ORIGINAL ARTICLE

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Effects of training intensity in electromyostimulation on human skeletal muscle

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Abstract

Purpose High-intensity neuromuscular electrical stimulation (NMES) training can induce muscle hypertrophy at the whole muscle and muscle fiber levels. However, whether low-intensity NMES training has a similar result is unknown. This study aimed to investigate whether low-intensity NMES training could elicit muscle hypertrophy at the whole muscle and muscle fiber levels in the human skeletal muscle.

Methods Eight untrained young males were subjected to 18 min of unilateral NMES training for 8 weeks. One leg received NMES at maximal tolerable intensity (HIGH); the other leg received NMES at an intensity half of that in the HIGH condition (LOW). Quadriceps muscle thickness (MT), muscle fiber cross-sectional area (CSA), and knee extension strength were measured before and after the training period.

Results The average training intensity throughout the intervention period in the HIGH and LOW conditions were $62.5 \pm 4.6\%$ maximal voluntary contraction (MVC) and $32.6 \pm 2.6\%$ MVC, respectively. MT, CSA, and muscle strength increased in both exercise conditions ($p < 0.05$); however, training effects in the LOW condition were lower than those in the HIGH condition ($p < 0.05$). The average training intensity showed a positive correlation with percent changes in muscle strength ($r = 0.797$, $p = 0.001$), MT ($r = 0.876$, $p = 0.001$), type I fiber CSA ($r = 0.730$, $p = 0.01$), and type II fiber CSA ($r = 0.899$, $p = 0.001$).

Conclusions Low-intensity NMES could increase MT, muscle fiber CSA, and muscle strength in healthy human skeletal muscles. However, the magnitude of increase is lower in low-intensity than in high-intensity NMES training.

Keywords Muscle fiber · Muscle thickness · Neuromuscular electrical stimulation · Maximal voluntary contraction

Abbreviations

ANOVA	Analysis of variance
BSA	Bovine serum albumin
CSA	Cross sectional area
MVC	Maximal voluntary contraction
MT	Muscle thickness
NMES	Neuromuscular electrical stimulation
PBS	Phosphate-buffered saline
SE	Standard error

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Introduction

Resistance training is recommended to increase muscle mass and strength (Ozaki et al. 2017). Neuromuscular electrical stimulation (NMES) has been used as an alternative way to induce muscle contraction, which could also induce muscle hypertrophy and strength gain in both healthy and injured skeletal muscles (Gondin et al. 2005, 2006; Ruther et al. 1995; Stevenson and Dudley 2001). Thus, NMES has been widely used as a complement to voluntary exercise in athletes (Maffiuletti et al. 2000) and in patients who could not perform conventional types of voluntary exercise because of various pathologies, such as heart failure (Deley et al. 2008;

Dobsak et al. 2006) and chronic obstructive pulmonary disease (Vivodtzev et al. 2008).

Numerous studies have investigated the effect of NMES training on muscle strength adaptation (Gondin et al. 2005, 2006; Lai et al. 1988; Laughman et al. 1983), and based on previous findings, strength gain following NMES training depends on the magnitude of electrically evoked force during the intervention period (Gondin et al. 2011b). However, information on NMES-induced muscle hypertrophy is mainly limited to the previous results of a relatively high-intensity NMES (> 60% MVC) (Gondin et al. 2005, 2011a; Ruther et al. 1995; Stevenson and Dudley 2001) and whether NMES at a lower intensity could also induce muscle hypertrophy remains unclear. The main limitation of high intensity NMES training is strong discomfort associated with the application of electrical stimuli over skin (Delitto et al. 1992). High electrically evoked force levels are not always achievable even in a healthy population. Indeed, the maximal tolerable levels of electrically evoked force differ greatly between individuals; force evoked by NMES in previous studies ranged from 12 to 95% (Gondin et al. 2005; Jubeau et al. 2008). Thus, exploring whether low-intensity NMES induce muscle hypertrophy at the whole muscle and muscle fiber levels would be useful for the population that cannot tolerate high-intensity NMES.

In typical resistance exercise, slow-twitch fibers are activated initially according to the "size principle", while larger motor units associated with fast-twitch fibers are recruited as external load increases (Pearson and Hussain 2014). In NMES, a unique recruitment pattern of motor units exists, which is characterized as nonselective, spatially fixed, and temporally synchronous (Bickel et al. 2011; Gregory and Bickel 2005; Jubeau et al. 2007). Thus, contrarily to voluntary contraction, NMES can recruit both slow-twitch and fast-twitch fibers even at low force levels. However, the number of recruited muscle fibers during electrical stimulation depends on the magnitude of electrically evoked force (Adams et al. 1993). Moreover, additional muscle fiber recruitment to compensate for muscle force reduction is not caused by a certain stimulation conditions, even if fatigue occurs in the muscle fibers recruited at the start of stimulation (Maffiuletti et al. 2011). Thus, a higher intensity would elicit greater recruitment of any muscle fiber type, when stimulation conditions other than stimulation intensity (electrical flow intensity) is constant. Thus, we hypothesized that higher intensity NMES training would result in greater muscle hypertrophy and strength gain.

The aims of this study were to compare the effects of low- and high-intensity NMES training on muscle size and strength and to investigate whether the magnitude of the hypertrophic effect is correlated with the magnitude of electrically evoked force during the intervention period at both the whole muscle and muscle fiber levels.

Methods

Subjects

Eight untrained young males (means ± standard error; age 28 ± 1 years, stature 1.75 ± 0.02 m, body mass 68.1 ± 2.5 kg) volunteered to participate in this 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. All subjects were free of overt chronic diseases according to their medical history. Subjects 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 in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee for Human Experiments of Juntendo University, Japan.

Study design

To compare the effects of training intensity on muscle size and strength, one leg was randomly assigned to receive high-intensity NMES (HIGH), while the other leg was assigned to receive low-intensity NMES (LOW). The maximal isometric strength and muscle thickness of both legs was measured 5 days before the first training session. In the HIGH condition, the maximum tolerable NMES intensity was applied to the quadriceps for 8-weeks training period. In the LOW condition, the NMES applied to the quadriceps was half of the percentage of MVC of the HIGH condition throughout the training session by modulating electrical current. Maximal isometric strength and muscle thickness were re-measured 5 days after the last NMES training session. Biopsy samples were obtained from the vastus lateralis of both legs for evaluating muscle fiber CSA at least 1 week before the beginning of the NMES training period and 1 week after the last NMES training session.

Neuromuscular electrical stimulation training

The subjects attended a total of 24 training sessions for 8 weeks (three training sessions per week). Each training session comprised 40 electrically evoked isometric contractions. During the stimulation, the subjects were seated on an isokinetic dynamometer (Biodex System 4; Biodex Medical Systems, Shirley, NY, USA) and underwent 18 min of involuntary muscle contractions of knee extensors at a fixed knee joint angle of 75° (where 0° corresponds to a full knee extension). The quadriceps muscles were stimulated using bipolar electrodes linked to a portable battery-powered

neuromuscular electrical stimulator (Compex Sport; Medicompex SA, Ecublens, Switzerland). Three self-adhesive electrodes (2-mm thickness) were placed over each thigh. The large electrode (10×5 cm) was positioned proximally or approximately 5 cm below the inguinal crease. Two small electrodes (5×5 cm) were placed as close as possible to the central motor points of vastus lateralis and the distal motor points of medialis muscles (Botter et al. 2011). The motor points were identified by stimulating the skin surface with a pen electrode and a large reference electrode placed over the skin, with the stimulatory current being gradually increased by the operator until a clear muscle twitch was observed. The positions of the motor points were marked, and the electrodes were applied at the same sites throughout the intervention period. The commercially available stimulator discharged biphasic rectangular pulses lasting 400 μs. The stimulation frequency and duty cycle were 75 Hz and 6.25 s of stimulation followed by a pause lasting 20 s (duty cycle, 24%).

In every exercise session, the HIGH condition was performed before the LOW condition. For the HIGH condition, the current amplitude was increased to the maximal tolerable intensity during the first 10 electrically isometric contractions and was maintained after that (for 30 contractions) in each session. All 40 evoked isometric torques were recorded by the isokinetic dynamometer and the percentage of MVC in each contraction was calculated from dividing the observed values by the MVC determined via pre-test. As shown in Fig. 1, maximal tolerable intensity (the mean value of the percentage of MVC in 40 contractions) gradually increased throughout the training period. In the LOW condition, the current amplitude was modulated by each contraction and the evoked isometric torque was monitored
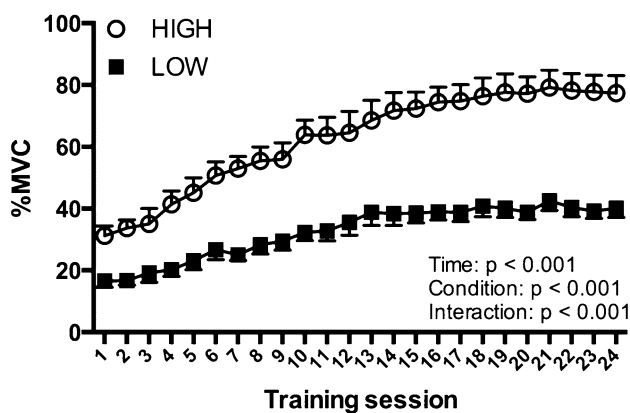
so that the percentage of MVC was the half of that in HIGH condition previously performed.

Muscle thickness

Muscle thickness (MT) on the anterior aspect of the thigh at the following eight sites was measured by B-mode ultrasound using a 5–18 MHz, 4×1 cm linear array probe (Noblus; Aloka, Tokyo, Japan) as an index of muscle hypertrophy: lateral, central, and medial surfaces at 30, 50, and 70% of the thigh length between the lateral condyle of the femur and greater trochanter (excluding the 30% medial point). Prior to all scans, the subjects rested quietly in a seated position for at least 30 min to avoid the influence of fluid shift within the muscle. We performed the measurements around the same time, and a single operator performed all measurements. Measurement sites were identified using a marker pen, as described previously (Kubo et al. 2008). Ultrasound measurements of MT were performed with subjects in the supine position, ensuring that the hip and ankle joint positions and the distance between both legs were similar in all the measurements. The scanning head was coated with a water-soluble transmission gel and transversely placed on each marked measurement site without depressing the dermal surface. The subcutaneous adipose tissue and muscle–bone interfaces were identified in the ultrasound images, and the distance between them was recorded as MT. The mean MT of the eight anterior sites was used in data analysis. In 10 subjects, test–retest reliabilities of the MT measurements were calculated using the intraclass correlation coefficient, SEM, and minimal difference (0.999, 0.21 mm, and 0.58 mm, respectively) in terms of anterior central 50% MT values (Natsume et al. 2015).

Maximal isometric strength

The maximal isometric strength of the knee extensors was determined using Biodex System 4 dynamometer. During the measurement, the subjects were seated on a chair with the hip joint angle positioned at 85° of flexion (where 0° is 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. The knee joint angle was at 75° (where 0° is full knee extension). Before the measurement, several warm-up knee extensions and one to two near maximal knee contractions were performed. During the measurement, the subjects were instructed to perform maximal isometric knee extension for approximately 5 s. Measurements were performed a total of three times and the peak torque was used in data analysis. Test–retest reliabilities of the strength measurements were calculated using the intraclass correlation coefficient, SEM, and minimal difference (0.988, 5.20,

Fig. 1 Changes in peak torque [% maximal voluntary contraction (MVC)] of knee extensors during each training session under HIGH (open circle) and LOW (filled square) conditions. Values are mean ± SE. HIGH, trained at maximum tolerable intensity; LOW, trained at half the intensity of that in the HIGH condition

and 14.41 Nm, respectively) in 10 subjects who performed maximal isometric knee extension (Natsume et al. 2015).

Muscle biopsy

Muscle samples (10–20 mg) were obtained from the vastus lateralis muscle approximately 15 cm above the patella in both legs of each subject by needle biopsy (14 gauge Max-Core; C.R. Bard, Covington, GA) under local anesthesia using 1% lidocaine, which is routinely used in our laboratory (Kakigi et al. 2011). Any visible non-muscle tissues (e.g., fat tissue) were removed from the biopsy samples. The obtained muscle samples were embedded in Tissue-Tek (OCT compound; Sakura Finetek Japan, Tokyo, Japan) and perpendicularly placed on a filter paper using a stereomicroscope (Leica MZ7$_5$, Leica Microsystems, Heerbrugg, Switzerland). The samples were immediately frozen in liquid nitrogen and stored at − 80 °C.

Immunohistochemistry

Immunohistochemical analysis was performed to identify type I and type II muscle fibers; cryostat was at − 20 °C. The samples were cut (10-µm thick) and mounted on microscope slides. The cross-sections were air-dried for 30 min and rinsed with phosphate-buffered saline (PBS) for 5 min. Subsequently, the slides were fixed in 4% paraformaldehyde in PBS for 10 min and washed with PBS for 5 min. Furthermore, the slides were blocked for 60 min with 5% bovine serum albumin (BSA) in PBS containing 0.5% Tween 20 (PBS-T), washed with PBS for 5 min, and incubated for 60 min with primary antibodies against myosin heavy chain type 1 (BA-D5, 1:100 dilution; Developmental Studies Hybridoma Bank, Iowa City, IA) and laminin (L9393, 1:500 dilution; Sigma-Aldrich, St. Louis, MO, USA). After washing with PBS for 5 min, the slides were further incubated with Alexa Fluor 555-conjugated goat anti-mouse IgG (1:500 dilution; Life Technologies, Eugene, OR, USA) and Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:500 dilution; Life Technologies, Eugene, OR, USA) for 60 min. All primary and secondary antibodies were diluted in 5% BSA in PBS-T. All incubations were at room temperature (23–25 °C). After the final washing with PBS, all slides were covered with a mounting medium (VECTASHIELD, Vector Laboratories, Burlingame, CA, USA).

Images were visualized and automatically captured at × 10 magnification with a fluorescent microscope (Leica DM5000 B, Leica Microsystems, Wetzlar, Germany). Microscope imaging software (LAS Ver4.5, Leica Microsystems, Wetzlar, Germany) was used for image acquisition. Quantitative analysis was performed using ImageJ version 1.50 g (National Institute of Health, MD). Individual fibers were traced inside the membrane stained with laminin, and a region-of-interest list was created listing all individual fibers. Subsequently, the muscle fiber cross-sectional area (CSA) was measured for each separate muscle fiber. As such, mean muscle fiber size (µm^2) was calculated separately for type I and type II muscle fibers. At least 300 muscle fibers were analyzed in the biopsy samples obtained at baseline and after 8 weeks of exercise intervention. For technical reasons, the analysis was performed on six subjects (two subjects were excluded from the immunohistochemical analysis).

Data analysis

Results are expressed as means and standard error (SE). Muscle strength, MT, muscle fiber CSA [condition (HIGH and LOW)×time (PRE and POST)], peak torque during NMES training [condition (HIGH and LOW)×training session (from 1 to 24)] were analyzed using two-way analysis of variance (ANOVA) for repeated measures. Effect size of two-way ANOVA for repeated measures was calculated using partial eta squared. If there was a significant interaction for muscle strength, MT, and muscle fiber CSA, post hoc tests were performed by Sidak test. Pearson's product moment correlations were performed to determine the relationship between training intensity (%MVC) and percent changes in muscle strength, MT, and muscle fiber CSA after NMES training. All baseline values (muscle strength, MT, and muscle fiber CSA) in the HIGH and LOW conditions and physical characteristics throughout the intervention period were analyzed using a paired t-test. All analyses were conducted using Prism 6 (GraphPad Software, La Jolla, CA, USA). A p value of 0.05 was used to determine statistical significance.

Results

No significant changes in body weight or body mass index were noted throughout the training period (Table 1), and no baseline differences in maximal isometric strength, MT, and CSA between type I and type II muscle fibers were found.

Figure 1 illustrates the changes in electrically evoked peak torque (%MVC) of the knee extensors over the 24 training

Table 1 Changes in physical characteristics

	PRE	POST	p
Age (years)	28 ± 1		
Stature (m)	1.75 ± 0.02		
Body mass (kg)	68.1 ± 2.5	68.3 ± 2.7	0.549
Body mass index (kg m^{-2})	22.3 ± 0.6	22.4 ± 0.7	0.571

Values are mean ± SD

PRE, before NMES training; POST, after NMES training for 8 weeks

sessions. Two-way ANOVA showed that the main effects of time ($p < 0.001$, F value $= 32.835$, ES $= 0.868$), condition ($p < 0.001$, F value $= 72.440$, ES $= 0.935$), and interaction ($p < 0.001$, F value $= 12.497$, ES $= 0.714$) were significant. The training intensity in the HIGH condition increased more than that in the LOW condition as the training advanced. The training intensity in HIGH ranged from 20.2 to 44.5% MVC at the first training session and 51.0–99.6% MVC at the last training session; this is consistent with previous research (Gondin et al. 2005). In the LOW condition, first and last session ranges were respectively, 11.9–23.4 and 26.4–49.5% MVC. The training intensity throughout the training period in the HIGH and LOW conditions were 62.5 ± 4.6 and 32.6 ± 2.6% MVC, respectively.

Figure 2 illustrates the changes in maximal isometric strength of the knee extensors. Two-way ANOVA showed that the interactions ($p < 0.05$, F value $= 9.202$, ES $= 0.568$) were significant. Muscle strength for HIGH ($p < 0.01$) and LOW conditions ($p < 0.01$) significantly increased after NMES training. Muscle strength in the HIGH condition significantly increased compared to the LOW condition after NMES training for 8 weeks ($p < 0.05$). Muscle strength of knee extensor for PRE and POST were 276.6 ± 16.4 and 340.5 ± 21.1 Nm in the HIGH condition and 280.8 ± 20.4 and 311.4 ± 18.9 Nm in the LOW condition, respectively.

Figure 3 shows the changes in MT of knee extensors throughout the training period. Two-way ANOVA showed that the interactions ($p < 0.01$, F value $= 23.889$, ES $= 0.773$) were significant. MT for HIGH ($p < 0.01$) and LOW conditions ($p < 0.01$) significantly increased after NMES training. MT in the HIGH condition significantly increased compared with that in the LOW condition after NMES training for 8 weeks ($p < 0.05$). MT of knee extensors for PRE and POST were 35.3 ± 0.8 and 38.5 ± 1.0 mm in HIGH condition and 35.5 ± 0.8 and 37.2 ± 0.9 mm in LOW condition, respectively.

Fig. 3 Changes in muscle thickness under HIGH and LOW conditions. Values are mean $\pm$ SE. HIGH, trained at maximum tolerable intensity; LOW, trained at half the intensity of that in the HIGH condition. **$p < 0.01$, vs. PRE; †$p < 0.05$, vs. HIGH

Figure 4 shows the changes in CSA of type I and II muscle fibers after the training period. Two-way ANOVA showed that the interaction (type I, $p < 0.05$, F value $= 6.725$, ES $= 0.574$; type II, $p < 0.01$, F value $= 43.183$, ES $= 0.896$) were significant. Both muscle fibers CSA for the HIGH (type I, $p < 0.01$; type II $p < 0.01$) and LOW conditions (type I, $p < 0.01$; type II $p < 0.01$) significantly increased after NMES training. Both muscle fibers CSA in the HIGH condition significantly increased compared with that in the LOW condition after NMES training for 8 weeks (type I, $p < 0.05$; type II $p < 0.05$). Muscle fiber CSA of type I for PRE and POST were 4173.2 ± 356.3 and 4762.4 ± 361.7 μm^2 in the HIGH condition and 4051.3 ± 459.1 and 4331.8 ± 475.8 μm^2 in the LOW condition, respectively. Muscle fiber CSA of type II for PRE and POST were 4626.0 ± 378.1 and 5675.7 ± 430.6 μm^2 in the HIGH condition and 4425.6 ± 543.8 and 4898.1 ± 587.4 μm^2 in the LOW condition, respectively.

Correlation between average training intensity (%MVC) throughout the training period and percent changes in muscle strength, MT, and muscle fiber CSA is shown in Fig. 5. Average training intensity showed a positive correlation with percent changes in muscle strength ($r = 0.797$, $p = 0.001$), MT ($r = 0.876$, $p = 0.001$), type I fiber CSA ($r = 0.730$, $p = 0.01$), and type II fiber CSA ($r = 0.899$, $p = 0.001$).

Discussion

This is the first study to demonstrate that NMES at the intensity of < 60% MVC can induce significant hypertrophy at the whole muscle and muscle fiber levels in the quadriceps in addition to increased knee extensor strength. The hypertrophic effects and strength gain induced by NMES were correlated with the mean training intensity during the training period: the training effects were higher in the HIGH condition than in the LOW condition. These results show
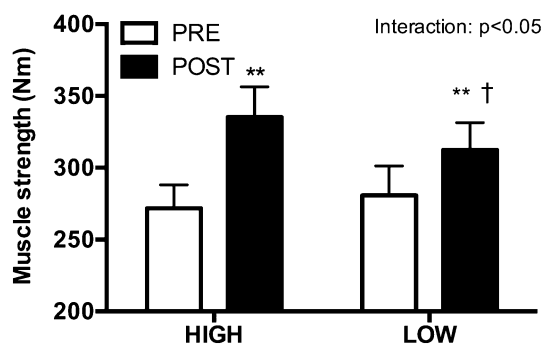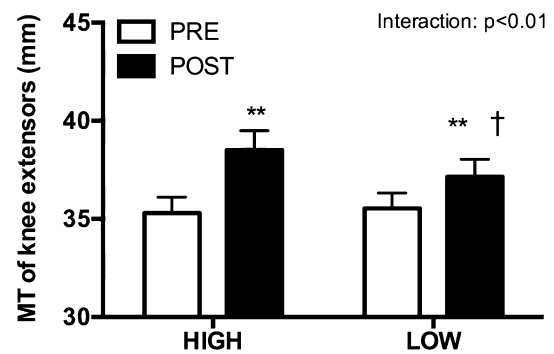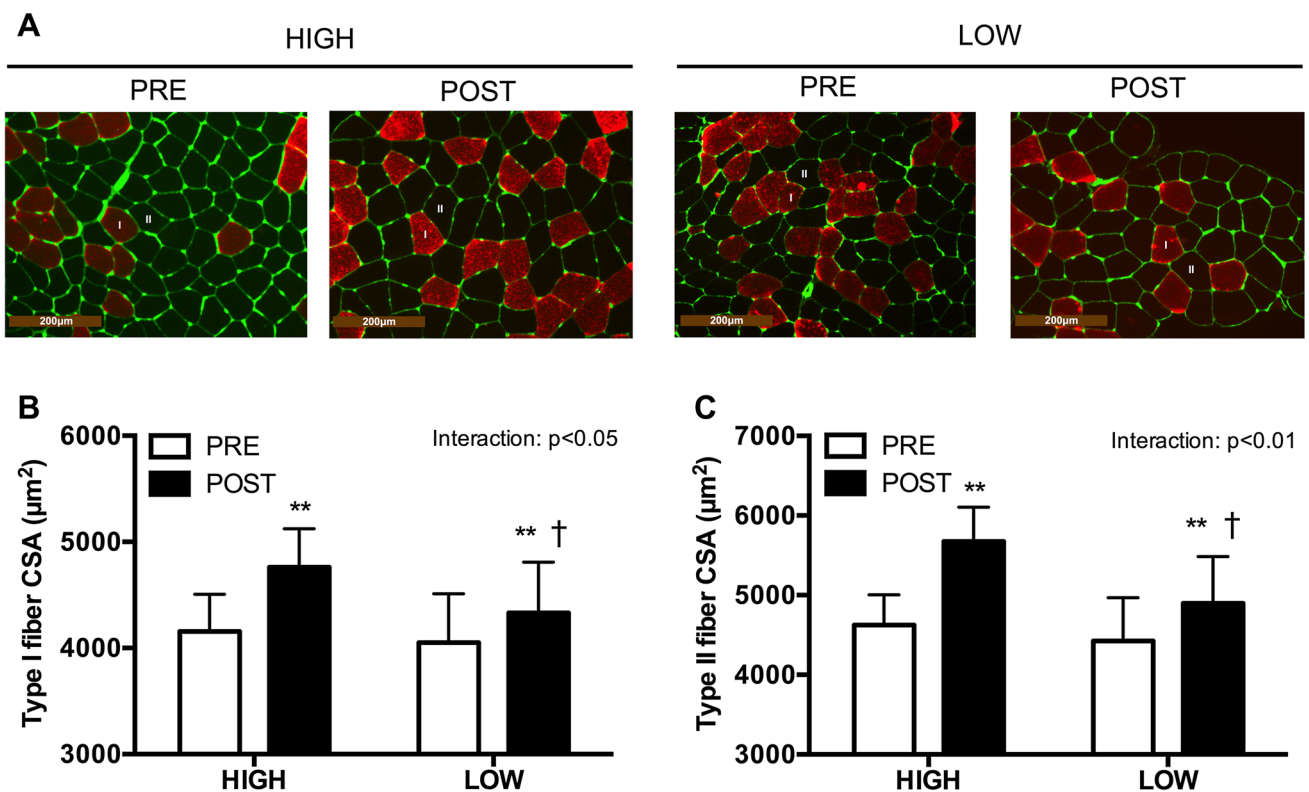

Fig. 2 Changes in maximal isometric strength of knee extensors under HIGH and LOW conditions. Values are mean $\pm$ SE. HIGH, trained at maximum tolerable intensity; LOW, trained at half the intensity of that in the HIGH condition. **$p < 0.01$, vs. PRE; †$p < 0.05$, vs. HIGH

Fig. 4 Representative photomicrographs (**a**) of type I (red) and type II (black) muscle fiber and changes in CSA of type I (**b**) and type II (**c**) muscle fibers under HIGH and LOW conditions. Values are mean $\pm$ SE. HIGH, trained at maximum tolerable intensity; LOW, trained at half the intensity of that in the HIGH condition. *CSA* cross-sectional area. $**p < 0.01$, vs. PRE; $^{\dagger}p < 0.05$, vs. HIGH

that training intensity is an important variable for induction of muscle hypertrophy in NMES training.

In this study, muscle strength increased in both the HIGH and LOW conditions after 8 weeks of NMES training. The results in the HIGH condition are consistent with previous results using NMES training in the quadriceps using an approximately similar intensity (Gondin et al. 2005, 2006, 2011a; Lai et al. 1988; Laughman et al. 1983). Moreover, the magnitude of the increase in muscle strength in the LOW condition is lower than the HIGH condition in our study. To our knowledge, only one study investigated the effects of training intensity (% of MVC) on muscle strength using NMES training and demonstrated that the strength gain for 25% MVC condition was approximately half that for 50% MVC condition (Lai et al. 1988). In addition, the muscle strength gain in this study has a positive linear relationship with training intensity, in accordance with previous results (Gondin et al. 2011b; Selkowitz 1985). Hence, it is plausible that the magnitude of strength gain induced by NMES training correlates with training intensity.

High intensity resistance exercise is widely used for inducing muscle hypertrophy (Garber et al. 2011). Thus, most of the previous studies investigating the hypertrophic effect of NMES training used a relatively high training intensity (Gondin et al. 2005, 2006, 2011a; Ruther et al. 1995). Some previous studies demonstrated that 8 weeks of NMES training at 60–70% of MVC increases thigh muscle CSA (Gondin et al. 2005, 2006, 2011a; Ruther et al. 1995). Gondin et al. also showed that 8 weeks of NMES training at 60% of MVC increases the CSA of both type I and type II muscle fibers (Gondin et al. 2011a). In this study, as in previous studies, muscle hypertrophy at the whole muscle and muscle fiber levels was observed in the HIGH condition. However, to the best of our knowledge, no study has demonstrated a hypertrophic effect of NMES training at low intensity, i.e., < 60% of MVC, on healthy human skeletal muscles, and this is the first study to show that NMES with approximately 30% of MVC is sufficient to induce muscle hypertrophy. Whether training intensity below 30% of MVC can achieve muscle hypertrophy is unknown. The topic warrants further research.

The precise mechanism of muscle hypertrophy and strength gain induced by NMES training is still poorly defined. Wall et al. suggested that transcutaneous NMES promotes muscle protein synthesis via mammalian target of rapamycin (mTOR) signaling pathway in human skeletal muscle, although the training intensity was not reported (Wall et al. 2012). Furthermore, a previous study has
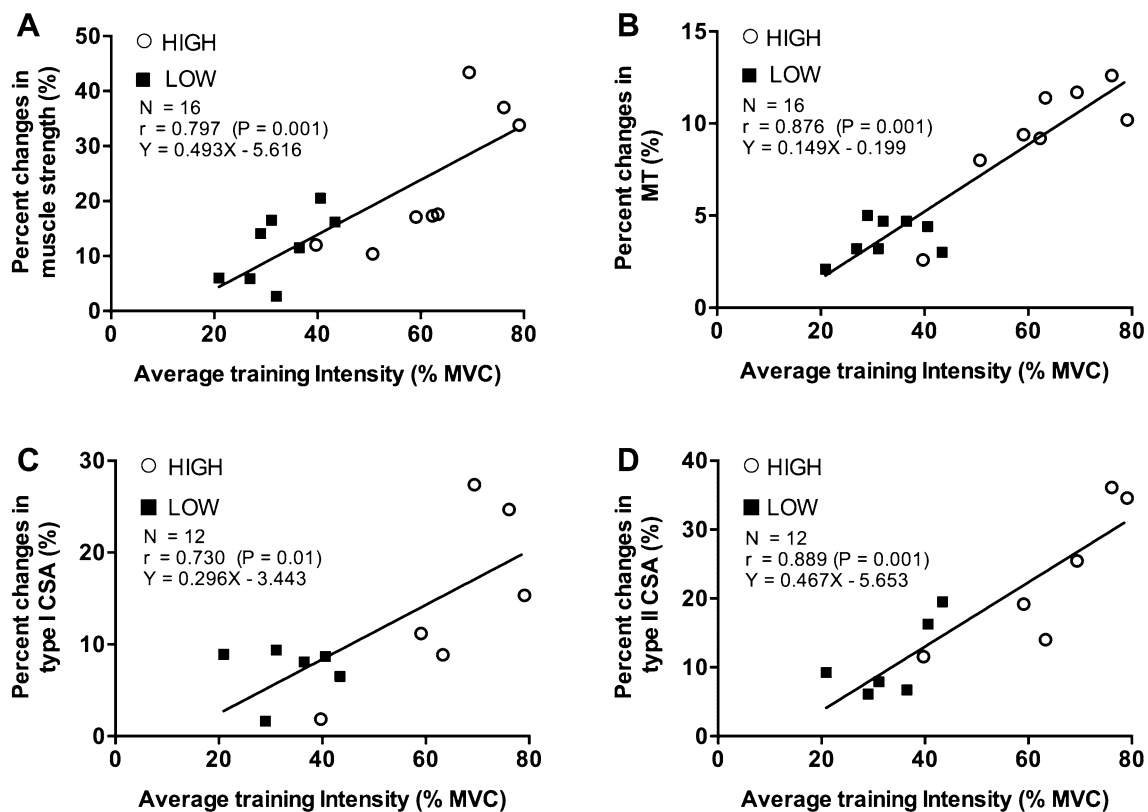
Fig. 5 Correlation between average training intensity (%MVC) and percent changes in muscle strength (**a**), MT (**b**), and CSA of type I (**c**) and type II (**d**) muscle fiber under HIGH and LOW conditions. Pear-son's correlation coefficients and p values are shown for each plot. HIGH, trained at maximum tolerable intensity; LOW, trained at half the intensity of that in the HIGH condition

reported that the application of transcutaneous NMES at approximately 36% of MVC increased insulin-like growth factor 1 binding proteins mRNA expression (Bickel et al. 2003), which would regulate the mTOR signaling pathway (Schiaffino and Mammucari 2011). Thus, even low-intensity NMES can activate muscle protein synthesis. Muscle hypertrophy observed in the LOW condition would at least partially contribute to strength gain.

A unique motor unit recruitment pattern is observed during NMES, which is characterized as random/nonselective, spatially fixed, and temporally synchronous (Gregory and Bickel 2005; Jubeau et al. 2007). Nevertheless, resistance exercise recruits from small to large motor units in accordance with size principle (Pearson and Hussain 2014). In addition, NMES induces continuous contractile activity of the same muscle fiber, in which additional recruitment to compensate for muscle force reduction is not caused by a fixed stimulation condition (Maffiuletti et al. 2011). Moreover, a previous study used magnetic resonance spectroscopy to show that during NMES at 30% of MVC, Pi splitting occurs (Jubeau et al. 2015). The Pi splitting indicates that oxidative and glycolytic muscle fibers were simultaneously recruited during NMES (Park et al. 1987). Therefore, our

study showed that NMES could recruit both slow and fast muscle fibers even at low force levels, thereby resulting in muscle hypertrophy of both muscle fiber types.

A limitation of our current study was that both legs were, respectively, assigned to the HIGH and LOW conditions, which cannot dispel the crossover effects of strength gain. However, considering the training effects of LOW condition is about half of HIGH condition and the linear relationship of training intensity and percent changes in muscle strength, the crossover effects may have not occurred. Interestingly, other previous studies showed that high external load might not be indispensable for the induction of muscle hypertrophy in conventional resistance-training modalities (Mitchell et al. 2012; Ozaki et al. 2016). Even a low external load of approximately 30% of MVC can induce comparable muscle hypertrophy when the exercise was performed until failure (Mitchell et al. 2012). In this study, the LOW condition was not performed until exercise failure. In the future, we need to carry out the verification of hypertrophic effects on NMES training, refining our understanding not only of training intensity but also training volume.

In conclusion, this is the first study to show that low-intensity NMES could induce muscular hypertrophy and

strength gain after 8 weeks of NMES training. We showed that low-intensity NMES could increase muscle strength, MT, and muscle fiber CSA in healthy human skeletal muscles. However, the magnitude of increase in low-intensity NMES training remains lower than that in high-intensity NMES training.

Author contributions TN conceived and designed of research. TN, HO, RK, and HK performed experiments. TN analyzed data. TN, HO, RK, HK, and HN interpreted results of experiments. TN prepared figures. TN and HO drafted manuscript. All authors read and approved final version manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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