Low-intensity exercise can increase muscle mass and strength proportionally to enhanced metabolic stress under ischemic conditions

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Low-intensity exercise can increase muscle mass and strength proportionally to enhanced metabolic stress under ischemic conditions. J Appl Physiol 113: 199–205, 2012. First published May 24, 2012; doi:10.1152/japplphysiol.00149.2012.—Skeletal muscle bulk and strength are becoming important therapeutic targets in medicine. To increase muscle mass, however, intensive, long-term mechanical stress must be applied to the muscles, and such stress is often accompanied by orthopedic and cardiovascular problems. We examined the effects of circulatory occlusion in resistance training combined with a very low-intensity mechanical load on enhancing muscular metabolic stress and thereby increasing muscle bulk. Muscular metabolic stress, as indicated by the increases in inorganic phosphate (Pi) and a decrease in intramuscular pH, was evaluated by 31P-magnetic resonance spectroscopy during unilateral plantar-flexion at 20% of the one-repetition maximum (1-RM) with circulatory occlusion for 2 min in 14 healthy, male untrained participants (22 yr) at baseline. Participants performed two sets of the same exercise with a 30-s rest between sets, 2 times/day, 3 days/wk, for 4 wk. The muscle cross-sectional area (MCA) of the plantar-flexors and the 1-RM were measured at baseline and after 2 and 4 wk of training. MCA and 1-RM were significantly increased after 2 and 4 wk (P < 0.05, respectively). The increase in MCA at 2 wk was significantly (P < 0.05) correlated with the changes in Pi (r = 0.876) and intramuscular pH (r = 0.601). Furthermore, the increases in MCA at 4 wk and 1-RM at 2 wk were also correlated with the metabolic stress. Thus enhanced metabolic stress in exercising muscle is a key mechanism for favorable effects by resistance training. Low-intensity resistance exercise provides successful outcomes when performed with circulatory occlusion, even with a short training period.

Both the metabolic and the bulk aspects of skeletal muscle affect mortality and quality of life, since muscle tissue serves as an important antioxidant system and an endocrine source of myokines (19a). Therefore, skeletal muscle bulk is increasingly becoming an important therapeutic target in applied medicine (2, 7). However, increases in muscle bulk and strength are not easily obtained in the clinical setting. To achieve this, typically high-intensity, all-out, long-term (usually ≥2 mo) resistance exercise must be performed. However, such an exercise protocol is frequently associated with orthopedic complications (17, 20). In addition, it has been reported that high-intensity resistance training [80% of one-repetition maximum (1-RM)] reduces central artery compliance (17). These findings suggest that effective resistance training to increase muscle mass and strength could worsen disease conditions in patients with severe cardiovascular disease and injured patients. Studies have shown that exercise under ischemic conditions may enhance training stress in exercising muscle and might provide favorable training effects without side effects such as vascular dysfunction and muscle injury (9, 16). Several researchers suggested that exercise under ischemia might produce muscle hypertrophy and strength gain by enhancing protein synthesis and increasing humoral factor secretion (1, 4).

During a transient exercise when circulatory occlusion was applied, our laboratory previously observed the remarkable enhancement of intramuscular metabolic stress followed by the accumulation of metabolic by-products, such as H+, inorganic phosphate (Pi), and ADP, and the increase of fast-twitch (FT) fiber recruitment in contracting muscle (23–25, 27). However, it remains unknown whether the effect of exercise training under ischemic condition is associated with metabolic stress. Therefore, the purposes of this study were to demonstrate the effects of short-term resistance training with a fixed, very low-intensity mechanical load combined with circulatory occlusion and to elucidate the causal relationship between training effects (e.g., muscle hypertrophy and enhanced strength) and muscular metabolic stress, along with FT fiber recruitment, during a single bout of training.

**METHODS**

Participants. Fourteen untrained, healthy male participants took part in the study. All participants were healthy and without orthopedic or cardiovascular diseases. Informed consent was obtained from all participants. The study was approved by the Ethics Committee of Hokkusho University (HOKUSHO-SPOR: 200704). Based on previous evidence (1) the reported change of MCA after 4 wk = 6%, standard deviation = 5%, a sample size of 10 participants was needed to detect the effect compared with the threshold change of 0 under the conditions of α error = 5% and power = 90%.

Exercise during the measurement of metabolic stress. Resistance exercise was comprised of unilateral plantar flexion (30 repetitions/min) at 20% of 1-RM with circulatory occlusion for 2 min. The blood pressure of the participants was measured twice after they had been resting in a sitting position for 20 min on average, and circulatory occlusion was applied using a pneumatic cuff around the thigh, with a pressure that was 1.3 times the systolic blood pressure.

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Short-term resistance training protocol. Short-term training involved performance of the same exercises at baseline measurements as 1 set, 2 sets/session (rest interval of 30 s), 2 sessions/day (at least 4 h between sessions), 3 days/wk, for 4 wk.

Measurement of training effects. Maximal muscle cross-sectional area (MCA) of plantar flexors was composed of medial gastrocnemius, lateral gastrocnemius, soleus, flexor hallucis longus, tibialis posterior, flexor digitorum longus, peroneus longus, and peroneus brevis at the proximal 30% of the leg length by using MRI (Magnetom Vision VB33G; Siemens, Erlangen, Germany) at 3 days after final training session to avoid overestimation due to muscular swelling (27). The exercise intensities used in the present study have been assigned to the level of low-intensity resistance training. The plantar-flexion exercises were carried out at 30 repetitions/min, and the weight was lifted 5 cm above the ground. Throughout the experiments, we carefully monitored the lifting height (5 cm) and repetitions (30 per minute) using a ruler and metronome, respectively, to avoid variance within and between participants. The 1-RM was determined to be a successful concentric-only contraction on the same plantar-flexion apparatus equipped with a magnetic resonance device. Participants were instructed to lift the load through the range of motion to prevent assistance from any other body part (e.g., the thigh). The 1-RM was determined to be a successful concentric-only contraction on the same plantar-flexion apparatus equipped with a magnetic resonance device. Participants were instructed to lift the load through the range of motion to prevent assistance from any other body part (e.g., the thigh). The 1-RM trials were designed using increments of 10 kg until 60–80% of the perceived maximum was reached (12, 23–25, 27). The load was then gradually increased by small weights (0.5–5 kg) until lift fail, which was defined as the participant’s failure to maintain proper form or to completely lift the weight. The last acceptable lift with the highest possible load was determined as 1-RM (23–25, 27). Circulatory occlusion pressure was set as 130% of resting systolic blood pressure (23–25, 27). Circulatory occlusion was carried out using a pneumatic rapid inflator (E-20 rapid cuff inflator, Hokanson) with an 18.5-cm-wide pressure cuff placed around the right thigh. The cuff was inflated for 10 s before the exercise protocol and was promptly released after the exercise was completed. The real-time cuff pressure was monitored digitally and was precisely maintained during exercise. Maximal MCA and 1-RM were measured at baseline, after 2 wk, and after 4 wk.

Metabolic stress during resistance exercise at baseline. Participants lay in the supine position on an original apparatus equipped with a magnetic resonance device, and the right foot was attached to the pedal by a Velcro strap. Phosphorus-magnetic resonance spectroscopy (31P-MRS) was also performed using a 55-cm-bore and a 1.5-T superconducting magnet (Magnetom Vision VB33G; Siemens) (23–25, 27). An 80-mm-surface coil was placed under the muscle belly of the right gastrocnemius. Shimming was adjusted using the proton signal from water. Spectra of high-energy phosphate were acquired at a pulse width of 500 μs, a transmitter voltage of 20 V, and a repetition time of 2,000 ms. The spectra were obtained at rest and every 30 s during exercise. Each spectrum consisted of an average of eight scans over 16 s before each time point. For the spectral postprocessing procedure, the standard software package (LUISE, Siemens) was applied. After Fourier transformation, Gauss filtering in the time domain, and phase- and baseline-correction of the spectral raw data, the peaks were identified and semiautomatically fitted for the integrals under the curve in the frequency domain. The millimolar concentration of intramuscular phosphocreatine (PCr) was assumed that [PCr] + creatine concentration ([Cr]) = 42.5 mM (6) and supposed that the [PCr] is equal to [Cr] (11). ADP was calculated by [P],-to-[PCr] ratio (5). Diprotonated phosphate (H2PO4- ) was calculated using the obtained [P], (14). Intramuscular pH was calculated from the chemical shift of P, relative to PCr (14). When distinct P, splitting was shown, the pH was calculated by standardizing the obtained individual pH on the basis of peaks corresponding to each P, (14, 19, 23–25, 27, 29).

RESULTS

Table 1 shows the study participants’ mean characteristics, resistance load, and applied circulatory occlusion blood pressure at baseline. None of the participant characteristics changed during any of the training periods (P > 0.05). Increases (% of baseline values) in MCA (Fig. 1A) and 1-RM (Fig. 1B) after 2 and 4 wk were significant (P < 0.001, respectively), whereas 1-RM/MCA (3.0 ± 0.8% increase, means ± SEM) tended to increase with respect to baseline after 2 wk of training (P = 0.09). Moreover, 4 wk after the onset of training, MCA, 1-RM, and 1-RM/MCA (8.3 ± 1.4% increase) had significantly increased compared with values seen after 2 wk of training (P < 0.05, respectively; Fig. 1). Figure 2 shows a typical muscle cross section at baseline and after 4 wk of training, as well as the baseline spectra associated with resistance exercise performed with circulatory occlusion. Baseline increases in P, and intramuscular pH decreases during exercise with respect to values obtained at rest were significantly correlated with MCA increases after 2 wk (Fig. 3, A and B) and 4 wk (P, = 0.876; intramuscular pH: r = 0.789) of training (P ≤ 0.001, respectively). Similarly, baseline increases in P, and decreases in intramuscular pH during exercise with respect to resting values were significantly correlated with increased 1-RM after 2 wk of training (P < 0.05, respectively; Fig. 3, C and D). However, the correlation between change in P, and intramuscular pH and increased 1-RM after 4 wk were not statistically significant (P, = 0.41, P = 0.15; intramuscular pH: r = 0.49, P = 0.08, respectively). Baseline intramuscular H2PO4- and ADP increases from a resting state, obtained during exercise, were significantly correlated with MCA increases after 2 (Fig. 4, A and B) and 4 wk (H2PO4- : r = 0.705; ADP: r = 0.769, respectively) of training (P = 0.01). Similarly, baseline H2PO4- and ADP increases from

Table 1. Physical and physiological characteristics at baseline

<table>
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<th>Baseline Characteristics</th>
<th>No. of participants</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>Body mass index, kg/m²</th>
<th>SBP, mmHg</th>
<th>MCA, cm²</th>
<th>1-RM, kg</th>
<th>1-RM/MCA, kg/cm²</th>
<th>20% 1-RM, kg</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>21.9 ± 2.3</td>
<td>170.8 ± 8.3</td>
<td>66.1 ± 10.2</td>
<td>110.0 ± 9.0</td>
<td>72.2 ± 11.5</td>
<td>47.7 ± 8.1</td>
<td>0.66 ± 0.02</td>
<td>9.5 ± 1.6</td>
</tr>
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</table>

Values are the means ± SEM. SBP, systolic blood pressure; MCA, muscle cross-sectional area; 1-RM, one-repetition maximum.

Statistical analyses. All data are presented as means ± SEM. Comparisons of MCA and strength before and after training (2 and 4 wk) were carried out by one-way ANOVA. Intergroup comparisons of the baseline characteristics between split P, and non-split P, groups were performed using Student’s unpaired t-test. Interaction effects in MCA and strength between the split P, and non-split P, groups before and after training (group × time) were examined by mixed-model, two-way ANOVA with repeated measures. Post hoc comparisons were examined by Bonferroni’s test. The relationship between variables was examined by linear regression analysis using Pearson’s test. All statistical analyses were performed with SPSS 17.0 software for Windows. The level of significance was set at P < 0.05.
resting values, seen during exercise, were correlated with 1-RM increases after 2 wk of training (P ≤ 0.05, respectively; Fig. 4, C and D); however, no significant correlations were seen after 4 wk of training (H₂PO₄⁻: r = 0.40, P = 0.16; ADP: r = 0.42, P = 0.14, respectively). There were no significant correlations between metabolic stress and participants’ physical characteristics (e.g., 1-RM, circulatory occlusion pressure at baseline, etc.) (P > 0.05).

A splitting of Pi peaks, which represents fast-twitch (FT) fiber recruitment (23–25, 27), was observed in 9 of 14 cases and was not observed in 5 of 14 cases. There were no significant differences between the split Pi group and the non-split Pi group in terms of physical characteristics, resistance load, or circulatory occlusion pressure (P > 0.05). Increases in MCA and 1-RM after training were significant at 2 wk in the split Pi group (P < 0.05; Fig. 5A) but not in the non-split Pi group, whereas the findings for both groups were significant after 4 wk of training (P < 0.05; Fig. 5B). However, no statistical difference between the two groups was observed in terms of increases in MCA and 1-RM after 2 or 4 wk of training (Fig. 5A: 2 wk, P = 0.24, 4 wk, P = 0.32; Fig. 5B: 2 wk, P = 0.18, 4 wk, P = 0.11, respectively).

DISCUSSION

Major findings. When combined with circulatory occlusion, resistance training with a fixed and atypically low mechanical
load was found to yield significant muscular hypertrophy and strength enhancement, even with a short-term exercise period. Elevated metabolic stress appears to be a crucial factor in achieving successful resistance training results. Evaluation of intramuscular metabolic stress during the measurement of a single bout of exercise predicts the extent of increases in muscle bulk and strength.

Enhanced intramuscular metabolic stress triggers muscle adaptations. Resistance training to achieve muscle hypertrophy and strength increases predominantly stresses the ATP-PCr system and is supported primarily by energy provided by ATP-PCr and glycolysis, with minor contributions from the oxidative phosphorylation system (13). One mechanism for obtaining training effects in resistance training, with or without circulatory occlusion, has been considered to be the accumulation of by-products such as P_i, ADP, H_2PO_4^- , and H^+ with muscle contraction and the enhanced secretion of growth factors via class III and IV afferents (22, 26, 30). Kraemer suggested that an enhancement of the ATP-PCr system, i.e., P_i increases, might be related to muscle hypertrophy (13). Schott and colleagues reported that an elevation in the concentration of metabolic by-products, especially H^+, with muscle contraction contributed to muscle bulking and strength increases via the enhanced secretion of growth hormone (22). In addition, Sutton and coworkers demonstrated that exercise under hypoxic conditions facilitates the accumulation of lactate and the secretion of growth hormone (26).

Alterations in muscle fiber recruitment. In addition to the accumulation of metabolites, increased FT fiber recruitment might have contributed to the present results. According to Henneman’s size principle (8), during low-level muscular activity, ST fibers with small motor units are predominantly recruited, whereas increasing muscular force causes FT fibers to gradually be recruited. However, several studies have suggested that early recruitment of FT fibers might occur to maintain muscular force during low-intensity resistance exercise with blood flow restriction caused by an inadequate oxygen supply for ST fibers (18). We previously reported a 31P-MRS study showing that low-intensity resistance exercise with circulatory occlusion enhanced metabolic stress and FT fiber recruitment in exercising muscle (23–25, 27). In the present study, the split P_i (FT fiber recruitment) group tended to obtain greater training effects than did the non-split P_i group (Fig. 5). It is well known that FT fiber recruitment is highly susceptible to muscle hypertrophy and strength increases from resistance training (15). However, in the present study, significant training effects were observed in the non-split P_i group after 4 wk of training. Therefore, it is likely that FT fiber recruitment is an important factor, but not a major factor, in muscle hypertrophy and strength increases in this context.

Implications of muscular hypoxia under conditions of circulatory occlusion. A previous study revealed that ischemic conditions induce compensatory hypertrophy in cardiac muscle
Fujita and coworkers reported the possibility that exercise under ischemic conditions increases lactate accumulation, which in turn enhances muscle protein synthesis in humans (4). In addition, Kawada and colleagues demonstrated that lactate accumulation also facilitates signaling for muscle hypertrophy (e.g., decreased myostatin, increased heat shock protein 72, increased nitric oxide synthase 1 mRNA) in an animal model under ischemic conditions (10). On the other hand, a couple of studies have also shown that the supplementation of circulatory occlusion reduced the rate of muscle atrophy (3, 28). Thus the findings to date suggest that muscle protein synthesis might be related to hypoxia and/or to increased metabolic stress induced by circulatory occlusion.

Clinical implications and perspective. We clearly showed that the increases in muscle mass and strength could be obtained by exercise training under circulatory occlusion, even if an extremely low-intensity load was applied. It has been reported that the training protocol in the present study is relatively safe (16). Therefore, this training might be effective in patients with chronic diseases such as heart failure and...
chronic obstructive pulmonary disease, and in injured patients who cannot perform recommended high-intensity resistance exercises. We need to do further experiments to clarify these issues.

Limitations
First, we did not collect blood samples or perform muscle biopsies in the present study. The data including humoral factors, the local expression of growth factors, genetic signaling, and detailed information about muscle hypertrophy obtained by these approaches may strengthen our findings. However, metabolic stress in exercising muscle could predict training effects without an invasive technique. It is very important to massage in exercise therapy and prescription.

Second, we studied only normal young subjects in the present study. Therefore, we do not know whether the same results would have been obtained by this approach in patients with muscular disease including chronic obstructive pulmonary disease and heart failure, or in the elderly.

Third, in the present study, we could not show that the increases in muscle mass and strength by our training method led to an improvement of overall performance.

In conclusion, a fixed protocol of low-intensity resistance training, when supplemented by circulatory occlusion, can provide significant muscular hypertrophy and strength increase, even in the short term and even when exercise is not performed in an all-out manner. Elevated metabolic stress appears to be a crucial factor in obtaining successful results from resistance training.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: S.T. and K.O. conception and design of research; S.T., T. Suga, M.O., T.K., T. Sato, M.T., Y.K.H., and N.M. performed experiments; S.T., T. Suga, M.O., M.T., Y.K.H., and M.H. analyzed data; S.T. prepared figures; S.T. and K.O. drafted manuscript; K.O., S.K., and H.T. experiments; S.T., T. Suga, M.O., M.T., T.Y., K.H., and M.H. analyzed data; DISCLOSURES appears to be a crucial factor in obtaining successful results from resistance training.

REFERENCES

