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

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20 **ABSTRACT**

21 **Purpose:** Low-load voluntary exercise can induce muscle hypertrophy and strength gain
22 when combined with blood flow restriction (BFR) in working muscles. However, it is
23 unknown whether such hypertrophy and strength gain can be induced by involuntary muscle
24 contractions triggered via low-intensity neuromuscular electrical stimulation (NMES),
25 combined with BFR. The purpose of this article was to investigate whether low-intensity
26 NMES combined with BFR could elicit muscle hypertrophy and strength gain in the
27 quadriceps. **Methods:** Eight untrained young males (means ± SEs; age 26.2±0.7 years, height
28 1.74±0.02 m, body weight 71.4±4.8 kg) received 23 min of unilateral low-intensity (5-10%
29 of maximal voluntary contraction) NMES, twice per day, 5 days per week, for 2 weeks, with
30 treatment of one leg being combined with BFR (NMES-BFR) and the other leg receiving
31 NMES alone (NMES-CON). Quadriceps muscle thickness (MT) and isometric and isokinetic
32 strength were measured before and every week throughout the training and detraining periods.
33 **Results:** In NMES-BFR legs, MT increased after 2 weeks of training (+3.9%) and decreased
34 after 2 weeks of detraining (-3.0%). NMES-BFR training also increased maximal knee
35 extension strength in isometric (+14.2%) and isokinetic (+7.0% at 90°/s, +8.3% at 180°/s)
36 voluntary contractions. In addition, maximal isometric strength decreased (-6.8%), whereas
37 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

45 **Introduction**

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

61 Given that such a hypertrophic effect is observed even under low-load voluntary
62 training using BFR, it is possible that even involuntary muscle contractions evoked by

63 low-intensity neuromuscular electrical stimulation (NMES) can induce muscle hypertrophy
64 and strength gain when combined with BFR. The significance of the present study is that
65 NMES is commonly used as a rehabilitative technique to prevent muscle atrophy during
66 immobilization periods (8). As the strength gain effect of NMES is correlated with the
67 electrically evoked force, higher-intensity NMES would be expected to be more effective (4).
68 However, the maximal tolerable levels of electrically evoked forces differ greatly between
69 individuals; the force evoked by NMES ranged from 12-95% of maximal strength in previous
70 studies (5, 9). Thus, an exploration of whether low-intensity (ca. 10 % maximal strength)
71 NMES induces strength gain and a hypertrophic effect would be useful to develop more
72 effective and well-tolerated exercise methods.

73 Therefore, the aim of the present study was to investigate the effects of
74 low-intensity NMES training combined with BFR on muscle size and strength. As it is
75 difficult to differentiate the effect of NMES training combined with BFR from that of other
76 rehabilitation programs in studies employing real patients, we enrolled untrained subjects
77 without apparent disease. Additionally, based on the previous studies demonstrating the
78 hypertrophic effect following 1-2 weeks of low-load BFR training [twice-daily training
79 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.

Methods

Subjects

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.

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

114 cuff width) below the inguinal crease, whereas the other two (positive) electrodes (5 × 5 cm)
115 were placed as close as possible to the motor points of the vastus lateralis and medialis
116 muscles. Muscle motor points were identified by stimulating the skin surface with a pen
117 electrode and a large reference electrode placed over the femoral area. The pen electrode was
118 moved slowly over the skin, with the stimulatory current being gradually increased by the
119 operator, until a clear muscle twitch was observed. The stimulator discharged biphasic
120 rectangular pulses. The stimulation frequency and duty cycle were approximately 30 Hz and
121 8 s of stimulation followed by a 3-s pause. The intensity of electrical flow was selected to
122 attain 5-10% of the MVC and the positions of the electrodes were marked. Throughout the
123 interventional period, the electrodes were applied at the same sites and the intensities of
124 electrical flow volume were held constant. The "rating of perceived exertion (RPE)" and the
125 "category ratio 10 scale (CR10)" were administered at the end of each training session. CR10,
126 which has a primary number range from 0 (nothing at all) to 10 (extremely strong), was used
127 to evaluate discomfort induced by NMES-BFR and NMES-CON based on the previous study
128 (19).
129
130 **Blood flow restriction**

131 A nylon cuff (MT-870 Digital Tourniquet; Mizuho, Tokyo, Japan) 105 mm wide
132 was applied tightly at the most proximal portion of the BFR leg. Before each session, all
133 subjects were seated on the Biodex System 4 and the thigh-mounted cuff was inflated to 100
134 mmHg. After 30 s the pressure was released for 10 s and then reinflated to a cuff pressure 20
135 mmHg higher than the previous for another 30 s. This process was repeated until the target
136 pressure was attained; this was calculated for each subject based on mid-thigh circumference,
137 as follows: <50 cm = 140 mmHg (n=3); 50-55 cm = 160 mmHg (n=4); >60 cm = 200 mmHg
138 (n=1). This is because arterial occlusion pressure is largely influenced by thigh circumference
139 (18). The subjects received four sets of BFR, each of 5 min, with 1-min rest intervals between
140 sessions. The cuff air pressure was released immediately upon completion of each session.
141
142 **Muscle thickness**
143 Muscle thickness (MT) was measured via B-mode ultrasound using a 5-MHz
144 scanning head (SSD-900; Aloka, Tokyo, Japan) at eight sites on the anterior aspect of the
145 thigh (at 30%, 50% and 70% of thigh length, and the central, lateral, and medial surfaces,
146 excluding the 30% medial point); and at two sites on the posterior aspect of the thigh (at 50%
147 and 70% of thigh length, and the central surface) 1-2 days before and every week throughout

148 training and de-training (PRE, MED, POST, POST2, and POST3). Prior to all scans, subjects
149 rested quietly in a seated position for at least 30 min. To avoid an influence of fluid shifts
150 within the muscle, the measurements were performed around the same time. Thigh
151 circumference was also measured at 50% of thigh length using a tape measure. Thigh length
152 was the distance between the lateral condyle of the femur and the greater trochanter. All
153 measurements were performed by the same operator. Measurement sites were marked using a
154 marker pen as described in a previous study (13). The ultrasound measurements of MT were
155 performed in the supine/prone position, with careful attention to ensure that hip and ankle
156 joint positions and the distance between both legs are the same in all the measurements. The
157 scanning head coated with a water-soluble transmission gel was placed on each marked
158 measurement site without depressing the dermal surface. The subcutaneous adipose
159 tissue-muscle interface and the muscle-bone interface were identified on the ultrasound
160 images, and the distance between the two interfaces was recorded as the MT. The mean MT
161 values of the eight anterior and two posterior sites were used in data analysis. The posterior
162 MTs of the NMES-CON and NMES-BFR legs were used to explore the effects of no
163 treatment at all, and the application of BFR alone, respectively; NMES was applied to only
164 the anterior aspect of the thigh. The test-retest (inter-session) reliabilities of MT

165 measurements were calculated using intraclass correlation coefficient (ICC), standard errors
166 of measurement (SEM), and minimal difference. These values were previously determined in
167 10 young subjects in terms of anterior central 50% MT values, and were 0.999, 0.21 mm, and
168 0.58 mm.
169
170 **Maximum isometric and isokinetic strengths**
171 The maximum voluntary isometric and isokinetic strengths of knee extensors and
172 flexors were determined using a Biodex System 4 dynamometer 1-2 days before and every
173 week throughout the training and de-training periods (PRE, MED, POST, POST2, and
174 POST3). Three or four days before baseline strength testing, participants were familiarized
175 with the strength-testing protocol. During testing, each participant was seated on a chair with
176 the hip joint angle positioned at 85° of flexion (0° = full hip extension). The center of rotation
177 of the knee joint was visually aligned with the axis of the dynamometer lever arm and the
178 ankle was firmly strapped to the distal pad of the lever arm. A knee joint angle of 0°
179 corresponded to full knee extension. Several warm-up contractions (4-5 submaximal
180 contractions and 1-2 near-maximal contractions at 180° per s) were performed before testing.
181 Participants were then instructed to perform maximal isometric knee extension for about 5 s

182 at a fixed knee joint angle of 75°, preceded by maximal isokinetic knee extension from 0° to
183 90°, at 90° and 180° per s. Next, they performed maximal isometric knee flexion for about 5 s
184 at a fixed knee joint angle of 30°. Two maximal efforts for each isometric measurement and
185 three maximal efforts for each isokinetic measurement were performed, and each peak torque
186 was used in data analysis. Maximal isometric knee flexion strengths of the NMES-CON and
187 NMES-BFR legs were recorded to explore the effects of no treatment and the application of
188 BFR alone, respectively. The test-retest (inter-session) reliabilities of strength measurements
189 calculated using the ICC, SEM, and minimal difference were previously determined in 10
190 young subjects performing maximal isometric knee extension, and were 0.988, 5.20 Nm, and
191 14.41 Nm.
192
193 **Statistical analyses**
194 All results are expressed as means with standard errors (SEs). Statistical analysis
195 featured two-way analysis of variance (ANOVA) with repeated measures [condition (with
196 and without BFR) × time (PRE, MED, POST, POST2, and POST3)]. All baseline values
197 for NMES-CON and NMES-BFR and measured knee flexor data variables were compared
198 using the paired t test. Statistical significance was set at $p<0.05$. Effect sizes (ESs) were

199 calculated as [(Post Mean – Pre Mean)/ Pre Standard Deviation](25). ESs < 0.20 were
200 considered trivial, 0.20-0.49 small, 0.50-0.79 moderate, and > 0.80 large (2).
201
202 **Results**
203 NMES training and BFR application did not give rise to any relevant side-effect such
204 as subcutaneous hemorrhage, numbness and cerebral anemia. All subjects tolerated training
205 well; the adherence rate was 100% for both training conditions. No significant difference in
206 the baseline values of MT or muscle strength was evident when the two training conditions
207 were compared. No significant change in body mass or BMI was noted throughout training
208 and detraining (Table 1).
209 Figure 1 illustrates changes in the MT of the knee extensors throughout the training
210 and detraining periods. Two-way repeated measures ANOVA showed that the condition ×
211 time interaction ($p<0.001$) was significant. Under the NMES-BFR condition, MT increased
212 after 2 weeks of training (+3.9%) and decreased after 2 weeks of detraining (-3.0%), whereas
213 no notable change was observed under the NMES-CON condition. The ESs were 0.18 and
214 0.03 for the NMES-BFR and NMES-CON conditions, respectively. The MTs of the knee
215 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.

233 CR10 fell more rapidly under NMES-BFR than NMES-CON condition early in the training
234 period, but similarly under either condition thereafter.
235
236 **Discussion**
237 The major finding of our present study was that low-intensity NMES training induced
238 muscular hypertrophy and a concomitant increase in isometric and isokinetic strength, when
239 combined with BFR.
240 Over the past decade, many peer-reviewed studies have found that low-load (10-30%
241 of maximum strength) voluntary exercise training of working muscles, combined with BFR,
242 can induce muscle hypertrophy and strength gains (20). The mechanisms underlying such
243 hypertrophy are not completely understood, but metabolic stress resulting from the
244 accumulation of metabolic by-products such as H^+ and Pi seems to play the dominant role in
245 creation of the hypertrophic effect under low-load resistance training with BFR, although
246 mechanical stress also plays a part (24). It has been suggested that metabolic stress triggers
247 secondary reactions including the recruitment of additional motor units to compensate for the
248 force loss (32), enhanced acute muscle cell swelling (34), and production of reactive oxygen
249 species (11). Such events may increase the rate of muscle protein synthesis by activation of

250 anabolic, and/or inhibition of catabolic signaling pathways (3, 7, 16, 21) as well as
251 proliferation of satellite cells (23), triggering hypertrophy. Furthermore, muscle hypertrophy
252 and strength gain during low-load BFR training is observable even if training periods are
253 short (1-2 weeks of twice-daily sessions; 10). Thus, it is not surprising that 2 weeks of NMES
254 training at 5-10% MVC, combined with BFR, induced muscle hypertrophy and strength gain.
255 However, the magnitudes of such improvements induced by NMES-BFR appear to be lower
256 than those attainable using other training modalities.

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

263 Additionally, we found that 2 weeks of detraining reduced MT (-3.0%, rate of change;
264 -0.2% per day) to the basal level. Yasuda et al. recently investigated the effects of short-term
265 (3 weeks) detraining following low-load BFR training on muscle size and found that muscle
266 size returned to the basal level after detraining (33). Also, Gondin et al. showed that cessation

267 of NMES training (at 68% MVC) for 4 weeks induced a significant decrease in muscle cross-
268 sectional area (6). Such results are consistent with our present data, suggesting that muscle
269 size returned toward basal levels when relatively short-term detraining followed NMES and
270 BFR training. We found that isometric strength decreased (-6.8%, rate of change; -0.5% per
271 day), but that no large decrease in isokinetic knee extension strength (-1.9% at 90°/s, -0.6% at
272 180°/s) was evident, throughout detraining. Marqueste et al. showed that the increase (14%
273 from the pre-training level) in concentric maximal strength of the knee extensor after 6 weeks
274 of NMES training was preserved (19% above the pre-training level) after 6 weeks of
275 detraining (22). In contrast, another study found a gradual decrease in isometric strength after
276 cessation of NMES training (6). Changes in muscle strength during detraining may depend
277 on the type of muscle contraction measured (i.e., dynamic vs. static strength). However, no
278 other studies have investigated the effects of detraining after NMES and BFR training on
279 increases in muscle size and strength. The topic warrants further work.

280 In general, BFR induction with a tourniquet may suppress the clearance of metabolites,
281 creating pain (30). We found that the NMES-BFR condition was associated with higher
282 CR10 and RPE scores than the NMES-CON condition. One previous study found that, when
283 subjects performed resistance exercise at moderate intensity (45-60% 1RM), the range of

284 RPE scores indicating perceived exhaustion was 13.0-17.0 (29), similar to the RPE scores
285 noted under the NMES-BFR condition. Additionally, the CR10 and RPE scores under the
286 NMES-BFR condition were lower than those recorded during knee extension exercise at 20%
287 1RM, with BFR (cuff width: 135 mm) (26). These results suggest that the NMES-BFR
288 training protocol used in the present study is generally well-tolerated.

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

295 Noted limitation of our current study was that the device inflating the nylon cuffs
296 did not allow an initial compressive force to be set although the cuffs were tightly wrapped
297 around the upper thigh. Thus, we have no data concerning the relationship between inflated
298 cuff pressure and compressive force on the skin under the cuff. Furthermore, some variables
299 of electrical stimulation were not recorded, although the extent of strength development
300 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.

Acknowledgements

This work was supported by Japan Society for the Promotion of Science (JSPS) KAKENHI Grant No. 24500870. The authors wish to express their sincere appreciation to Mr. Keisuke Watanabe for his technical assistance during the course of this study. We also gratefully acknowledge the co-operation of all subjects who volunteered. The Juntendo University Institute of Health and Sports Science Medicine also supported our research.

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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416 **Figure legends**

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

425

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

433 after the training period, POST2; 2 weeks after the training period, POST3.

434

435 **FIGURE 3**–Changes in RPE (A) and CR10 (B) scores after each training session under both

436 NMES-CON (□) and NMES-BFR (■) conditions. Data are presented as means ± SEs.

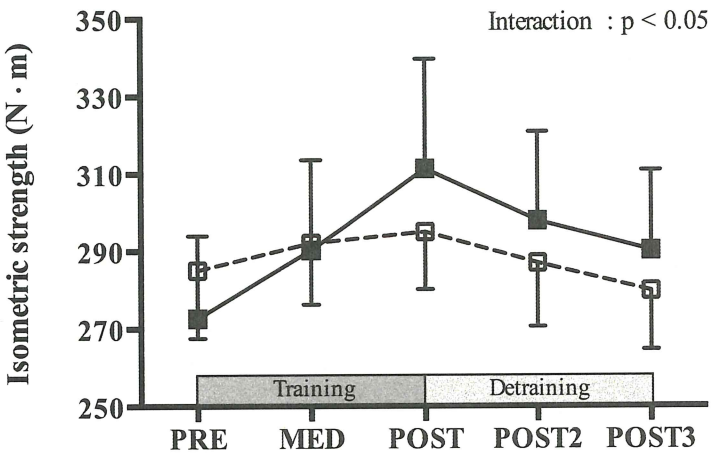
437 Abbreviations: rating of perceived exertion, RPE; category ration 10, CR10; neuromuscular

438 electrical stimulation, NMES-CON; neuromuscular electrical stimulation combined with

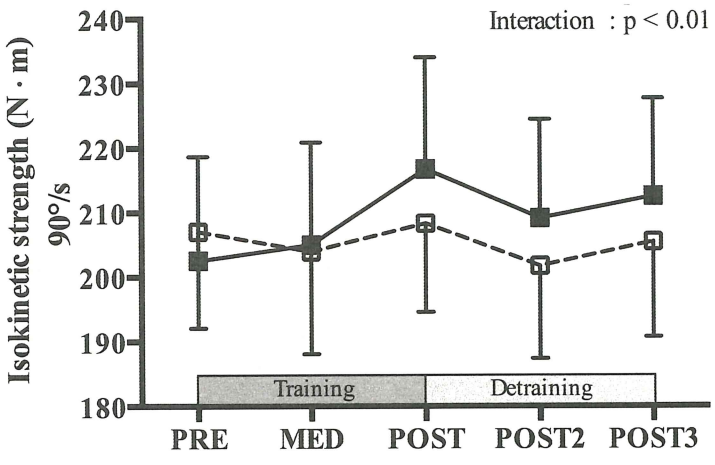
439 blood flow restriction, NMES-BFR.

	PRE	POST	POST3	p value
Age (yrs)	26.2 ± 0.7			
Height (m)	1.74 ± 0.02			
Body mass (kg)	71.4 ± 4.8	71.2 ± 4.6	71.4 ± 4.6	p = 0.801
BMI (kg/m^2)	23.4 ± 1.2	23.4 ± 1.2	23.5 ± 1.2	p = 0.900

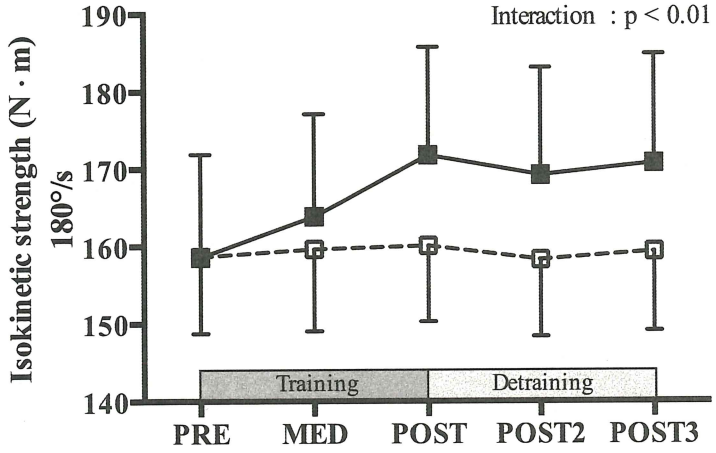
A

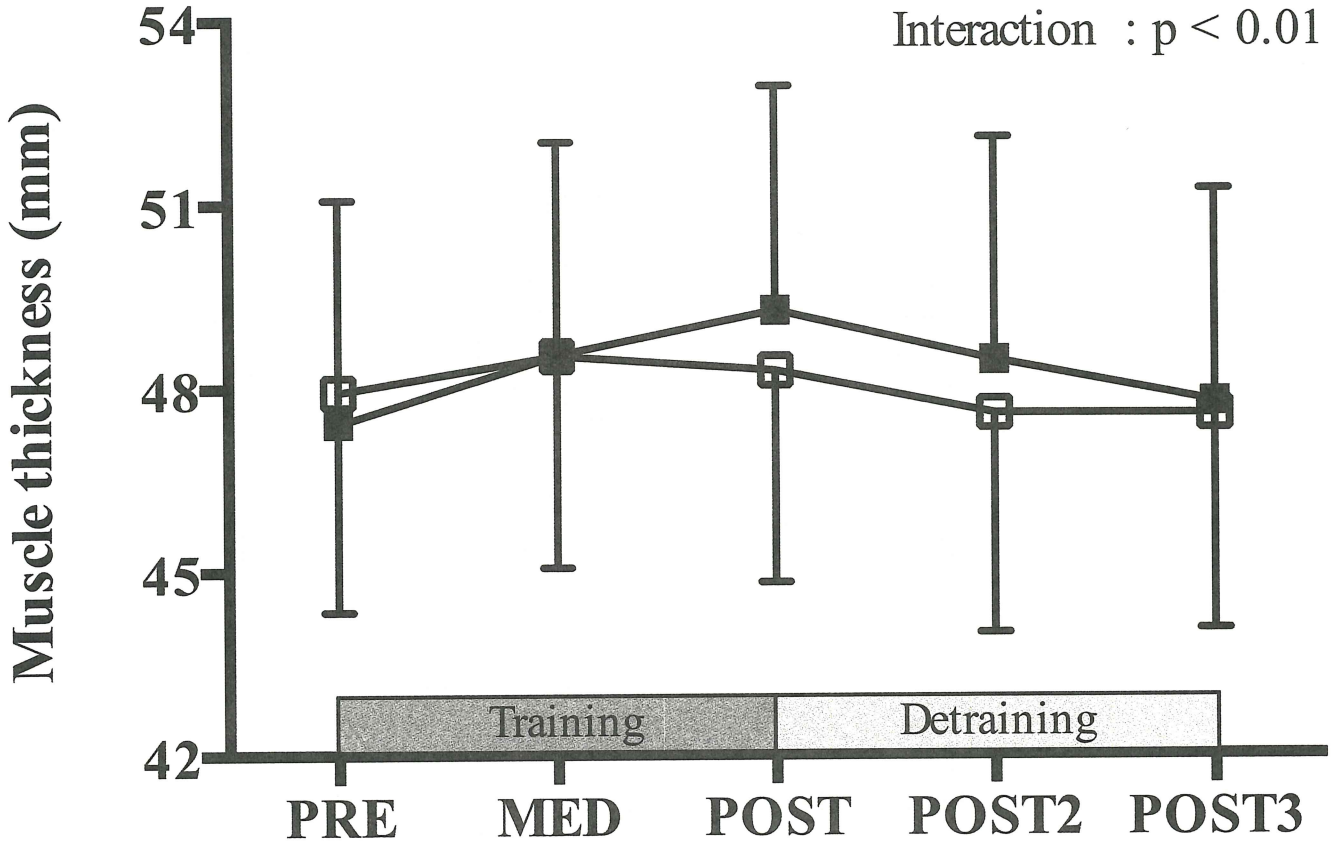

Isometric strength (N · m)
350
330
310
290
270
250
Interaction : p < 0.05
Training
Detraining
PRE
MED
POST
POST2
POST3

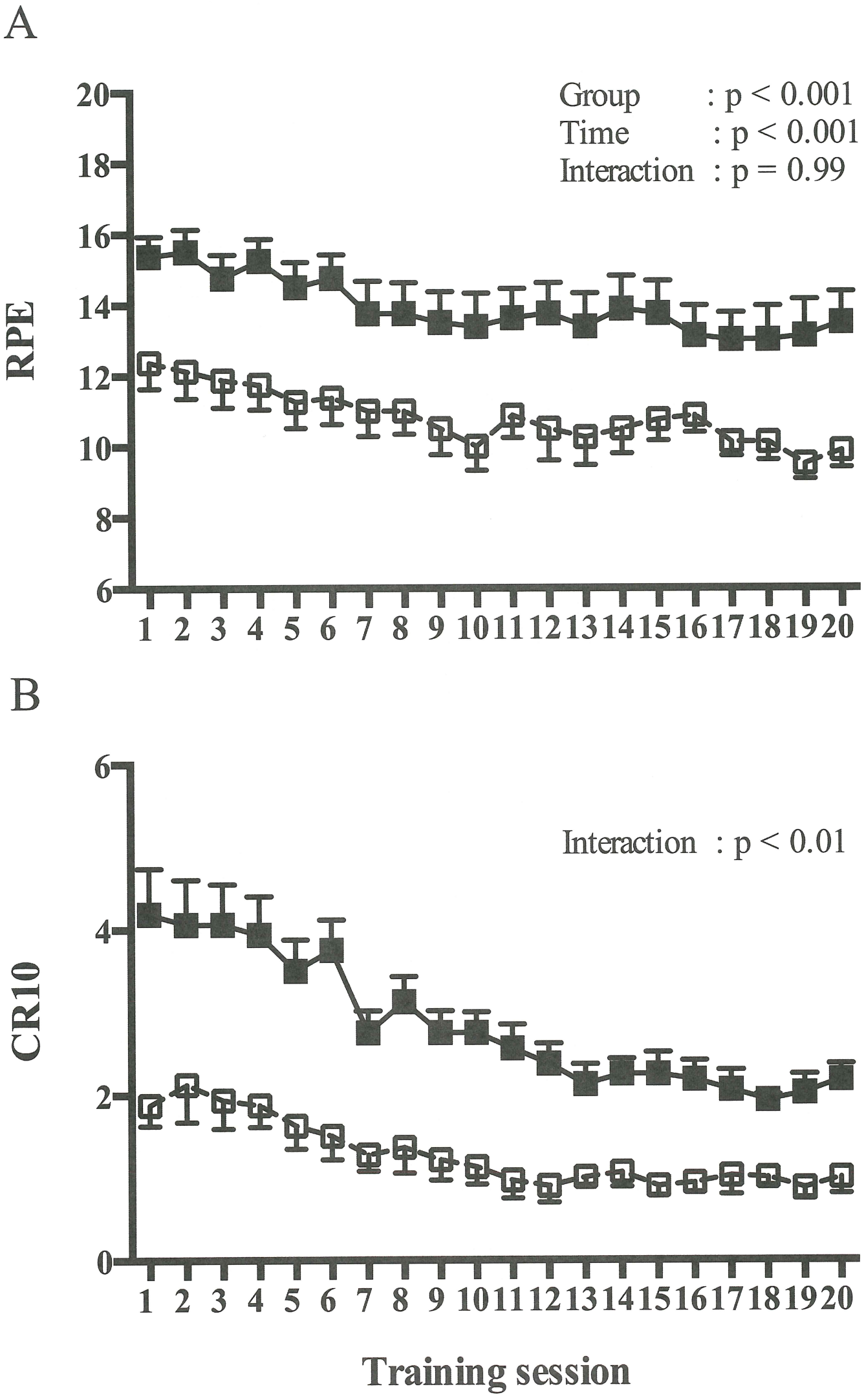
B


Isokinetic strength (N · m)
90°/s
240
230
220
210
200
190
180
Interaction : p < 0.01
Training
Detraining
PRE
MED
POST
POST2
POST3

C


Isokinetic strength (N · m)
180°/s
190
180
170
160
150
140
Interaction : p < 0.01
Training
Detraining
PRE
MED
POST
POST2
POST3


Interaction : p < 0.01
Muscle thickness (mm)
54
51
48
45
42
Training
Detraining
PRE
MED
POST
POST2
POST3


A
Group : p < 0.001
Time : p < 0.001
Interaction : p = 0.99
RPE
20
18
16
14
12
10
8
6
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
B
Interaction : p < 0.01
CR10
6
4
2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Training session