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Title

The effect of electrical muscle stimulation on quadriceps muscle strength and activation patterns in healthy young adults

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1 Abstract

The aim of the present study was to clarify the effect of electrical muscle 2 3 stimulation (EMS) on the spatial distribution pattern of electromyographic activity in healthy young adults using multi-channel surface electromyography 4 (SEMG). A total of 32 men (age =21-26 years) were randomly assigned to the 5 intervention group (n=18) and control group (n=14). Participants in the 6 intervention group performed EMS to stimulate the bilateral lower limb muscle 7 for four weeks (20 min/3 days/week). The control group received no EMS 8 intervention. To understand the effects of EMS, following measurements were 9 made at baseline and four weeks: knee extension torque, muscle mass, and spatial 10 distribution of neuromuscular activation during a target torques [10%, 30%, 50%, 11 and 70% of the maximal voluntary contraction (MVC)] using multi-channel 12 SEMG. The knee extension torque was significantly increased in intervention 13 group compared with control group (p < 0.0001). However, the muscle mass did 14 not show a significant difference between pre and post intervention in each group. 15

16	The muscle activation patterns of 50% and 70% MVC task showed significant
17	enhancement between baseline and four weeks in the intervention group.
18	Furthermore, a moderate correlation between Δ knee extension torque and Δ
19	spatial distribution pattern of electromyographic activity of 50% and 70% MVC
20	in the intervention group was observed. These results suggested EMS intervention
21	induced different distribution of muscle activity at high-intensity muscle
22	contraction compared with low-intensity muscle contraction.

24 Introduction

25	An improvement of muscle performance with training is due to the
26	adaptation of morphological and neural factors [1]. Especially, neural factors are
27	the major contributors to increase strength during the early phase of training [2].
28	Many previous studies reported that resistance training enhanced muscle strength
29	and muscle thickness [1, 3, 4]. In general, the morphological factors can be
30	assessed by the measurements of muscle mass with magnetic resonance imaging
31	[3, 5]. Recently, the change of neural factors during resistance training have been
32	reported by measurements of motor unit activity using multi-channel surface
33	electromyography (SEMG) [6]. This technique provides data on the spatial
34	distribution of SEMG within a muscle. Previous studies using this technique have
35	demonstrated that the spatial SEMG potential distribution pattern within a muscle
36	is altered by contractions or fatigue level [7, 8]. This phenomenon has been
37	explained by spatial inhomogeneity in the location of different types of muscle
38	fibers [9]. Previous studies have also demonstrated that changes in the spatial

39	distribution of multi-channel SEMG can be explained by the physiological
40	phenomenon of motor unit recruitment, which suggests that the spatial
41	distribution of multi-channel SEMG can be used to study changes in motor unit
42	recruitment [10, 11]. Although, previous studies reported that increase in the
43	SEMG amplitude due to resistance training [1, 3, 12], spatial distribution pattern
44	of muscle activation did not change during resistance training using multi-channel
45	SEMG [6]. These results suggested that the spatial distribution of neuromuscular
46	activation is not influenced by resistance training.
47	It is widely known that electrical muscle stimulation (EMS) interventions
48	can improve muscle performance and muscle thickness [13-18]. The previous
49	studies speculated that the EMS intervention induced non-physiological
50	recruitment order and synchronous discharge of motor units [19-21]. In general,
51	according to the size principle, voluntary motor unit recruitment describes the
52	progressive recruitment of small, typically slow motor units followed in order of
53	increasing size to the large, typically motor units [22, 23]. However, EMS recruits

54	motor units randomly in relation to axon diameter [24]. It indicates that muscle
55	activation differs between voluntary and electrically-induced contraction.
56	The purpose of the present study was to clarify the effect of EMS
57	intervention on spatial distribution of neuromuscular activation in healthy young
58	adults. We hypothesized that EMS intervention induced improvement of muscle
59	strength and different distribution of muscle activity.
60	
61	Materials and Methods
62	Participants
63	A total of 32 males were randomly assigned to the intervention group ($n =$
64	18; age = 21–26 years; height = 171.1 \pm 4.4 cm; body mass = 65.1 \pm 7.7 kg) and
65	control group (n = 14; age = 21–27 years; height = 170.3 ± 4.9 cm; body mass =
66	63.8 ± 5.9 kg). Our previous study showed that compared with healthy young
67	male, healthy young female exhibited greater differences in the spatial
68	distribution patterns of muscle activation using multi-channel SEMG during

69	sustained isometric contraction [25]. This finding suggests that it is necessary to
70	differentiate between males and females when assessing muscle activation pattern
71	using multi-channel SEMG. Therefore, we included only male in the current study.
72	The exclusion criteria were patients with neuromuscular disease, cardiovascular
73	disease, and diabetes mellitus. All procedures were performed in accordance with
74	the Declaration of Helsinki and were approved by Hiroshima University's
75	Committee on Ethics in Research (C-273). All participants signed an informed
76	consent form and consented to the publication of this work. The following
77	measurements were made at baseline and 4 weeks for all participants: muscle
78	strength, muscle mass, and multi-channel SEMG measurement.
79	
80	Experimental design
81	Participants in the intervention group underwent EMS of the quadriceps
82	muscle of both legs for 4 weeks. Muscles were stimulated at a frequency of 20

83 Hz with a monophasic square-wave pulse of 250 μs duration using an EMS device

84	(AUTO TENS PRO Rehabili Unit, Homer Ion Co., Ltd., Tokyo, Japan). The
85	stimulation intensity was individually set to the maximal level without discomfort
86	in each subject. EMS was performed a lying position for 3 days per week. The
87	device was positioned at the trunk and mid-point of the femur and thigh for 20
88	min once per day. Participants were instructed not to actively contract muscles
89	during the stimulation. Those in the control group did not undergo any
90	intervention. Although the EMS intervention was applied to both legs, the
91	following test measurements were made on a single test leg (e.g., dominant side).
92	To estimate the effects of EMS, the following measurements were made at
93	baseline and four weeks: maximal voluntary knee extensor contraction strength,
94	vastus lateralis (VL) muscle spatial distribution of neuromuscular activation
95	during a submaximal isometric knee extension using multi-channel SEMG, and
96	muscle mass.

98 Maximal voluntary strength

99	All participants performed maximal voluntary contractions (MVCs) during
100	isometric knee extension at baseline and 4 weeks. Isometric knee extension was
101	performed using a Biodex system (Biodex System 4; Biodex Medical Systems,
102	Shirley, NY, USA). During contraction, both the hip and knee extension angles
103	were fixed at 90°. The MVC involved a gradual increase in knee extension torque
104	exerted by the knee extensor muscles from 0 to maximum over 3 s, with the
105	maximum torque held for 2 s [18, 26]. The participants performed at least two
106	MVC trials with a 2 minutes rest between trials, and a warm up for 10 min,
107	including indoor walking and lower limb stretching before MVC measurement.
108	The highest MVC torque was used to calculate the MVC torque and target torque
109	for sustained contractions. All participants performed submaximal isometric
110	contractions at 10%, 30%, 50%, and 70% MVC in a randomized order with a 2
111	minutes rest between trials. The contractions at 10% and 30% were sustained for
112	20 sec, 50% MVC was sustained for 15 sec, 70% MVC was sustained for 10 sec,
113	and the rising phase and decline phase for 5 sec[27]. This assessment was

114 performed at baseline and 4 weeks.

115

116	Measures	of muscle	mass
T T O	11100000000	of musere	mass

Measurements of muscle mass were performed using direct segmental 117 multifrequency bioelectrical impedance analysis (InBody S10, InBody Japan, 118 Tokyo, Japan). It is a validated method for estimating skeletal muscle mass 119 comparable to dual-energy X-ray absorptiometry [28]. All participants were 120 attached electrode on bilateral thumb, middle finger, and ankle, and asked to lie 121 down in supine position with straightened arms and legs whenever able and to lie 122 as still as possible during the measurements. This assessment was performed at 123 baseline and 4 weeks. 124

125

126 SEMG recording

127 The participant performed a maximum isometric knee extension contraction.
128 During the maximal contraction, multi-channel SEMG signals were detected from

129	the dominant VL muscle using a semi-disposable grid of 64 electrodes
130	(ELSCH064NM2; OT Bioelettronica, Torino, Italy) according to the same
131	procedure used in previous studies [18, 25, 29]. The grid consisted of 13 columns
132	and five rows of electrodes (diameter, 1 mm; inter-electrode distance, 8 mm in
133	each direction), with one missing electrode in the upper left corner. The
134	participants hair was removed, the skin was cleaned with alcohol, and the
135	electrode was attached to the skin with a bi-adhesive sheet (KITAD064NM2; OT
136	Bioelettronica) after applying conductive paste (Elefix Z-181BE; NIHON
137	KOHDEN, Tokyo, Japan) corresponding to the placement of the electrodes. The
138	center of the electrode grid was attached at the center of the line between the
139	superior lateral edge of the patella and the greater trochanter protuberance. The
140	columns of the electrode grid were placed parallel to the longitudinal axis of the
141	VL muscle. Participants were made a mark on the position where the electrode
142	was attached with an oil-based marker and asked to keep the mark until the end
143	of the study period. The site of the missing electrode was placed proximal to the

145 All procedures were performed by the same investigator.

146	Monopolar multi-channel SEMG signals were amplified by a factor of 1000,
147	sampled at 2048 Hz per channel, and converted to digital data using a 12-bit
148	analog-to-digital converter (EMG-USB2+; OT Bioelettronica). The recorded
149	monopolar multi-channel SEMG signals were off-line bandpass-filtered (10-500
150	Hz) and transferred to software for analysis (MATLAB 2018b; Math Works GK,
151	Natick, MA, USA). Bipolar multi-channel SEMG signals ($n = 59$) along the
152	columns were divided from the 64 electrodes. To calculate the root mean square
153	(RMS) of multi-channel SEMG signals, the signals were sampled at the
154	submaximal voluntary contraction task (e.g., 10%, 30%, 50%, and 70% MVC
155	task). To control for inter-participant variability, we normalized the RMS
156	measures to the values obtained at MVC.
157	To characterize the heterogeneity in the spatial multi-channel SEMG

158 potential distribution, we determined the modified entropy, coefficient of

159	variation (CoV), and the correlation coefficient of spatial RMS estimates. The
160	modified entropy of the spatial distribution of the SEMG amplitude was
161	calculated for 59 RMS values of single differential signals computed over a 1-s
162	period taken at the time of the contraction during the isometric sustained
163	contraction. Using the methods of Farina et al., [7] modified entropy was
164	calculated for 59 RMS measurements and average of 59 RMS measurement.
165	The CoV of spatial RMS estimates was defined as the quotient of the standard
166	deviation of the 59 RMS measurements and the average of 59 RMS measurement.
167	For submaximal voluntary contraction, modified entropy and CoV of RMS at 50%
168	of contraction time, and correlation coefficients between the spatial distribution
169	pattern of SEMG at 10% and 100% of contraction time were calculated. A
170	decrease in the modified entropy and an increase in the CoV of spatial RMS
171	estimates indicated increased heterogeneity in the spatial multi-channel SEMG
172	potential distribution within the electrode grid [30]. We calculated the percent
173	change in modified entropy, CoV and correlation coefficient measures from

baseline at 4 weeks.

175

176 Statistical analysis

Statistical analyses were performed using GraphPad Prism 8 (GraphPad 177 Software Inc, San Diego, CA, USA). The continuous data are presented as the 178 mean \pm standard deviation or the median (minimum, maximum). Before the 179 analysis, the normal distribution of data was confirmed using the Shapiro-Wilk 180 test. Age, height, body mass, and muscle mass were compared between the 181 intervention and control groups using unpaired *t*-tests. Statistical differences in 182 maximal knee extension torque, muscle mass, absolute value of RMS, modified 183 entropy, CoV of RMS, and correlation coefficient of submaximal voluntary 184 contraction tasks (e.g., 10%, 30%, 50%, and 70% MVC task) were analyzed using 185 two-way (group (intervention and control) vs. period (pre and post)) analysis of 186 variance (ANOVA) with repeated measure. The differences between each group 187 and/or period were analyzed by Bonferroni post hoc test. Pearson's correlation 188

189	coefficients were computed to assess bivariate correlations between the Δ knee
190	extension torque and Δ modified entropy, Δ CoV, and Δ correlation coefficient.
191	The correlation coefficients were qualitatively interpreted according to the
192	following thresholds: $0.2 < 0.4$, small; $0.4 < 0.7$, moderate; $0.7 < 0.9$, strong; 0.9
193	< 1.0, very strong. Two-tailed p values < 0.05 were considered statistically
194	significant.
195	
196	Results
197	The general characteristics of the participants are presented in Table 1.
198	There were no significant differences between groups in terms of the
199	anthropometric parameters.
200	The knee extension torque showed significant interaction between group
201	and period (F (1, 30) = 32.09, $p < 0.0001$). The intervention group showed
202	significantly higher knee extension torque in the post intervention than in the pre
203	intervention ($p < 0.0001$), but not in the control group (Fig.1A and B). The muscle 14

mass did not show a significant interaction between group and period (F (1, 30) 204 = 0.05526, p = 0.8158), and not a significant difference between pre and post 205 206 intervention in each group (Fig. 1C and D). RMS of 50% and 70% MVC task showed significant interaction between 207 group and period (F (1, 30) = 10.40, p = 0.0030, F (1, 30) = 25.45, p < 0.0001, 208 respectively). The intervention group showed significantly higher RMS value in 209 the post intervention than in the pre intervention of 50% and 70% MVC, but not 210 in the control group (p = 0.9908 and p = 0.9964 respectively, Fig. 2). 211 The modified entropy, CoV, and correlation coefficient of 50% MVC task 212 showed significant interaction between group and period (F (1, 30) = 9.784, p =213 0.0039, F (1, 30) = 20.13, p < 0.0001, F (1, 30) = 37.33, p < 0.0001, respectively). 214 The intervention group showed significantly higher CoV and lower modified 215 entropy and correlation coefficient (p < 0.0001, respectively) in the post 216 intervention than in the pre intervention of 50% MVC task (Fig. 3 G-I). The 217 modified entropy, CoV, and correlation coefficient of 70% MVC task did not show 218

significant interaction between group and period (F (1, 30) = 2.523, p = 0.1227, 219 F(1, 30) = 2.879, p = 0.1001, F(1, 30) = 3.253, p = 0.0813, respectively). Results 220 221 of two-way ANOVA, the modified entropy, CoV, and correlation coefficient of 70% MVC task showed significant group factor (F (1, 30) = 5.775, p = 0.0226, F 222 (1, 30) = 5.888, p = 0.0215, and F (1, 30) = 0.0016, respectively, Fig. 2 J-L). The 223 modified entropy, CoV, and correlation coefficient of 10% and 30 % MVC task 224 did not show significant interaction between group and period, and not significant 225 difference between pre and post intervention (Fig. 3 A-F). 226 Moderate correlations were observed between Δ knee extension torque and 227 Δ modified entropy (r = -0.5741, p = 0.0160 and r = -0.5612, p = 0.0191), Δ CoV 228 (r = 0.4904, p = 0.0457 and r = 0.6015, p = 0.0083), and Δ correlation coefficient 229 (r = -0.4913, p = 0.0452 and r = -0.7286, p = 0.0006) of 50% and 70% MVC task 230 (Fig. 4). The 10% and 30 % MVC task show did not correlation between Δ knee 231 extension torque and Δ modified entropy, Δ CoV, and Δ correlation coefficient for 232 each group. 233

235 Discussion

236 The present study examined the effects of EMS on muscle strength, spatial distribution of neuromuscular activation, and muscle mass in healthy young adults. 237 The principal results of the present study were that EMS of the lower limbs 238 increased muscle strength, and led to more inhomogeneity in spatial muscle 239 distribution patterns at 50% and 70% MVC tasks. 240 The results of the present study showed that knee extensor torque was 241 significantly increased in the intervention group. On the other hand, muscle mass 242 did not change by EMS for 4 weeks in the intervention group. It is widely known 243 that EMS intervention can improve muscle performance [13-17]. Furthermore, 244 several studies reported that increase in the muscle strength and muscle thickness 245 occurred after EMS intervention [12, 31, 32]. In accordance with the results of 246 the present study, which compared a control group with an intervention group 247 show significantly increased knee extension torque. However, we did not observe 248

249	the change of muscle mass in the intervention group. We considered that the
250	intervention period is a key factor. Intervention periods of previous studies were
251	set longer than the present study (e.g., 6 weeks [15], 16 weeks [12], and 20 weeks
252	[32]). Furthermore, Singer and Breidhal demonstrated that there were no
253	significant gross morphological changes in the image of computed tomography,
254	following a four-week program of EMS intervention [33]. Therefore, our results
255	suggest early neural adaptations enhanced muscle strength by EMS intervention.
256	Strength performance depends not only on the quantity and quality of the involved
257	muscles, but also upon the ability of the nervous system to appropriately activate
258	the muscles [34]. Consequently, it is considered that muscle strength increased
259	through neural adaptations without enhanced muscle mass.
260	In addition to the increases in knee extension torque, the results of the
261	present study showed that spatial muscle distribution patterns of 50% and 70%
262	MVC tasks were changed by EMS interventions. We used modified entropy and
263	CoV of spatial RMS estimates to assess the spatial distribution of neuromuscular

264	activation. A decrease in the modified entropy, correlation coefficient and an
265	increase in the CoV of spatial RMS estimates is consistent with increased
266	heterogeneity in the spatial multi-channel SEMG potential distribution within the
267	electrode grid [30]. The spatial distribution of SEMG is altered by contraction
268	levels or fatigue during isometric contraction [7, 35]. Results of the present study
269	showed significantly higher CoV and lower modified entropy and correlation
270	coefficient ($p < 0.0001$, respectively) in the post intervention than in the pre
271	intervention of 50% and 70% MVC tasks in the intervention group. Furthermore,
272	our results showed that significant correlation between Δ knee extension torque
273	and EMG variables (modified entropy, CoV of RMS, and correlation coefficient)
274	of 50% and 70% MVC tasks. In general, according to the size principle, the
275	progressive recruitment of small, typically slow motor units followed in order of
276	increasing size to the large [22, 23]. Many previous studies also reported that
277	EMS intervention induced non-physiological recruitment order and synchronous
278	discharge of motor units [19-21]. Chasiotis et al., reported that EMS induced more

279 activity of metabolic function (glycogen phosphorylase, high energy phosphates, and lactates) on fast twitch muscle fiber than that on slow twitch muscle fiber 280 281 [36]. Walters et al., reported that EMS induced more increase cross-sectional area on fast muscle fiber than on slow muscle fiber [37]. These findings suggested that 282 EMS is likely to affect fast muscle fibers. Therefore, it is considered that the 283 change of muscle activity distribution pattern occurred in the 50% and/or 70% 284 MVC tasks which requires the activity of fast muscle fiber, as compared with 10% 285 and/or 30% MVC tasks. 286 The present study has several limitations. First, participants performed 287

EMS intervention only 4 weeks. Previous study showed that significant strength changes can be produced with 3–5 weeks of training without significant morphological changes such as muscular hypertrophy [1]. Further study needs a longer intervention period than 5 weeks. Second, the present study assessed only isometric muscle strength as a physical performance. Therefore, it is considered that it is possible to clarify the impact of EMS intervention on physical

294	performance in the future study by adding dynamic evaluation (e.g., jump and
295	balance assessments). Third, the present study examined only the SEMG method.
296	SEMG amplitude estimates provide only crude estimates of MU recruitment [38].
297	Therefore, we were unable to evaluate MU recruitment properties in detail.
298	Several recent studies predicted MU recruitment from multi-channel SEMG using
299	convolution kernel compensation [27, 39]. Further studies using additional
300	analysis methods are needed to elucidate the detailed mechanisms underlying the
301	effect of EMS intervention on MU recruitment patterns.
302	
303	Conclusions
304	We investigated that effects of EMS on muscle strength and activation
305	patterns in young adults. The results of the present study indicate that EMS
306	intervention induced enhance muscle strength and different distribution of muscle
307	activity at 50% and 70% MVC tasks.

309 Conflicts of Interest

- 310 The authors declare no conflict of interest and that no companies or
- 311 manufacturers will benefit from the results of this study.

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426	Figure	legends
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427 Fig. 1

Results of knee extension torque and muscle mass at pre and post intervention in 428 each group. 429 430 Fig. 2 431 Results of root mean square of all channels during submaximal voluntary 432 contraction (10%, 30%, 50%, and 70%). Data are presented as mean \pm SD. * p <433 0.05. 434 435 Fig. 3 436 Results of modified entropy, coefficient of variation, and correlation coefficient 437 of submaximal voluntary contraction at pre and post intervention in each group. 438 * *p* < 0.05. 439

- 441 Fig. 4
- 442 Correlation coefficient between Δ knee extension torque and Δ modified entropy,
- 443 Δ coefficient of variation, and Δ correlation coefficient of 50% (A-C) and 70%
- 444 (D-F) maximal voluntary contraction task in the intervention group.



0.25 0.25 * Coot mean square (mV) Root mean square (mV) 0.20-0.15-0.15-0.10-0.05-0.00 0.00

10%

30%

50%

Control group

70%

Pre 🗖 Post

10%

30%

50%

Intervention group

70%



