM) at the final stage of experiment, with the range between 200 and 260 mm Hg.

The subjects in control group followed the same daily schedule inclusive of dietary program as that for the experimental group (Fig. 1). According to the protocol for the experimental group, an occlusion cuff was placed on their thigh for 37 min but without inflation (sham operation).

**MRI.** To obtain cross-sectional images of the thigh, MRI was performed by using a 0.5 T superconducting system (Gyroscan T5 II, Philips Medical Systems International, Best, The Netherlands) with a wraparound body coil. The coil covered the whole thigh, including markers attached to the skin. Twelve serial sections were acquired with a 6- to 10-mm sectional thickness and a 0.6- to 1.0-mm intersection gap. The field of view was 160–350 mm. Pulse sequences for spin-echo T1-weighted images were performed with a repetition time of 500–552 ms and an echo time of 20–25 ms. Two signal acquisitions were used. The scan matrix and reconstruction matrix were 205 × 256 and 256 × 256, respectively. The image acquisition was started immediately after the subject took the supine position to minimize the effect of gravity-induced fluid shift. The time required for the whole sequence was about 4–6 min.

For each subject, the range of serial sections was deliberately determined on longitudinal images along femur to obtain sections of identical portions before and after the experimental period. Among the photographs of 12 cross-sectional images obtained, those of two portions near the midpoint of femur (distal to the occluded site) were chosen for the measurements of muscular cross-sectional area (CSA). Photographic negatives were digitized into an 8-bit gray scale at a space resolution of 144 pixels per inch, and stored in a Macintosh 8100 AV computer with an Epson ART-8500G scanner.

Determinations of tissue outlines and measurements of CSAs for muscles and other tissues were made by using the NIH image (ver. 1.25). The measurements were repeated three times for each image and their mean values were used. Deviation in these three sets of measurement was less than 2%.

**Statistical analysis.** Unless otherwise stated, variables were expressed with means ± SEM. Because of the small $N$ values, comparisons between variables obtained from control group and those obtained from experimental group were made with the nonparametric, Mann-Whitney $U$-test. Changes in variables within the same individuals were examined with Wilcoxon signed-ranks test. In both tests, $P < 0.05$ was considered significant.

## RESULTS

Changes in the CSAs of knee extensors (quadriceps), flexors, and femur are summarized in Table 2. In both the control and experimental groups, the CSAs of knee extensors and flexors on the 14th day after the operation were significantly smaller than those on the 3rd day after the operation ($P < 0.05$, Wilcoxon signed-ranks test), whereas the CSA of femur showed no significant change. Because Wilcoxon singed ranks test requires $N > 5$ for a group, changes in CSAs within the same individuals were not examined separately for male and female subjects.

Figure 2 shows the percentage changes in CSAs during the 14-d experimental period. The CSA of knee extensors decreased by 20.7 ± 2.2 and 9.4 ± 1.6% in the control ($N = 8$) and experimental ($N = 8$) groups, respectively. The

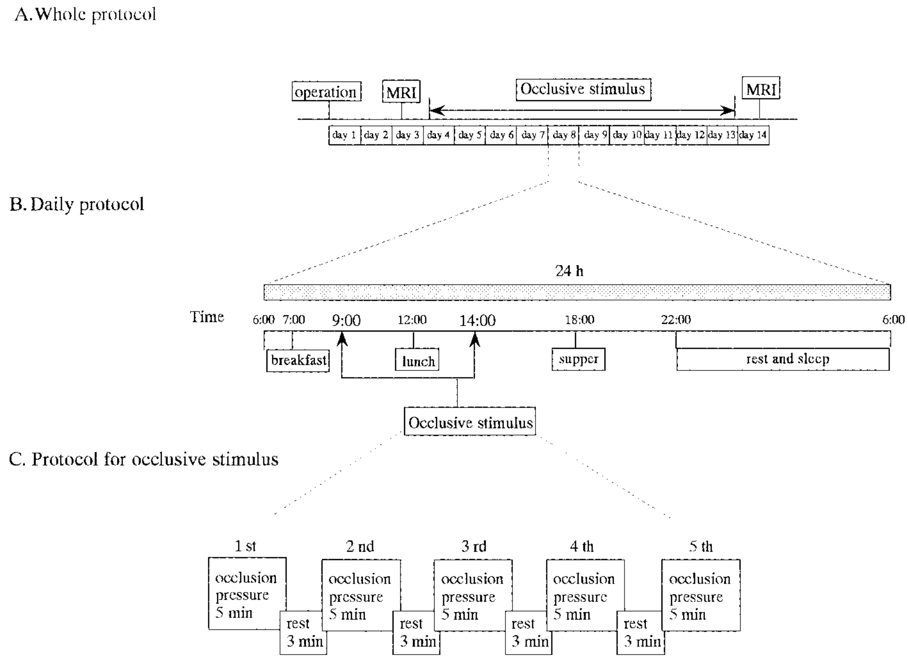


**Figure 1—Schematic representation of the experiment procedure.**

extent of decrease was significantly smaller in the experimental group than in the control group ($P = 0.046$, Mann-Whitney *U*-test). On the other hand, the CSA of knee flexors decreased by 11.3 ± 2.6, and 9.2 ± 2.6% in the control and experimental groups, respectively. These were not significantly different ($P = 0.69$).

The significant effect of occlusive stimuli in diminishing the extent of decrease in the CSA of knee extensors was also seen when the data from male and female subjects were separately analyzed ($N = 4$ for each group): male subjects in control group, 17.6 ± 3.7%; male subjects in experimental group, 8.9 ± 2.1% ($P = 0.043$); female subjects in control group, 23.8 ± 2.0%; female subjects in experimental group, 11.2 ± 2.5% ($P = 0.021$).

There appeared no regional difference in the responses of the muscles to the occlusive stimuli. The muscular CSAs in the region near the occluded site (the uppermost position within the range of image acquisition) changed substantially in a manner similar to that of the CSAs in the mid portion of the thigh, although cross sectional images for the same position were not obtained consistently from all of the subjects (data not shown).

## DISCUSSION

The present study indicates that the periodic applications of occlusive stimuli, even without any exercise stimulus combined, are effective in diminishing the disuse atrophy of the knee extensors after surgical operation. Because the application of occlusive stimuli was limited for only the initial 2 wk after the operation, no apparent effect was observed on the healing process of the injured ACL. However, our preliminary study in which the occlusive stimuli combined with light exercise were continuously given to the patients during the subsequent period of rehabilitation

TABLE 2. Changes in cross-sectional area (CSA) of muscles and bone in both experimental and control groups.

A. Experimental group (N = 8)

| | Male (N = 4) | | Female (N = 4) | | Male + Female (N = 8) | |
|---|---|---|---|---|---|---|
| | 3rd Day After Op. | 14th Day After Op. | 3rd Day After Op. | 14th Day After Op. | 3rd Day After Op. | 14th Day After Op. |
| Total cross-sectional area ($cm^2$) | 171.0 ± 9.1 | 162.9 ± 7.4 | 164.0 ± 8.0 | 148.1 ± 11.2 | 167.5 ± 5.1 | 156.3 ± 6.5* |
| Extensor ($cm^2$) | 57.8 ± 6.1 | 52.4 ± 4.6 | 50.1 ± 2.0 | 44.5 ± 2.4 | 54.0 ± 3.0 | 48.5 ± 2.6* |
| Flexor ($cm^2$) | 50.8 ± 4.6 | 44.8 ± 3.3 | 41.6 ± 1.7 | 38.8 ± 3.2 | 46.2 ± 2.6 | 41.7 ± 2.2* |
| Femur ($cm^2$) | 7.5 ± 0.3 | 7.5 ± 0.3 | 6.7 ± 0.5 | 6.7 ± 0.4 | 7.0 ± 0.3 | 7.0 ± 0.2 |

B. Control group (N = 8)

| | Male (N = 4) | | Female (N = 4) | | Male + Female (N = 8) | |
|---|---|---|---|---|---|---|
| | 3rd Day After Op. | 14th Day After Op. | 3rd Day After Op. | 14th Day After Op. | 3rd Day After Op. | 14th Day After Op. |
| Total cross-sectional area ($cm^2$) | 168.0 ± 9.4 | 149.8 ± 10.6 | 154.5 ± 3.0 | 125.1 ± 2.0 | 161.0 ± 4.7 | 137.5 ± 6.4* |
| Extensor ($cm^2$) | 59.5 ± 1.4 | 49.1 ± 3.2 | 46.0 ± 4.8 | 35.1 ± 4.0 | 52.8 ± 3.3 | 42.1 ± 3.4* |
| Flexor ($cm^2$) | 51.8 ± 5.0 | 48.4 ± 5.8 | 41.3 ± 3.6 | 34.9 ± 3.4 | 49.1 ± 4.7 | 44.8 ± 5.4* |
| Femur ($cm^2$) | 7.5 ± 0.6 | 7.6 ± 0.6 | 5.3 ± 0.1 | 5.3 ± 0.1 | 6.4 ± 0.5 | 6.5 ± 0.5 |

Values are means ± S.E.M.

Symbols denote statistically significant changes from CSAs measured on the 3rd day after the operation (*, $P < 0.05$ Wilcoxon signed-ranks test).

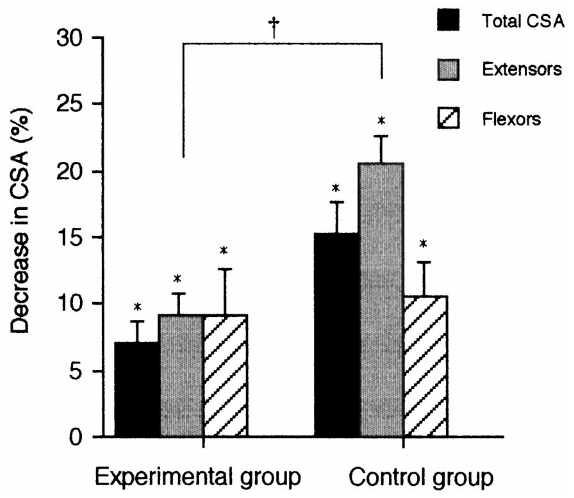


**Figure 2—Relative decreases in muscular cross-sectional area during the period between 3rd and 14th days after the operation. Symbols just above the columns denote statistically significant changes from CSAs measured on the 3rd day after the operation (* $P < 0.05$ Wilcoxon signed-ranks test), whereas † denotes significant difference between control and experimental groups ($P < 0.05$, Mann-Whitney *U*-test).**

showed a marked shortening of the period required for a full recovery to the original activity level.

The present effect of occlusive stimuli appears to be similar to that of exercises in attenuating decrease in muscular strength during the bed rest. Duvoisin et al. (2) showed that isometric muscular exercises (4 $d \cdot wk^{-1}$, 4 $sessions \cdot d^{-1}$) effectively reduced the extent of decrease in knee extension strength from 19% to 9% after the 30-d bed rest. However, the exercises were without effect in diminishing the decrease in knee flexion strength. The mechanism for such a differentiated response to exercise between knee extensors and flexors is unknown, though it may be related to the difference in their functions against gravity and/or muscle-fiber composition (10).

The cuff inflation compress the muscular tissue beneath the occlusion cuff. This localized mechanical stress does not seem to have a direct, large effect on the muscle protein metabolism, because the atrophy-attenuating effect was seen in the portion much distal to the occluded site. However, it may cause a slight stretch of some muscles extending toward the distal region through the occluded site. This possibility should not be ignored, because keeping muscles stretched has been shown to diminish effectively the disuse muscular atrophy in animal models (6,11). Such an effect of stretch may, at least, be either additional to or closely related to the effects of vascular occlusion stated below, although both the extent and the duration of stretch may not be sufficient to produce large trophic effect in the present experimental condition.

The pressure level of 180–238 mm Hg used in the present study was based on the previous study on acute effects of vascular occlusion (mean pressure, 210 mm Hg; duration, 5 min), which has shown marked, sequential increases in plasma concentrations of lactate, noradrenaline and growth hormone within a few tens of minutes after the removal of occlusive pressure (18). During the occlusive stimulus, the subjects kept their upper body inclined at 45°, so that the position of the heart was higher by 30 cm (gravitational pressure equivalent of 20 mm Hg) than that of the cuff. However, even if these factors and mechanoreflex-induced increase in arterial blood pressure (21) are taken into consideration, the present occlusive pressure is thought to inhibit effectively both the arterial inflow and venous outflow of blood through the occluded site. Consequently, blood pooling, hypoxia, and the accumulation of metabolic subproducts such as lactate would successively occur in the portion distal to the occluded site, as has been shown in arm (17) and leg muscles (18).

The intramuscular accumulation of metabolites has been shown to stimulate the sympathetic nerve and the hypothalamus-pituitary system through actions of muscular metaboreceptors and cause the increase in plasma concentrations of noradrenaline, adrenaline (8,14,18,19), and growth hormone (18). In addition, the application of strong occlusive pressure may directly stimulate the sympathetic nerve through the intramuscular pressure-sensitive mechanoreflex (3,21). These nervous and hormonal responses may be involved in the mechanisms underlying the present atrophy-attenuating effect of occlusive stimulus. In particular, it should be noted that the stimulation of adrenergic $\beta_2$-receptors promotes in rat muscles a selective hypertrophy of fast, Type II fibers, by possibly suppressing protein catabolism (9,22).

In addition to these mechanical, neural, and hormonal factors, changes in the intramuscular oxygen environment may play a part in the present effect of occlusive stimulus. It has been shown that the muscular xanthine oxidase activity is elevated in a hypoxic condition and produces reactive oxygen species (ROS) during the subsequent reperfusion (7). Although the ROS has been shown to often cause lethal damages in small, mononucleated cells, it has also been shown in some tissues to promote the signal transduction for growth and proliferation by possibly modifying the redox state of regulatory proteins (13,15,16).

These effects of metabolites and ROS suggest that the occlusion-induced hypoxic stress affects either directly or indirectly the protein metabolism of muscle. Indeed, a hypertrophy with the increase in numerical proportion of Type II fibers has been shown to occur sometimes in leg muscles of patients with heart failure, chronic obstructive lung diseases, and peripheral vascular diseases such as intermittent claudication (4,5,12). The similar mechanism may operate in the exercise-induced muscular hypertrophy, because strong muscular contractions have been shown to cause in the muscle an ischemia followed by a reactive hyperaemia (20).

In conclusion, the present occlusive stimuli can effectively diminish the disuse atrophy of thigh muscles, although effect may be specific to muscle types. Therefore, it would be potentially highly useful in the postoperation rehabilitation and also for improving muscular function in bedridden old people. However, further studies are required to obtain an insight into the exact mechanism underlying the effect of occlusive stimulus on the muscular protein metabolism, and also to clarify any side effect associated with the occlusive stimulus on the circulatory functions.

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