Cooperative Effects of Exercise and Occlusive Stimuli on Muscular Function in Low-Intensity Resistance Exercise with Moderate Vascular Occlusion

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Abstract: To obtain insight into the relative contributions of exercise and occlusive stimuli to these muscular adaptations, the present study investigated the short- and long-term effects of varied combinations of low-intensity exercise and vascular occlusion. The subjects were separated into 3 groups ($n = 6$ for each group): low-intensity with vascular occlusion (LIO), low-intensity without vascular occlusion (LI), and vascular occlusion without exercise (VO). LIO and LI groups performed bilateral knee extension exercises in seated positions with an isotonic extension machine. In the LIO group, both sides of the thigh were pressure-occluded at the proximal end by means of a tourniquet during the entire session of exercise (~10 min), whereas only the occlusion with the same pressure and duration was given in the VO group. The mean occlusion pressure was 218 ± 8.1 mmHg (mean ± SE). The exercise session consisted of five sets of exercise at an intensity of 10–20% 1RM and was performed twice a week for 8 wk. After the period of exercise training, isometric and isokinetic strengths at all velocities examined increased significantly in the LIO group ($p < 0.05$), whereas no significant change in strength was seen in the LI and VO groups. The increase in muscular strength in LIO was associated with a significant increase in the cross-sectional area of knee extensor muscles by $10.3 \pm 1.6\%$. The plasma growth hormone concentration measured 15 min after the session of exercise showed a marked increase only in LIO. The results showed that the low-intensity exercise and occlusive stimuli have cooperative effects in the long-term adaptation of muscle and an acute response to growth hormone. [The Japanese Journal of Physiology 54: 585–592, 2004]

Key words: growth hormone, occlusion, muscular hypertrophy.

Skeletal muscles adapt themselves to varied exercise stimuli in a manner so that they respond appropriately to the new mechanical and metabolic demands. Intense resistance exercises generally cause increases in muscular size and strength [1], whereas exercises with a much smaller load and a larger volume (low-intensity endurance exercises) result in an increase in the muscle oxidative capacity without much increase in muscular size [2]. For the particular purpose of muscular hypertrophy and concomitant increase in strength, it has been believed that intensity higher than 65% of one repetition maximum (1RM) is to be used [1].

On the other hand, we have shown that a low-intensity resistance exercise (30–50% 1RM) combined with vascular occlusion induced marked increases in size and strength in elbow flexor muscles of old women, even if the intensity of exercise was much lower than expected to promote muscular hypertrophy [3]. The mechanisms underlying such an effect of externally applied occlusive stimulus have been interpreted as follows: (i) additional recruitment of fast-twitch fibers in a hypoxic condition [3, 4]; (ii), moderate production of reactive oxygen species including nitric oxide (NO) that may promote tissue growth [5–9]; (iii), stimulated secretions of growth hormone (GH) and

norepinephrine [4]. Furthermore, we showed that repeated applications of the occlusive stimulus with no exercise combined during bed rest effectively diminish disuse atrophy of knee extensor muscles [10]. This suggests that the above processes (i) and (iii) after occlusive stimuli *per se* play roles in the protein metabolism of muscle.

However, because applications of occlusive stimuli without exercise caused neither hypertrophy nor complete abolition of disuse atrophy during bed rest also suggests the requirement of exercise stimuli for muscular hypertrophy as an indispensable factor [10]. At present, the exercise intensity that can be used with vascular occlusion ranges from >0 to 50% 1RM, and a minimal intensity required for muscular hypertrophy in this intensity continuum remains unclear. With regard to the endocrine responses, knee extension exercises at an intensity as low as 20% 1RM have been shown to cause a dramatic increase in the plasma concentration of growth hormone (GH) when combined with vascular occlusion [4]. This level of force can be sustained for >1 h with no substantial symptom of muscular fatigue [11] so that it would not be far from the level of force required for daily activity.

The present study investigated the long-term effects of a low-intensity resistance exercise (10–20% 1RM) combined with vascular occlusion on muscular function in humans to see if such a low level of force production can effectively cause muscular hypertrophy and increase in strength when combined with vascular occlusion. Also, the relative contributions of exercise and occlusive stimuli to the long-term adaptation of muscle and an acute GH response were studied.

METHODS

Subjects and general procedures. Eighteen young male athletes volunteered for the study. The subjects were divided into three groups ($n = 6$ for each group): low-intensity exercise with vascular occlusion (LIO, aged 21.3 ± 0.6 yr, mean ± SD); low-intensity exercise without vascular occlusion (LI, aged 21.8 ± 0.8 yr); vascular occlusion without exercise (VO, aged 22.2 ± 0.8 yr). Their physical characteristics are shown in Table 1. None had previous experience with any specialized resistance exercise training. Before and after the 8 weeks of training, the strength of the knee extensor muscles and muscular cross-sectional area of the midthigh were measured. The load of exercise was determined relative to the maximal weight that could be lifted throughout the whole range of movement (one-repetition maximum: 1RM). All subjects were previously informed well about the experimental procedure to be utilized as well as the purpose of the study, and their informed consent was obtained. The study was approved by the Ethical Committee for Human Experiments, University of Tokyo.

Regimes for exercise training. The subjects for both LIO and LI performed bilateral knee extension exercises in seated positions with an isotonic leg extension machine (Nautilus Group Japan Inc., Tokyo, Japan). The range of knee joint motion in the exercise was from 0 to 90° (0° at full extension). In LIO, both sides of their thighs were pressure-occluded at the proximal ends by means of specially designed tourniquets (width, 90 mm; length, 700 mm) during the session of exercise, and the pressure was released immediately after the exercise session. The mean occlusion pressure given by the tourniquet throughout the period of training was 218 ± 8.1 mmHg (mean ± SEM), the level of which has been shown to induce a marked increase in plasma concentration of lactate during a low-intensity exercise [4]. The occlusion was maintained throughout the entire session of exercise, which lasted for ~10 min, and was released immediately after the end of the session. In VO, only the vascular occlusion without exercise was given to both thighs, as in LIO, for 10 min, which was equal to the duration of exercise in LIO. The magnitude of occlusive pressure in VO was almost the same as that in LIO.

The exercise was performed twice a week and lasted for 8 wk, including the period for instruction and orientation (16 sessions in all). In each exercise session, the subjects performed five sets of exercise with an interval of 1 min. The intensity of exercise was

Table 1. Physical characteristics of subjects.

	LIO		LI		VO	
	Pre-training	Post-training	Pre-training	Post-training	Pre-training	Post-training
Age (yrs)	21.3 ± 0.6		21.8 ± 0.8		22.2 ± 0.8	
Height (cm)	171.4 ± 2.9	171.7 ± 2.9	173.4 ± 3.2	172.8 ± 3.3	171.7 ± 1.5	171.8 ± 1.5
Body mass (kg)	69.3 ± 5.2	70.1 ± 5.2	72.4 ± 6.8	71.8 ± 6.5	67.9 ± 2.9	67.8 ± 3.0

Values are means ± SEM; $n = 6$ for low-intensity exercise with occlusion (LIO), $n = 6$ for low-intensity exercise without occlusion (LI), $n = 6$ for vascular occlusion without exercise (VO).

~20% 1RM for both LIO and LI. They were determined in the initial stage of exercise training and kept unchanged throughout the period of training. In LIO, the subjects repeated the lifting movement until failure in each set of exercises in order to induce the additional recruitment of fast-twitch fibers in a hypoxic condition as shown in our previous studies [12, 13], whereas in LI they were instructed to match the number of repetitions performed by LIO. The mean repetition in each set of exercises was 16.8 ± 2.1 (mean ± SEM). The mean intensity and repetitions for each set of exercise are shown in Table 2. The total volume of exercise for LIO was similar to that for LI. The subjects in both LIO and LI were instructed to lift and lower the load at an approximately constant velocity, taking about 2 s for each concentric and eccentric action (15 repetitions/min). In VO, the vascular occlusion for 10 min without exercise was given to the subjects twice a week for 8 weeks. All the exercise sessions were preceded by a 10 min warm-up on a bicycle ergometer at about 50% of the physical work capacity and a stretching of the major muscle groups to be trained.

Measurements of muscular strength. Isokinetic torque-angular velocity relations of knee extensors of the nondominant side were examined by using an isokinetic dynamometer (Myoret, Kawasaki Industry, Co. Ltd., Tokyo, Japan). The subjects were familiarized with the testing procedure on several occasions before the measurements. They sat on a chair with their backs upright and with their left legs firmly attached to the lever of the dynamometer. The pivotal point of the lever was visually aligned with the rotation axis of the knee joint maintained at that position during all movements. The isokinetic strength (IST) was measured at preset angular velocities of 30, 60, and 180°/s. The range of angular movement of the knee joint was limited between 0 and 90°. The value of peak torque was measured regardless of where it was developed within the range of movement. Three trials were made at each angular velocity, and the highest value obtained was used for further analyses. Maximal isometric torque (MVC) was measured at the knee angle of 80°.

Magnetic resonance imaging. Changes in the cross-sectional areas of thigh muscles were measured with a magnetic resonance imaging (MRI) 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 350 mm. Pulse sequences for spin-echo T1-weighted images were performed with a repetition time of 500 to 552 ms and an echo time of 20 to 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 assumed a supine posture to minimize the effect of gravity-induced fluid shift. The time required for the whole sequence was 4 to 6 min. The range of serial sections was deliberately determined on longitudinal images along the femur to obtain sections of identical portions before and after the period of exercise training. To reduce errors in measurement associated with a slight mismatch between the sectional portions obtained before and after the period of exercise training and incidental deformations of muscles during the processes of MRI, two sections around the midportion of the femur, each separated by ~20 mm, were selected from 12 serial sections, and a mean tissue cross-sectional area (CSA) was obtained from these two sections. Photographic negatives were digitized into an 8-bit gray scale at a space resolution of 144 pixels per inch and stored in a computer with an Epson ART-8500G scanner. The determinations of tissue outlines and measurements of CSAs for muscles and other tissues were made by using National Institute of Health Image (version 1.25) software. The measurements were repeated three times for each image, and their mean values were used. The deviation in these three sets of measurements was less than 2%.

Measurement of plasma growth hormone (GH). Before exercise training, the effects of the present exercise regimens and the vascular occlusion

Table 2. Intensity and repetitions for each set of exercise with occlusion.

	1st	2nd	3rd	4th	5th	Mean
Intensity, %1RM	21.6 ± 2.7	20.2 – 2.4	18.8 ± 2.9	16.3 ± 2.5	16.4 ± 2.6	17.6 ± 2.0
Repetitions	23.3 ± 1.1	18.4 – 0.8	17.2 ± 0.6	16.3 ± 0.7	15.2 ± 1.0	18.2 ± 0.6

Values are means ± SEM; *n* = 6 subjects for LIO. In LI, each subject performed exercise without occlusion at the same intensity and repetitions for each set. 1RM, one repetition maximum.

without exercise on the plasma concentration of GH were examined in 6 of 18 subjects, as previously described [4]. Venous blood samples (20 ml) were obtained 15 min after an exercise session, then processed and stored at $-20°C$ until analysis. Plasma concentrations of GH were determined by two-site immunoradiometric assay (GH kit Daiichi; Daiichi Radioisotope Laboratories, Ltd., Tokyo, Japan) [14]. This assay employed two anti-GH monoclonal antibodies, one of which was labeled with ^{125}I, and the other was coupled to a solid phase (bead). The standards were calibrated against WHO GH 66/217. The detection limit was 0.1 ng/ml, and interassay coefficients of variation with GH concentrations of 1.9, 7.5, and 30.2 ng/ml were 1.4, 2.0, and 2.1%, respectively.

Statistical analysis. Unless otherwise stated, the variables were described as means ± SEM. A Wilcoxon signed ranks test was used to compare differences between pre- and posttraining variables within the same subjects. In comparisons among the effects of LIO, LI, and VO, examinations of statistical significance were based on a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. For all statistical analyses, $P < 0.05$ was regarded as significant.

RESULTS

Changes in muscle cross-sectional area after exercise training. Typical examples of the cross-sectional MRIs of an identical midportion of the thigh are shown in Fig. 1. These images were taken before (A) and after (B) exercise training with occlusion (8 wk) and exhibit a marked increase (by ~16%) in the cross-sectional area (CSA) of knee extensors after the training. The low-intensity exercise with occlusion caused significant ($P < 0.01$) increases in

A   **B**
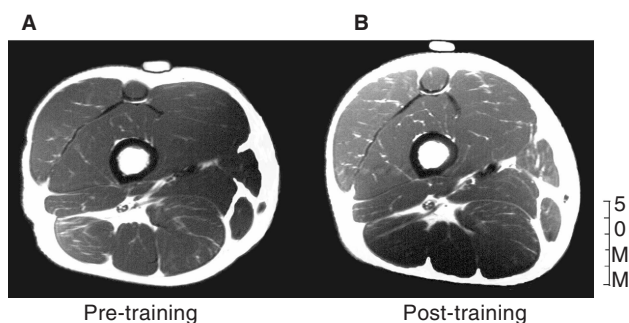

Pre-training   Post-training

Fig. 1. Typical magnetic resonance images showing transverse sections of the midthigh taken before (A) and after (B) exercise training with occlusion lasted for 8 wk. The images show identical midportions along the femur in the same subject. The bar indicates 50 mm.

the CSA of knee extensors from those before the exercise training (Fig. 2). The percent increase in CSA of extensors after LIO was 10.3 ± 1.6%, whereas no significant changes were observed in those of knee flexors and femur. In contrast, no significant changes occurred in CSA of knee extensors in both LI and VO.

Changes in muscular strength after exercise training. Figure 3 shows changes in torque-velocity relations after the 8-wk training period. All values of IST were normalized to the pretraining values of MVC. The LIO induced significant increases in isometric and isokinetic strengths at all velocities examined (Fig. 3A), whereas no changes in strength were observed after LI and after VO (Fig. 3, B and C). When averaged throughout all velocities, percent increases in strength after the exercise training was 9.2 ± 2.2% in the LIO group, 3.1 ± 1.4% in the LI group, and 2.8 ± 1.8% in the VO group. In the LIO group, however, maximal isometric torque per unit CSA was unchanged (in $N\ m^{-1}$) after the exercise training: pretraining, 3.3 ± 0.2; posttraining, 3.2 ± 0.2. These results indicate that the LIO is substantially effective in inducing muscular hypertrophy and a concomitant increase in strength.

Plasma GH concentration. Figure 4 shows plasma GH concentrations measured for 10 min before and 15 min after either session of exercise (LIO and LI) or vascular occlusion (VO). The plasma GH concentration increased significantly only after LIO, the magnitude of which was much larger than that reported by Kraemer *et al.* [15] for a high-intensity resistance exercise with a short rest period. Furthermore, the magnitude of GH response appeared to be much larger than the sum of those after LI and VO, suggesting the cooperativeness between exercise and occlusive stimuli. Our previous study with similar exercise protocols has shown that plasma GH concentration reaches a peak 15 to 30 min after the exercise and then gradually declines toward its resting level [4]. Therefore the present result quite likely represents the difference between all the responses of GH in LIO, LI, and VO.

DISCUSSION

The present study showed that a low-intensity resistance exercise causes muscular hypertrophy and a concomitant increase in isometric and isokinetic strengths when combined with moderate vascular occlusion. The percent increase in CSA of knee extensors was 10.3 ± 1.6%, and the average percent increases in isometric and isokinetic strengths at all velocities examined was 9.2 ± 2.2% after an 8-wk training
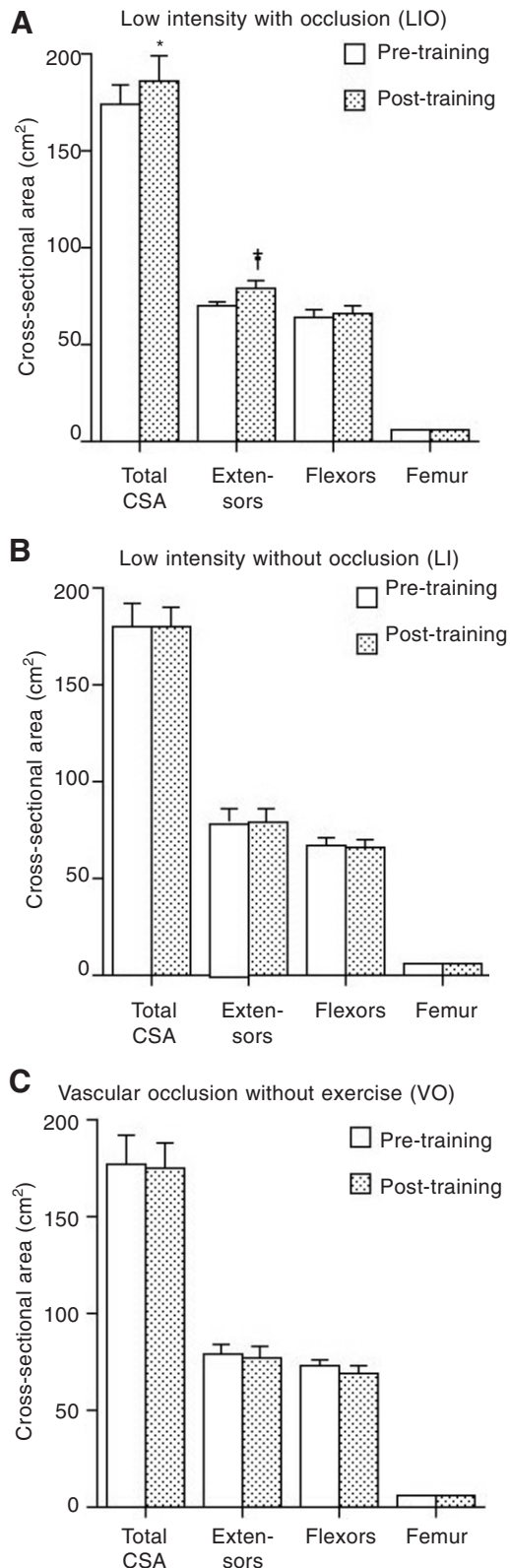
Fig. 2. CSAs of knee extensor, flexor, and femur measured before (open bars) and after the period of exercise training (filled bars). A: exercise at low intensity with occlusion (LIO; $n = 6$); **B**: exercise at low intensity without occlusion (LI; $n = 6$); **C**: vascular occlusion without exercise (VO, $n = 6$). The values are means ± SE. * and † denote statistically significant differences at $P < 0.05$ and $P < 0.01$, respectively.

exercise in LIO. Also, the maximal isometric torque per unit CSA was unchanged (in N m^{-1}): pretraining, $3.3 ± 0.2$; posttraining, $3.2 ± 0.2$. Thus the increases in isometric and isokinetic strengths could be considered to be mainly caused by muscular hypertrophy. The intensity used in the LIO was as low as 10 to 20% 1RM, which generally induces an improvement of muscular endurance, but is much smaller than expected to promote increases in muscular size and strength [1]. Indeed, the LI made at the same intensity and volume caused no increases in muscular CSA and isometric and isokinetic strengths.

Comparing the effects of LIO and LI protocols implies that the occlusive stimulus has a primary role in inducing the muscular adaptations to LIO. However, the applications of only vascular occlusion (VO) also resulted in no substantial increase in muscular size and isometric and isokinetic strengths. These results indicate that the combination of exercise and vascular occlusion is important to promote muscular adaptation, and some cooperativeness exists between the effects of exercise and occlusive stimuli.

The combination of exercise and vascular occlusion may cause an enhanced change in intramuscular oxygen environment. It has been known that the hypoxic environment stimulates the productions of hypoxia-inducible factors (HIFs) and vascular endothelial growth factor (VEGF), thereby promoting vascular neoformations within skeletal muscles [16]. Therefore an increase in vascular volume may contribute to the present increase in muscular size after LIO. However, this effect is quite likely minor, if any, because the MVC/unit CSA did not change after LIO.

The presence of the cooperative effects of exercise and occlusive stimuli was also shown in an acute response of plasma GH (Fig. 4). The plasma GH increased significantly only after LIO with both exercise and occlusive stimuli, the magnitude of which was much greater than that reported to occur after the typical exercise (high intensity, short rest period) widely used for gaining muscular size [15]. The interpretation of the present results needs much precaution because several studies have shown that immunoreactive forms of GH do not necessarily represent bioassayable, active isoforms of GH [17]. However, as far as the increase in serum concentration of immunoreactive GH after a strenuous exercise is concerned, it has been shown to correlate with the degree of muscle fiber hypertrophy for both type I and type II fibers [18]. This suggests that total GH released after exercise contains a sizable proportion of bioactive forms of GH under the normal condition, and the increase in plasma GH concentration will be
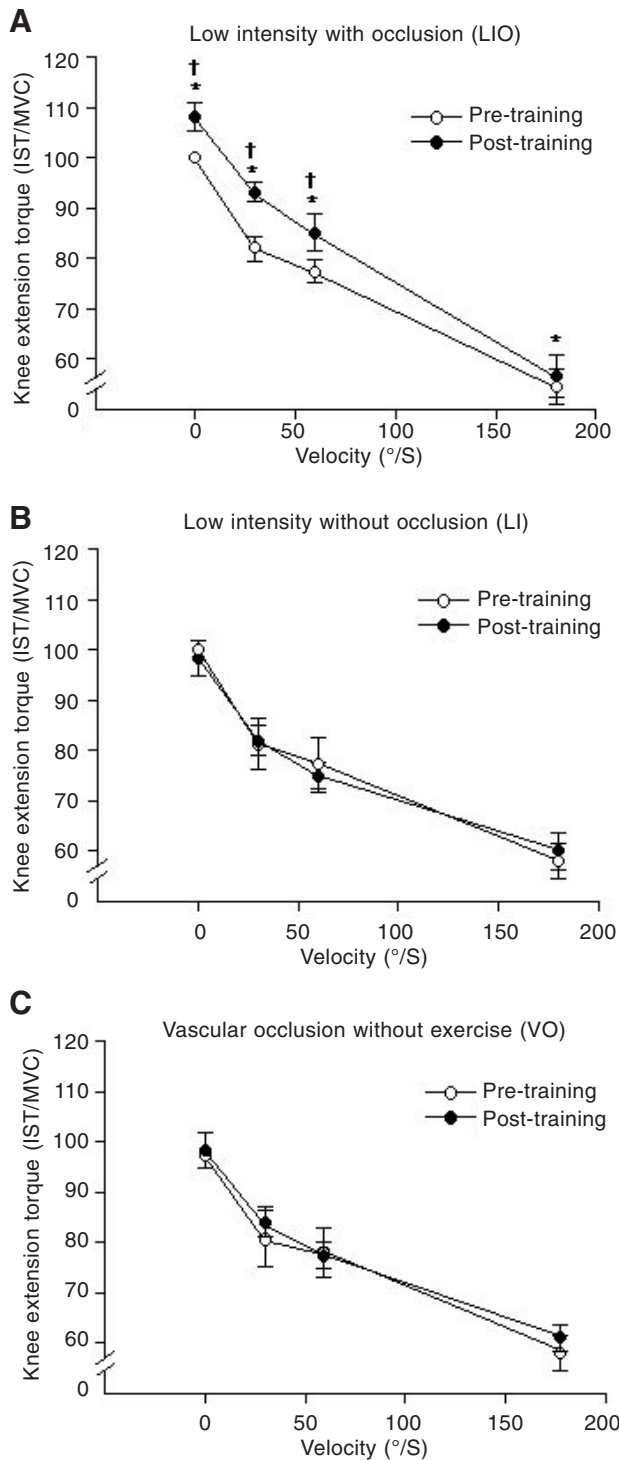
A Low intensity with occlusion (LIO)

B Low intensity without occlusion (LI)

C Vascular occlusion without exercise (VO)

Fig. 3. Effects of exercise training on torque-velocity relations. Isokinetic torque-angular velocity relations of knee extensor were obtained before (open circle) and after (filled circle) exercise training. All values of knee extension torque were normalized to the pretraining values of iso-metric (velocity = 0) torque, and means ± SE were plot-ted. **A**: exercise at low intensity with occlusion (LIO; *n* = 6); **B**: exercise at low intensity without occlusion (LI; *n* = 6); **C**: vascular occlusion without exercise (VO; *n* = 6). *Statistically significant changes from pretraining values (P < 0.05). †Statistically significant differences between LIO, LI, and VO (P < 0.05).
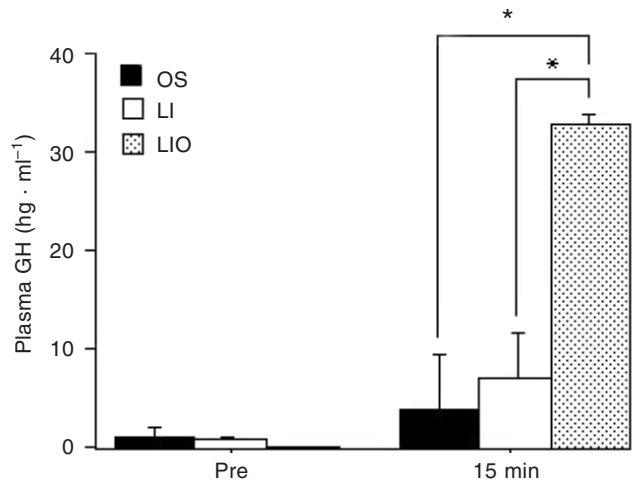

Fig. 4. Effects of occlusion on plasma concentration of growth hormone measured before and 15 min after exer-cises at a low (20% 1RM × 15 repetitions) intensity either with (LIO; *n* = 6) or without occlusion (LI; *n* = 6) and after vascular occlusion without exercise for 10 min (VO; *n* = 6). Values are means ± SE. *Statistically significant dif-ferences between groups (P < 0.05).

able to be regarded as an initial indicator of the mus-cle-trophic effect of a variety of resistance-exercise protocols in the present study, as has been done in the previous studies [15, 19].

Although lines of evidence have been accumulat-ed showing phenomenological relations between GH and muscular hypertrophy, the role played by GH in muscular growth remains unclear. Recent studies have shown that such growth factors as systemic IGF-I [19], mechanosensitive IGF-I [20], and myostatin [21] play crucial roles not only in the development of mus-cle during embryogenesis, but also in the adaptation of muscle to resistance exercise training in adult organisms. Circulating GH may stimulate the synthesis and secretion of IGF-I within the muscle, which then acts on the muscle itself to promote growth [13, 22, 23]. Alternatively, IGF-I production within the mus-cle may be independent of the postexercise increase in circulating GH [24]. On the other hand, some *in vitro* studies have shown that GH stimulates myoblasts directly and promote their proliferation and differen-tiation [24]. Furthermore, the synthesis of GH recep-tors may be stimulated by IGF-I in either myoblasts or muscle satellite cells [24]. Although it has been controversial whether administrations of exogenous GH and IGF-I stimulate muscular growth in adult humans [12, 25–29], combinations of GH application and exercise stimuli have been shown to evoke inter-active, positive effects in potentiating muscular hyper-trophy in humans and in rats [30–32]. Therefore the stimulated secretion of GH may play a part in the pre-

sent effects of low-intensity exercise with occlusion on muscular hypertrophy.

If GH plays any part in the exercise-induced muscular hypertrophy, the present changes in GH can account for the increases in muscular size and isometric and isokinetic strengths seen only after the LIO protocol. The circulating level of GH is primarily regulated by the hypothalamus-pituitary axis [33], which has been postulated to be affected by both central motor commands and peripheral chemoreceptions of metabolic subproducts [33, 34]. We have shown that the external application of vascular occlusion significantly increased the fMRI-measured brain activation of the primary motor cortex during handgrip exercises at 20% MVC (Takarada Y. and Taira M., in preparation). Furthermore, it has been previously shown that the resistance exercise with vascular occlusion causes an additional recruitment of motor units and accumulations of metabolic subproducts in muscles, such as lactate and H^+ [4], both of which may have resulted from the suppressions of oxygen supply to and clearance of metabolites from the muscle. If these two factors are to be satisfied simultaneously and sufficiently, exercise with a certain level of muscular activity and a resulting increase in energy metabolism would be required in combination with the vascular occlusion.

Apart from the regulatory mechanism of systemic hormones, changes in local metabolic conditions may directly stimulate the adaptation of muscle. For instance, a sustained exertion of isometric force has been shown to cause increases in intramuscular concentrations of inorganic phosphate and H^+ and to lead to an enhanced muscular hypertrophy [35]. The similar mechanism may also be involved in the present effects of exercise and vascular occlusion because the restriction of intramuscular blood flow may suppress aerobic metabolism through a limited gas exchange and an accumulation of metabolic subproducts such as lactate.

On the other hand, it has been shown that muscular xanthine oxidase activity is elevated in a hypoxic condition and produces reactive oxygen species (ROS) during the subsequent reperfusion after the restriction of blood flow [36]. Although 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 reduction-oxidation (redox) state of regulatory proteins [6, 8, 9]. Moreover, recent studies have shown that nitric oxide (NO) formed by muscle fibers themselves plays an important role in the adaptations of muscle to exercise and mechanical stress [5, 7]. These effects of ROS may not operate in the present exercise with vascular occlusion, but they may have operated in the previous study in which the repeated applications of occlusive stimuli given during bed rest have effectively attenuated the disuse atrophy of leg muscles without any combined exercise stimulus [10].

In conclusion, a moderate vascular occlusion and a low-intensity resistance exercise gave rise to significant increases in muscular size and strength only when combined, suggesting their cooperative effects. Although a similar cooperative effect between exercise and occlusive stimuli was seen in an acute response of GH, the role played by GH in the muscle hypertrophy remains unclear. Further studies focusing on the local regulators of muscular growth, such as growth factors and reactive oxygen species, are to be conducted to elucidate the mechanism for the present cooperative effects of exercise and occlusive stimuli.

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