Effects of electrostimulation with blood flow restriction on muscle size and strength

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ABSTRACT

Purpose: Low-load voluntary exercise can induce muscle hypertrophy and strength gain when combined with blood flow restriction (BFR) in working muscles. However, it is unknown whether such hypertrophy and strength gain can be induced by involuntary muscle contractions triggered via low-intensity neuromuscular electrical stimulation (NMES), combined with BFR. The purpose of this article was to investigate whether low-intensity NMES combined with BFR could elicit muscle hypertrophy and strength gain in the quadriceps. Methods: Eight untrained young males (means ± SEs; age 26.2±0.7 years, height 1.74±0.02 m, body weight 71.4±4.8 kg) received 23 min of unilateral low-intensity (5-10% of maximal voluntary contraction) NMES, twice per day, 5 days per week, for 2 weeks, with treatment of one leg being combined with BFR (NMES-BFR) and the other leg receiving NMES alone (NMES-CON). Quadriceps muscle thickness (MT) and isometric and isokinetic strength were measured before and every week throughout the training and detraining periods. Results: In NMES-BFR legs, MT increased after 2 weeks of training (+3.9%) and decreased after 2 weeks of detraining (-3.0%). NMES-BFR training also increased maximal knee extension strength in isometric (+14.2%) and isokinetic (+7.0% at 90°/s, +8.3% at 180°/s) voluntary contractions. In addition, maximal isometric strength decreased (-6.8%), whereas no large fall (-1.9% at 90°/s, -0.6% at 180°/s) in isokinetic maximal strength was evident.
after 2 weeks of detraining. In NMES-CON legs, no prominent change was observed; there
was a negligible effect on isometric strength. Conclusion: Low-intensity NMES combined
with BFR induces muscle hypertrophy and strength gain in untrained young males.

Key Words: Muscle adaptations, Training, Electrically evoked force, Occlusion, Knee
extensors
Introduction

Application of blood flow restriction (BFR) alone to the lower body in the absence of exercise has been shown to attenuate muscle atrophy after anterior cruciate ligament (ACL) reconstruction (27) and cast immobilization (14, 15). The mechanism of the attenuating effect of BFR alone on disuse atrophy is unclear. However, acute muscle cell swelling caused by BFR may favorably influence the net protein balance via activation of the mechanistic target of rapamycin (mTOR) and mitogen-activated protein kinase (MAPK) signaling pathways. This may explain the observed attenuating effects (17). Although BFR alone appears to attenuate muscle atrophy, BFR has not been shown to provide a stimulus adequate to elicit muscle hypertrophy (28). However, low-load exercise training can induce hypertrophy of working muscles when combined with BFR (20). BFR accelerates the development of metabolic fatigue, which seems to play the dominant role in inducing muscle hypertrophy, and is considered an alternative method to increase training efficacy in the absence of high mechanical stress (24). A review article demonstrated that about 10% of the maximum strength appears to be the minimum exercise intensity required to achieve hypertrophy of limb muscles under BFR, based on previous intervention studies (1).

Given that such a hypertrophic effect is observed even under low-load voluntary training using BFR, it is possible that even involuntary muscle contractions evoked by
low-intensity neuromuscular electrical stimulation (NMES) can induce muscle hypertrophy and strength gain when combined with BFR. The significance of the present study is that NMES is commonly used as a rehabilitative technique to prevent muscle atrophy during immobilization periods (8). As the strength gain effect of NMES is correlated with the electrically evoked force, higher-intensity NMES would be expected to be more effective (4). However, the maximal tolerable levels of electrically evoked forces differ greatly between individuals; the force evoked by NMES ranged from 12-95% of maximal strength in previous studies (5, 9). Thus, an exploration of whether low-intensity (ca. 10 % maximal strength) NMES induces strength gain and a hypertrophic effect would be useful to develop more effective and well-tolerated exercise methods.

Therefore, the aim of the present study was to investigate the effects of low-intensity NMES training combined with BFR on muscle size and strength. As it is difficult to differentiate the effect of NMES training combined with BFR from that of other rehabilitation programs in studies employing real patients, we enrolled untrained subjects without apparent disease. Additionally, based on the previous studies demonstrating the hypertrophic effect following 1-2 weeks of low-load BFR training [twice-daily training sessions (10)], we designed a novel training program, which may provide insightful
information that this method could be used even in short-term rehabilitative program.

82 Methods

83 Subjects

84 Eight untrained young males (means ± SEs; age 26.2±0.7 years, height 1.74±0.02 m, body weight 71.4±4.8 kg) volunteered to participate in the study. The subjects were recruited through printed advertisements and by word-of-mouth. None had participated in any regular aerobic or resistance training during the previous year. The subjects were instructed to avoid other physical activities and not to change dietary patterns during the interventional period. All subjects were free of overt chronic disease as assessed by medical history-taking. Potential candidates who were past or present smokers or who were taking any medication were excluded. All subjects were informed of the methods, procedures, and risks, and signed an informed consent form before participating in the study. This study was conducted according to the Declaration of Helsinki and was approved by the Ethics Committee for Human Experiments of Juntendo University, Japan.

96 Neuromuscular electrical stimulation training
One week before the beginning of the training period, subjects participated in a practice session to familiarize themselves with NMES training. Next, they attended two NMES sessions per day, 5 days per week, for 2 weeks, to make a total of 20 sessions, and also completed 2 weeks of detraining. During all sessions, subjects were seated on an isokinetic dynamometer (Biodex System 4; Biodex Medical Systems, Shirley, NY) and underwent 23 min of unilateral involuntary muscle contractions of the knee extensors, triggered by NMES, at a fixed knee joint angle of 75°. The morning and afternoon sessions were approximately 4-5 h apart. During each session, one leg (determined by randomization) underwent NMES training combined with BFR (NMES-BFR) and the other NMES training only (NMES-CON). The dominant limb was randomized into the NMES-BFR or NMES-CON. All training sessions were under the direct supervision of persons technically familiar with NMES and BFR training. During all sessions, participants were instructed to relax their thigh muscles as much as possible.

The quadriceps muscles were stimulated using bipolar electrodes linked to a portable battery-powered neuromuscular electrical stimulator (Compex Sport Energy; Medicompex, Ecublens, Switzerland). Three self-adhesive electrodes (2 mm thick) were placed over each thigh. The negative electrode (10 × 5 cm) was positioned proximally, about 11 cm (the BFR
cuff width) below the inguinal crease, whereas the other two (positive) electrodes (5 × 5 cm) were placed as close as possible to the motor points of the vastus lateralis and medialis muscles. Muscle motor points were identified by stimulating the skin surface with a pen electrode and a large reference electrode placed over the femoral area. The pen electrode was moved slowly over the skin, with the stimulatory current being gradually increased by the operator, until a clear muscle twitch was observed. The stimulator discharged biphasic rectangular pulses. The stimulation frequency and duty cycle were approximately 30 Hz and 8 s of stimulation followed by a 3-s pause. The intensity of electrical flow was selected to attain 5-10% of the MVC and the positions of the electrodes were marked. Throughout the interventional period, the electrodes were applied at the same sites and the intensities of electrical flow volume were held constant. The "rating of perceived exertion (RPE)" and the "category ratio 10 scale (CR10)" were administered at the end of each training session. CR10, which has a primary number range from 0 (nothing at all) to 10 (extremely strong), was used to evaluate discomfort induced by NMES-BFR and NMES-CON based on the previous study (19).

Blood flow restriction
A nylon cuff (MT-870 Digital Tourniquet; Mizuho, Tokyo, Japan) 105 mm wide was applied tightly at the most proximal portion of the BFR leg. Before each session, all subjects were seated on the Biodex System 4 and the thigh-mounted cuff was inflated to 100 mmHg. After 30 s the pressure was released for 10 s and then reinflated to a cuff pressure 20 mmHg higher than the previous for another 30 s. This process was repeated until the target pressure was attained; this was calculated for each subject based on mid-thigh circumference, as follows: <50 cm = 140 mmHg (n=3); 50-55 cm = 160 mmHg (n=4); >60 cm = 200 mmHg (n=1). This is because arterial occlusion pressure is largely influenced by thigh circumference (18). The subjects received four sets of BFR, each of 5 min, with 1-min rest intervals between sessions. The cuff air pressure was released immediately upon completion of each session.

Muscle thickness

Muscle thickness (MT) was measured via B-mode ultrasound using a 5-MHz scanning head (SSD-900; Aloka, Tokyo, Japan) at eight sites on the anterior aspect of the thigh (at 30%, 50% and 70% of thigh length, and the central, lateral, and medial surfaces, excluding the 30% medial point); and at two sites on the posterior aspect of the thigh (at 50% and 70% of thigh length, and the central surface) 1-2 days before and every week throughout
training and de-training (PRE, MED, POST, POST2, and POST3). Prior to all scans, subjects
rested quietly in a seated position for at least 30 min. To avoid an influence of fluid shifts
within the muscle, the measurements were performed around the same time. Thigh
circumference was also measured at 50% of thigh length using a tape measure. Thigh length
was the distance between the lateral condyle of the femur and the greater trochanter. All
measurements were performed by the same operator. Measurement sites were marked using a
marker pen as described in a previous study (13). The ultrasound measurements of MT were
performed in the supine/prone position, with careful attention to ensure that hip and ankle
joint positions and the distance between both legs are the same in all the measurements. The
scanning head coated with a water-soluble transmission gel was placed on each marked
measurement site without depressing the dermal surface. The subcutaneous adipose
tissue-muscle interface and the muscle-bone interface were identified on the ultrasound
images, and the distance between the two interfaces was recorded as the MT. The mean MT
values of the eight anterior and two posterior sites were used in data analysis. The posterior
MTs of the NMES-CON and NMES-BFR legs were used to explore the effects of no
treatment at all, and the application of BFR alone, respectively; NMES was applied to only
the anterior aspect of the thigh. The test-retest (inter-session) reliabilities of MT
measurements were calculated using intraclass correlation coefficient (ICC), standard errors
of measurement (SEM), and minimal difference. These values were previously determined in
10 young subjects in terms of anterior central 50% MT values, and were 0.999, 0.21 mm, and
0.58 mm.

**Maximum isometric and isokinetic strengths**

The maximum voluntary isometric and isokinetic strengths of knee extensors and
flexors were determined using a Biodex System 4 dynamometer 1-2 days before and every
week throughout the training and de-training periods (PRE, MED, POST, POST2, and
POST3). Three or four days before baseline strength testing, participants were familiarized
with the strength-testing protocol. During testing, each participant was seated on a chair with
the hip joint angle positioned at 85° of flexion (0° = full hip extension). The center of rotation
of the knee joint was visually aligned with the axis of the dynamometer lever arm and the
ankle was firmly strapped to the distal pad of the lever arm. A knee joint angle of 0°
corresponded to full knee extension. Several warm-up contractions (4-5 submaximal
contractions and 1-2 near-maximal contractions at 180° per s) were performed before testing.
Participants were then instructed to perform maximal isometric knee extension for about 5 s
at a fixed knee joint angle of 75°, preceded by maximal isokinetic knee extension from 0° to 90°, at 90° and 180° per s. Next, they performed maximal isometric knee flexion for about 5 s at a fixed knee joint angle of 30°. Two maximal efforts for each isometric measurement and three maximal efforts for each isokinetic measurement were performed, and each peak torque was used in data analysis. Maximal isometric knee flexion strengths of the NMES-CON and NMES-BFR legs were recorded to explore the effects of no treatment and the application of BFR alone, respectively. The test-retest (inter-session) reliabilities of strength measurements calculated using the ICC, SEM, and minimal difference were previously determined in 10 young subjects performing maximal isometric knee extension, and were 0.988, 5.20 Nm, and 14.41 Nm.

**Statistical analyses**

All results are expressed as means with standard errors (SEs). Statistical analysis featured two-way analysis of variance (ANOVA) with repeated measures [condition (with and without BFR) × time (PRE, MED, POST, POST2, and POST3)]. All baseline values for NMES-CON and NMES-BFR and measured knee flexor data variables were compared using the paired t test. Statistical significance was set at p<0.05. Effect sizes (ESs) were
calculated as [(Post Mean – Pre Mean)/ Pre Standard Deviation](25). ESs < 0.20 were considered trivial, 0.20-0.49 small, 0.50-0.79 moderate, and > 0.80 large (2).

Results

NMES training and BFR application did not give rise to any relevant side-effect such as subcutaneous hemorrhage, numbness and cerebral anemia. All subjects tolerated training well; the adherence rate was 100% for both training conditions. No significant difference in the baseline values of MT or muscle strength was evident when the two training conditions were compared. No significant change in body mass or BMI was noted throughout training and detraining (Table 1).

Figure 1 illustrates changes in the MT of the knee extensors throughout the training and detraining periods. Two-way repeated measures ANOVA showed that the condition × time interaction (p<0.001) was significant. Under the NMES-BFR condition, MT increased after 2 weeks of training (+3.9%) and decreased after 2 weeks of detraining (-3.0%), whereas no notable change was observed under the NMES-CON condition. The ESs were 0.18 and 0.03 for the NMES-BFR and NMES-CON conditions, respectively. The MTs of the knee flexors did not change under either BFR or CON conditions.
Figure 2 shows the changes in isometric and isokinetic knee extension strengths throughout training and detraining. Two-way repeated measures ANOVA showed that the condition × time interaction (p < 0.05 isometrically, p < 0.01 at 90°/s, p < 0.01 at 180°/s) was significant for all angle velocities. The NMES-BFR condition showed maximal voluntary strength improvements under isometric (+14.2%) and isokinetic (+7.0% at 90°/s, +8.3% at 180°/s) conditions after the 2 weeks of training were completed. In addition, isometric maximal strength (Fig. 2A) decreased (-6.8%), but no large decreases (-1.9% at 90°/s, -0.6% at 180°/s) in isokinetic maximal strength (Figs. 2B, C) were observed after 2 weeks of detraining. Under the NMES-CON condition, no noticeable change was observed except for a negligible effect on isometric strength. ESs were calculated for the NMES-BFR condition, and were 0.64 isometrically, 0.31 at 90°/s, and 0.35 at 180°/s; and for the NMES-CON condition, being 0.20 isometrically, 0.03 at 90°/s, and 0.05 at 180°/s. Neither the isometric nor isokinetic knee flexion strength changed under either the CON or BFR condition.

Figure 3 shows the changes in RPE and CR10 after each training session. For RPE, the main effects of condition and training session were significant (p < 0.001). The interaction between condition and training session was significant for CR10 (p < 0.01). RPE after NMES-CON and NMES-BFR treatments fell to similar extents as training advanced.
CR10 fell more rapidly under NMES-BFR than NMES-CON condition early in the training period, but similarly under either condition thereafter.

Discussion

The major finding of our present study was that low-intensity NMES training induced muscular hypertrophy and a concomitant increase in isometric and isokinetic strength, when combined with BFR.

Over the past decade, many peer-reviewed studies have found that low-load (10-30% of maximum strength) voluntary exercise training of working muscles, combined with BFR, can induce muscle hypertrophy and strength gains (20). The mechanisms underlying such hypertrophy are not completely understood, but metabolic stress resulting from the accumulation of metabolic by-products such as $\text{H}^+$ and $\text{Pi}$ seems to play the dominant role in creation of the hypertrophic effect under low-load resistance training with BFR, although mechanical stress also plays a part (24). It has been suggested that metabolic stress triggers secondary reactions including the recruitment of additional motor units to compensate for the force loss (32), enhanced acute muscle cell swelling (34), and production of reactive oxygen species (11). Such events may increase the rate of muscle protein synthesis by activation of
anabolic, and/or inhibition of catabolic signaling pathways (3, 7, 16, 21) as well as proliferation of satellite cells (23), triggering hypertrophy. Furthermore, muscle hypertrophy and strength gain during low-load BFR training is observable even if training periods are short (1-2 weeks of twice-daily sessions; 10). Thus, it is not surprising that 2 weeks of NMES training at 5-10% MVC, combined with BFR, induced muscle hypertrophy and strength gain. However, the magnitudes of such improvements induced by NMES-BFR appear to be lower than those attainable using other training modalities.

Previous studies found that the ESs of isometric strength gain and muscle hypertrophy were 1.08 and 0.41 for low-load BFR training (20), but 1.25 (25) and 0.35-1.23 (12, 31) for high-load resistance training, respectively. Compared to the latter type of training, the strength gain effects we noted were about half (0.31-0.64) and the hypertrophic effects less than half (0.18) upon NMES-BFR training. The small ESs of NMES-BFR training may be attributable to the short interventional period in addition to differences among exercise types.

Additionally, we found that 2 weeks of detraining reduced MT (-3.0%, rate of change; -0.2% per day) to the basal level. Yasuda et al. recently investigated the effects of short-term (3 weeks) detraining following low-load BFR training on muscle size and found that muscle size returned to the basal level after detraining (33). Also, Gondin et al. showed that cessation
of NMES training (at 68% MVC) for 4 weeks induced a significant decrease in muscle cross-
sectional area (6). Such results are consistent with our present data, suggesting that muscle
size returned toward basal levels when relatively short-term detraining followed NMES and
BFR training. We found that isometric strength decreased (-6.8%, rate of change; -0.5% per
day), but that no large decrease in isokinetic knee extension strength (-1.9% at 90°/s, -0.6% at
180°/s) was evident, throughout detraining. Marqueste et al. showed that the increase (14%
from the pre-training level) in concentric maximal strength of the knee extensor after 6 weeks
of NMES training was preserved (19% above the pre-training level) after 6 weeks of
detraining (22). In contrast, another study found a gradual decrease in isometric strength after
cessation of NMES training (6). Changes in muscle strength during detraining may depend
on the type of muscle contraction measured (i.e., dynamic vs. static strength). However, no
other studies have investigated the effects of detraining after NMES and BFR training on
increases in muscle size and strength. The topic warrants further work.

In general, BFR induction with a tourniquet may suppress the clearance of metabolites,
creating pain (30). We found that the NMES-BFR condition was associated with higher
CR10 and RPE scores than the NMES-CON condition. One previous study found that, when
subjects performed resistance exercise at moderate intensity (45-60% 1RM), the range of
RPE scores indicating perceived exhaustion was 13.0-17.0 (29), similar to the RPE scores noted under the NMES-BFR condition. Additionally, the CR10 and RPE scores under the NMES-BFR condition were lower than those recorded during knee extension exercise at 20% 1RM, with BFR (cuff width: 135 mm) (26). These results suggest that the NMES-BFR training protocol used in the present study is generally well-tolerated.

NMES alone had no effect on MT or isokinetic strength, and only a negligible effect on isometric strength, in the present study. To achieve both muscle hypertrophy and strength gain via NMES, a training period of 1-2 months appears to be required even when the training intensity is high (i.e., 68% MVC) (5). Therefore, it is possible that a short training period (2 weeks) featuring low-intensity electrical current (5-10% MVC) did not greatly affect skeletal muscle size or strength in the present study.

Noted limitation of our current study was that the device inflating the nylon cuffs did not allow an initial compressive force to be set although the cuffs were tightly wrapped around the upper thigh. Thus, we have no data concerning the relationship between inflated cuff pressure and compressive force on the skin under the cuff. Furthermore, some variables of electrical stimulation were not recorded, although the extent of strength development during training was similar (5-10% MVC) for each subject. Additional research is needed to
address these issues.

In conclusion, the present study is the first to show that low-intensity NMES training induces muscular hypertrophy and concomitant increases in isometric and isokinetic strengths when combined with BFR in stimulated muscles. Our results indicate that addition of BFR to current NMES protocols affords potential benefits that are clinically relevant and thus warrant further investigation in patients who are immobilized. Further work is needed to define the stimulation conditions maximizing muscle hypertrophy when electrical stimulation is combined with BFR.

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Conflict of interest
No commercial company or manufacturer has any professional relationship with any author involved in this work, and the results of this work will not confer any commercial benefit on any author.

The results of the present study do not constitute endorsement by the American College of Sports Medicine.
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Figure legends

FIGURE 1–Changes in thigh MT under NMES-CON (□) and NMES-BFR (■) conditions measured before (PRE), during (MED), and immediately after (POST) the training period; and during (POST2) and after (POST3) the detraining period. Data are presented as means ± SEs. Abbreviations: muscle thickness, MT; neuromuscular electrical stimulation, NMES-CON; neuromuscular electrical stimulation combined with blood flow restriction, NMES-BFR; before the training period, PRE; 1 week after the beginning of training, MED; immediately after the training period, POST; 1 week after the training period, POST2; 2 weeks after the training period, POST3.

FIGURE 2–Changes in maximal isometric (A) and isokinetic knee extension strengths (B, C) under NMES-CON (□) and NMES-BFR (■) conditions measured before (PRE), during (MED), and immediately after (POST) the training period; and during (POST2) and after (POST3) the detraining period. Data are presented as means ± SEs. Abbreviations: neuromuscular electrical stimulation, NMES-CON; neuromuscular electrical stimulation combined with blood flow restriction, NMES-BFR; before the training period, PRE; 1 week after the beginning of training, MED; immediately after the training period, POST; 1 week after the beginning of training, POST2; 2 weeks after the training period, POST3.
after the training period, POST2; 2 weeks after the training period, POST3.

**FIGURE 3**—Changes in RPE (A) and CR10 (B) scores after each training session under both NMES-CON (□) and NMES-BFR (■) conditions. Data are presented as means ± SEs.

Abbreviations: rating of perceived exertion, RPE; category ration 10, CR10; neuromuscular electrical stimulation, NMES-CON; neuromuscular electrical stimulation combined with blood flow restriction, NMES-BFR.
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<td>Body mass (kg)</td>
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<td>BMI (kg/m²)</td>
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<td>23.4 ± 1.2</td>
<td>23.5 ± 1.2</td>
<td>p = 0.900</td>
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A

Interaction: p < 0.05

Isometric strength (N·m)

250 310 330 350

PRE MED POST POST2 POST3

Training Detraining

B

Interaction: p < 0.01

Isokinetic strength (N·m)

180 210 230 240

PRE MED POST POST2 POST3

Training Detraining

C

Interaction: p < 0.01

Isokinetic strength (N·m)

140 150 160 180

PRE MED POST POST2 POST3

Training Detraining
Interaction: $p < 0.01$
Group : $p < 0.001$
Time : $p < 0.001$
Interaction : $p = 0.99$

Interaction : $p < 0.01$