

1 **Title:** Ischemic Conditioning Increases Strength and Volitional Activation of Paretic
2 Muscle in Chronic Stroke: A Pilot Study

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30 **Abstract**

31 Ischemic conditioning (IC) on the arm or leg has emerged as an intervention to improve
32 strength and performance in healthy populations, but the effects on neurologic
33 populations are unknown. The purpose of this study was to quantify the effects of a
34 single session of IC on knee extensor strength and muscle activation in chronic stroke
35 survivors. Maximal knee extensor torque measurements and surface EMG were
36 quantified in 10 chronic stroke survivors (>1 year post-stroke) with hemiparesis before
37 and after a single session of IC or Sham on the paretic leg. IC consisted of five minutes
38 of compression with a proximal thigh cuff (inflation pressure = 225 mmHg for IC or 25
39 mmHg for Sham) followed by five minutes of rest. This was repeated five times.
40 Maximal knee extensor strength, EMG magnitude, and motor unit firing behavior were
41 measured before and immediately after IC or Sham. IC increased paretic leg strength
42 by 10.6 ± 8.5 Nm while no difference was observed in the Sham group (change in Sham
43 = 1.3 ± 2.9 Nm; $p = 0.001$ IC vs. Sham). IC-induced increases in strength were
44 accompanied by a $31 \pm 15\%$ increase in the magnitude of muscle EMG during maximal
45 contractions and a 5% decrease in motor unit recruitment thresholds during sub-
46 maximal contractions. Individuals who had the most asymmetry in strength between
47 their paretic and non-paretic legs had the largest increases in strength ($r^2 = 0.54$). This
48 study provides evidence that a single session of IC can increase strength through
49 improved muscle activation in chronic stroke survivors.

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51 **New and Noteworthy:** Current rehabilitation strategies for chronic stroke survivors do
52 not optimally activate paretic muscle, and this limits potential strength gains. Ischemic
53 conditioning of a limb has emerged as an effective strategy to improve muscle
54 performance in healthy individuals, but has never been tested in neurologic populations.
55 In this study we show that ischemic conditioning on the paretic leg of chronic stroke
56 survivors can increase leg strength and muscle activation while reducing motor unit
57 recruitment thresholds.

58 **Key Words:** Stroke Rehabilitation, Ischemic Conditioning, Muscle Strength,
59 Electromyography
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72 **Introduction**

73 The aim of this study was to quantify gains in paretic muscle strength and muscle
74 activation due to ischemic conditioning. Diminished ability to generate paretic muscle
75 force contributes to long term motor deficits and disability in chronic stroke survivors (6,
76 26, 37). Fundamentally, damage to cortical structures limits a stroke survivor's ability to
77 optimally activate paretic motoneuron pools, thereby reducing force development (21,
78 27, 28), even during brief maximal efforts. Stroke rehabilitation interventions are
79 currently not optimized because stroke survivors are unable to adequately activate the
80 paretic muscle, and functional gains in response to traditional therapies have been
81 moderate at best (32, 35, 43). Interventions that optimize residual paretic muscle
82 activation and strength are needed to achieve greater functional gains.

83 In healthy populations, ischemic conditioning (IC) has emerged as a
84 neuroadaptive technique which results in improved motor performance. IC was first
85 described in 1986 as a vascular stimulus to protect vital organs from ischemic injury
86 (36). Subsequent studies in humans have shown that both local IC (performed on
87 tissue of interest) and remote IC (performed on a remote limb) improves motor learning,
88 muscle performance and delays muscle fatigue. Specifically, in healthy individuals,
89 brief, repeated 5 minute bouts of limb ischemia (using a blood pressure cuff inflated to
90 225 mmHg on the arm or leg) improve stability on a tilted platform balance task (8), task
91 duration during handgrip exercise (4), 5 km running time (3), and maximal power output
92 (10). In these studies, IC was shown to enhance force generation and muscle activation
93 and the authors propose a potential mechanism of engagement of autonomic centers in
94 the brainstem sensitive to ischemia and exercise. Given the positive effects on motor

95 output in individuals with intact nervous systems and optimal motor function, it is likely
96 that IC may have a larger neuroadaptive effect on clinical populations with impaired
97 neural activation of muscle and diminished motor function. At this time, the effects of
98 ischemic conditioning on motor recovery in patient populations such as stroke are
99 unknown and quantifying the effects may lead to a new treatment strategy to optimize
100 strength gains and function.

101 In this pilot study, we quantified the effects and tolerance of a single session of
102 IC on paretic leg strength and muscle activation in chronic stroke survivors. We
103 hypothesize that IC will be well-tolerated, increase the magnitude of the maximal
104 voluntary contraction of the knee extensor muscles of the paretic leg, and that this
105 increase will be accompanied by increased *vastus lateralis* activity as measured by
106 electromyography (EMG). Interpretive measures of resting twitch responses were made
107 to understand the effects of IC on muscle contractile properties.

108 **Methods**

109 *Subjects*

110 This study was a single-blinded, randomized, controlled trial with paired analysis.
111 All subjects were studied twice with a minimum of one week between study sessions.
112 All activities in this study were approved by the Institutional Review Boards of Marquette
113 University and the Medical College of Wisconsin (PRO19103). All participants gave
114 written informed consent prior to study participation. Ten participants with chronic stroke
115 (≥ 1 year post-stroke) participated in this study (see Table 1 for participant
116 characteristics). Stroke subject inclusion criteria were: 1) history of a single, unilateral

117 stroke and 2) residual hemiparesis. Stroke subject exclusion criteria: 1) history of
118 multiple strokes, 2) brainstem stroke, 3) any uncontrolled medical condition, 4) lower
119 extremity contractures, 5) uncontrolled hypertension, 6) inability to follow 2-3 step
120 commands, 7) deep vein thrombosis, 8) peripheral arterial grafts in the lower extremity,
121 and 9) any condition in which tissue ischemia is contraindicated.

122 *Torque Measurements*

123 Participants were positioned in a dynamometer chair (Biodex Medical Systems,
124 Inc, Shirley New York) with their test knee and hip at 90° of flexion. Subjects had a belt
125 placed around their trunk and waist to reduce movement during knee extensor
126 contractions. Knee extension torque was sampled at 2048 Hz and acquired by an EMG-
127 USB2+ amplifier (256-channel regular plus 16-auxiliary channels, OT Bioelettronica,
128 Turin, Italy) and acquired using the OT Biolab software.

129 *Surface Electromyography Measurements*

130 Surface EMGs were obtained using a 64 channel 2-D electrode array (13 rows, 5
131 columns). A double-sided adhesive sticker designed for and compatible with the array
132 was placed over the array. The holes within the adhesive sticker were filled with a
133 conductive electrode paste (Ten20, Weaver and Company, Aurora, Co). The array was
134 placed over the belly of the *vastus lateralis*, midway between the patella and the greater
135 trochanter, after rubbing the subject's skin with an alcohol swab to remove superficial
136 dead skin. The signals for each channel were differentially amplified between 1000 and
137 5000 v/v (subject dependent) and bandpass filtered between 10 and 500 Hz using the

138 EMG-USB2+ amplifier. The signals were sampled at 2048 Hz and acquired with the OT
139 Biolab software throughout the duration of the experimental protocol.

140 *Ischemic Conditioning*

141 IC treatments were performed in accordance with other studies which have used
142 IC as an intervention (31, 39, 45). Briefly, in a supine position, a rapid inflation cuff
143 (Hokanson SC12 thigh cuff) was placed around the proximal thigh and inflated to 225
144 mmHg for five minutes, then released for a five minute recovery period, and five cycles
145 of inflation/recovery were performed. For the IC Sham, the cuff was inflated to 25
146 mmHg. This level of inflation was chosen because participants still perceive the cuff
147 tightness, however the inflation pressure is not high enough to occlude arterial blood
148 flow or venous return. Subjects were blinded to the purpose of the different cuff inflation
149 pressures. A minimum of one week between test sessions was given, and the order of
150 IC vs. Sham IC was randomized.

151 *Electrical Stimulation*

152 In a subset of six participants, resting twitch torque responses were elicited to
153 quantify the effects of IC on muscle contractile properties as done in other studies (24,
154 49, 50). Following each MVC, a brief constant-current stimulator (Digitimer DS7AH,
155 Welwyn Garden City, UK) delivered a rectangular pulse of 100 μ s duration with
156 maximum amplitude of 400 V, which was used to percutaneously stimulate the
157 quadriceps muscle. The stimulation intensity (200 mA to 500 mA) was set at 20% above
158 the level required to produce a maximal resting twitch amplitude.

159 *Experimental Protocol*

160 Subjects first performed baseline isometric maximum voluntary contractions
161 (MVCs) of the knee extensor muscles (See Fig. 1A for protocol summary). Subjects
162 were given visual and verbal encouragement. MVC efforts were repeated until there
163 was less than a 5% difference in torque between two subsequent MVCs. A minimum of
164 five MVCs were performed. At least 1 min rest was given between subsequent MVCs.
165 Resting twitch responses were elicited following each MVC. Next, subjects performed a
166 submaximal ramp and hold isometric contraction equal to 40% of their MVC (4 second
167 graded contraction, 5 second hold at 40% of MVC, 4 second graded relaxation) with
168 visual feedback. Subjects then underwent either the IC or IC sham protocol.
169 Immediately following completion of the IC or IC-Sham protocol (within 10 minutes),
170 subjects repeated the MVC, resting twitch, and sub-maximal ramp and hold contractions
171 using identical positioning within the dynamometer chair. Surface EMG measurements
172 of the *vastus lateralis* were made continuously throughout the pre and post motor
173 testing. An example of MVC torque traces from a single subject before and after either
174 IC Sham or IC (see below) are shown in Figs. 1B and 1C, respectively.

175 *Data Processing*

176 Knee extensor torque signals were zero phased lowpass filtered at 15 Hz using a
177 2nd order Butterworth filter prior to analysis and processed using custom Matlab
178 (MathWorks, Natick, MA) scripts. Peak torque was calculated for each MVC trial and the
179 resting twitch responses. To determine how IC affected force steadiness, the knee
180 extensor torque coefficient of variation ($((\text{standard deviation torque}/\text{mean torque}) * 100)$)
181 was determined for a 4 second window during the hold portion of the ramp contraction
182 as previously described (25).

183 *Surface Electromyography*

184 Single motor unit action potential trains during the sub-maximal ramp and hold
185 contractions were detected with a multichannel blind source separation using
186 convolution kernel compensation (CKC) for the high density surface EMG signal
187 decomposition as described and validated previously (22, 38). Individual motor units
188 were tracked between the pre and post measurements and mean firing rates during a 4
189 second window in the hold phase of the sub-maximal contraction were calculated as
190 well as the torque at which each motor unit was recruited and de-recruited.

191 For global surface EMG measurements, the mean root mean square of the EMG
192 for each of the channels during the 4 second hold of the MVC and during the 4 second
193 window during the hold portion of the ramp contraction was calculated using a sliding
194 window of 200 ms. To understand how IC effects the variability of the EMG measurement
195 from each channel (48), the mean coefficient of variation (coefficient of variation =
196 standard deviation of RMS/mean RMS*100) was calculated during the 4 second window
197 of the MVC. A decrease in coefficient of variation would indicate that the EMG activity is
198 more consistent (less variable) during the hold portion of the MVC irrespective of
199 magnitude. In order to understand how IC effects the homogeneity of the spatial
200 activation of the muscle, modified entropy was calculated
201 ($Entropy = -\sum_{i=1}^{59} p^2(i) \log_2 p^2(i)$) (where $p^2(i)$ is the square of the RMS value at
202 electrode i normalized by the summation of the squares of all RMS values for each
203 channel). Modified entropy is the normalized power of the EMG signal across the array
204 and reflects the homogeneity of the muscle activity. Higher values occur if the energy
205 were the same across all channels – i.e. if the muscle activity is very homogenous (14,

206 30). Measures of coefficient of variation and entropy provide insight into how the
207 nervous system is spatially activating the paretic muscle irrespective of magnitude.

208 *Statistical Analyses*

209 Separate, two way repeated measures ANOVAs were performed on the following
210 variables: MVCs and resting twitch responses amplitudes. Main effects of time (Pre,
211 Post) and condition (IC, IC Sham) and interaction effects of time x condition were
212 determined. A Bonferroni post-hoc test was used to test for differences between
213 individual means. Because the coefficient of variation data were not normally
214 distributed, a Friedman's Test was performed. Linear regression and goodness of fit
215 analysis was performed to determine if there was a correlation between the percent
216 increase in paretic leg strength following IC and baseline motor function (assessed as
217 either symmetry of leg strength, walking speed, or Lower Extremity Fugl-Meyer score).

218 Because there was no detected effect of the Sham IC condition on torque
219 generation, EMG measurements were only evaluated for the IC condition. Separate
220 paired t-tests were performed to detect pre- and post-IC differences on the following
221 EMG variables: coefficient of variation, force recruitment threshold, modified entropy,
222 and magnitude of the RMS. All statistical tests were performed using an alpha level of
223 0.05 for significance. Data are reported as the mean \pm standard deviation.

224

225 **Results**

226 Knee extensor strength and muscle activation were measured in ten individuals
227 with chronic stroke before and after a single session of IC or IC sham. Consistent with
228 previous studies performed in chronic stroke subjects from our group (13) and others

229 (34), the paretic leg was weaker than the non-paretic leg (paretic vs. non-paretic MVC:
230 88.8 ± 50.2 Nm vs. 139.0 ± 78.6 Nm, respectively; $p = 0.012$, paired t-test). Following
231 IC, 9/10 individuals had increased strength in their paretic leg knee extensor muscles,
232 with an observed mean increase in MVC of 10.6 ± 8.5 Nm (Fig. 2A; $p=0.001$ vs. pre-IC,
233 two-way repeated measures ANOVA). No difference in knee extensor MVC was
234 observed after the Sham IC treatment (mean difference post Sham IC: 1.3 ± 2.9 Nm;
235 $p=0.65$; Fig. 2B). Relative to each individual's baseline strength, a 16.1 ± 14.5 %
236 increase in strength was observed in the IC group vs. a relative change in strength of -
237 0.04 ± 11.76 % in the Sham IC group ($p = 0.04$ IC vs. Sham IC, paired t-test; Fig. 2C).
238 Pre-test MVCs were similar for all subjects between both the Sham and IC treatment
239 groups ($p = 0.79$, paired t-test), demonstrating the test/re-test reliability of the MVC
240 measurement across multiple sessions.

241 There was a significant positive correlation between baseline asymmetry in knee
242 extensor strength and percent change in MVC following IC, whereby those individuals
243 whose paretic leg had the greatest difference in strength compared to their non-paretic
244 leg had the greatest relative increase in knee extensor MVC following IC (Fig. 3A; $p =$
245 0.014 ; $R^2 = 0.55$). Subjects who had the lowest Lower Extremity Fugl Meyer score (a
246 performance-based index to assess the sensorimotor impairment in stroke survivors)
247 also showed the greatest increase in knee extensor strength following IC (Fig. 3B; $p =$
248 0.008 ; $R^2 = 0.61$). Finally, there was a moderate correlation between baseline self-
249 selected walking speed and improvement following IC whereby subjects who walked the
250 slowest also tended to show the largest IC-induced improvements in knee extensor
251 MVC (Fig 3C, $R^2 = 0.33$), however this result was not statistically significant ($p = 0.08$).

252 With respect to the magnitude of muscle activation, there was a significant
253 increase in the root mean square (RMS) magnitude of vastus lateralis EMG during
254 MVCs following IC (Fig. 4A; $p=0.01$; paired t-test), which resulted in an overall
255 $30.7\pm 15\%$ increase in total EMG signal. Fig. 4B, shows a single subject example of the
256 change in EMG RMS between pre and post MVCs. Modified entropy increased from
257 4.19 ± 0.9 to 5.12 ± 0.3 ($p=0.02$; paired t-test; Fig. 4C) which reflects an increase in the
258 homogeneity of the spatial EMG potential distribution. Consistent with this, the
259 coefficient of variation of the EMG RMS decreased from $19.4 \pm 9.5\%$ to $10.6 \pm 8.2\%$
260 ($p=0.02$; paired t-test; Fig. 4D) which reflects an overall decrease in the variability in
261 individual EMG channels.

262 During the 40% submaximal ramp and hold contractions, there was a decrease
263 in the motor unit force recruitment thresholds from $25.0 \pm 1.7 \%$ to $21.8 \pm 1.7 \%$ of the
264 MVC (See single subject example Fig. 5A, Fig. 5B; $p < 0.01$; paired t-test). Sub-maximal
265 torque regulation during the ramp and hold contractions was not diminished by IC as
266 there was no significant change in the coefficient of variation of the torque trace in
267 response to the IC or IC sham (IC pre = $4.6\% \pm 2.4 \%$ vs post = $2.8 \pm 1.5 \%$; IC sham
268 pre = $4.4 \% \pm$ vs post= $3.9 \pm 2.4\%$). The coefficient of variation tended to decrease but
269 the effect was not significant ($p = 0.06$).

270 Finally, as shown in Fig. 6, the mean amplitudes of the resting twitch torque
271 responses were not different pre-post for either the IC or IC sham condition (IC pre-
272 post: 37 ± 13 Nm vs. 35 ± 13 Nm, respectively; Sham IC pre-post: 28 ± 14 Nm vs. $31 \pm$
273 13 Nm, respectively; $p=0.60$, two-way RM ANOVA), indicating that IC had no effect on
274 muscle contractile properties.

275 **Discussion**

276 There are three novel findings from this pilot study. First, our data support the
277 hypothesis that a single session of IC is a feasible, well-tolerated intervention that can
278 increase strength in the paretic leg of chronic stroke survivors. Second, increases in
279 EMG magnitude and unchanged resting twitch responses to electrical stimulation of the
280 muscle indicate that the increased strength is due to improved neural activation of the
281 muscle as opposed to changes in muscle contractile properties. Finally, we show a
282 positive relationship between the response to IC and baseline physical function,
283 whereby individuals whose lower extremity motor function is most affected by the stroke
284 show the largest improvement in leg strength following IC. This finding provides insight
285 into which individuals may benefit the most from IC intervention.

286 Very recently, two studies have shown that repetitive, remote IC performed on
287 the arm prevents recurrence of stroke (33) and that daily remote IC over the course of
288 one year slows cognitive decline in patients with cerebral small-vessel disease–related
289 mild cognitive impairment (47). We present the first study to our knowledge to apply IC
290 as an intervention to improve motor function post-stroke - specifically increased
291 maximal force generating capabilities in the paretic leg. As other groups have shown,
292 IC can improve motor performance by 2.5 – 11.2% in healthy subjects (2, 5, 45), who
293 presumably have optimal neural activation of their skeletal muscle and thus have a
294 ceiling effect when it comes to IC-induced improvements in motor function. In this study
295 we report, on average, an increase in strength of 16% in the paretic leg of chronic
296 stroke survivors. Furthermore, we show that those subjects with the largest degree of
297 motor deficits had the largest improvement (Fig. 3). Together, these findings suggest

298 that subjects who have the greatest impairments will benefit the most from IC, and that
299 IC has the potential to produce large strength gains in neurologic populations.

300 Although multifactorial (7), the neural mechanisms of IC have been linked to the
301 engagement of the autonomic nervous system. For example, in animal models, the
302 cardioprotective effects of IC can be abolished with spinal cord section, bilateral
303 vagotomy or blockade of muscarinic cholinergic receptors (12). One mechanism by
304 which IC is believed to act centrally is through stimulation of muscle afferents sensitive
305 to ischemia (group III and IV afferents) which in turn engage brainstem centers that
306 release neuromodulators such as serotonin and norepinephrine (42, 44). Importantly,
307 these neuromodulators are known to increase the excitability of spinal motoneurons (18,
308 19, 42). Moreover, there is evidence that the group III and IV pathways in the paretic leg
309 are hyperexcitable post stroke (20), which may amplify the potential response to IC in
310 this patient population. Thus, in individuals without stroke, IC enhances the gain of
311 descending excitatory commands by increasing the excitability of motoneuron pools,
312 thereby improving torque output. Our data are consistent with this mechanism, as
313 maximum voluntary contractions post-IC resulted in increased torque generation and
314 global EMG magnitude. Further, the increased homogeneity (48) of the EMG signal is
315 consistent with a more coordinated and consistent activation of the paretic muscle.
316 Finally, the decrease in the force recruitment thresholds of the matched motor units is
317 also consistent with increased excitability of the motoneuron pools (17). Thus, it is
318 plausible that post-stroke the benefits of IC may be larger as compared to
319 neurologically intact individuals given the decreased volitional ability to fully activate
320 paretic muscle.

321 Volitional engagement of the nervous system during strength training (as
322 opposed to electrical stimulation of the muscle) is important for the neural adaptations
323 that precede muscle hypertrophy and facilitate motor learning (1, 15). Recently, in
324 persons with spinal cord injury, transient hypoxia has been used to increase the
325 excitability of the nervous system and increase affected muscle activation for
326 therapeutic training (9, 16, 29, 46). Similar to IC, investigators attribute the priming
327 effects of hypoxia to engagement of neuromodulatory centers in the brainstem (11, 40)
328 and forebrain (23). Although intermittent hypoxia may be advantageous for some, IC
329 might be a strong alternative because it is non-invasive, cost effective, and easier to
330 implement in the clinic and in the community because it requires only inflation of a cuff
331 similar to a blood pressure cuff.

332 *Study Limitations and Future Directions*

333 We recognize several study limitations and propose future study directions based
334 on our pilot study. First, we recognize the small sample size of 10 subjects as a study
335 limitation, but our data clearly show that IC is well tolerated in stroke subjects and that it
336 caused an improvement in knee extensor strength in 9/10 of our test subjects. A
337 second limitation is that we did not test the effects of IC on the non-paretic leg, or the
338 remote effects of IC, (i.e. to perform IC on the non-paretic limb and test the paretic limb)
339 and recognize these as important future study directions. Third, we did not test how
340 long the positive effects of IC are sustained. Decades of research on the
341 cardioprotective effects of IC indicate there is both a short (0-24 hours) and long (24-48
342 hours) phase of IC-induced cardioprotection, and these phases are mediated by
343 different mechanisms (41). Future studies examining the time-course of IC-induced

344 improvements on motor function are necessary to determine how long the
345 improvements in strength last, and whether there are different mechanisms mediating
346 the improvements. We also did not test the effects of multiple sessions of IC to
347 determine if there is an additive effect. Finally, we only performed our study in
348 individuals with chronic stroke. Given that, on average, we saw an increase in strength
349 of 16% following IC in subjects who were many years post stroke, future studies
350 examining the effects of IC on subacute stroke patients (days to weeks post-stroke) who
351 are in a highly plastic recovery stage and undergoing physical therapy are warranted.

352 As our data show, IC is effective at increasing paretic muscle activation in stroke
353 survivors. There are several important, non-trivial advantages of IC as an interventional
354 adjunct to stroke rehabilitation: 1) a wide range of patients can benefit because the
355 technique does not require high levels of physical activity or function, 2) IC is non-
356 invasive, well-tolerated, and safe in cardiovascular populations, and 3) IC can be
357 accomplished with inexpensive equipment at home or in the clinic in less than 60
358 minutes. We propose that IC has the potential to be an ideal adjunct to physical therapy
359 in patients with hemiplegia because it “primes” the nervous system to more fully activate
360 the paretic muscle during exercise and is clinically feasible.

361

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370

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374 SM performed experiments; AH, FN, JN, MD, SM analyzed data; AH, BS, DG, FN, MD,
375 SM interpreted results of experiments; AH, MD, SM prepared figures; AH, MD drafted
376 manuscript; AH, BS, DG, MD edited and revised manuscript; AH, BS, DG, FN, JN, MD,
377 SM approved final version of manuscript.

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554 **Figure Legends**

555 **Figure 1.** (A) Protocol summary of the ischemic conditioning (IC) protocol. Subjects
556 performed a series of isometric maximum voluntary contractions (MVC) of the knee
557 extensor muscles followed by a submaximal contraction equal to 40% of their maximum
558 using a Biodex dynamometer. After the initial contractions were completed, the
559 subjects moved to a bed where the ischemic conditioning protocol was performed. The
560 subjects laid in the supine position and a blood pressure cuff was placed around the
561 proximal thigh of paretic leg and inflated to either 225 mmHg (IC condition) or 25 mmHg
562 (Sham condition) for 5 minutes. After 5 minutes of inflation, the cuff was deflated for 5
563 minutes, and this was repeated for 5 cycles. Following the IC or Sham protocol,
564 subjects were placed back in the Biodex dynamometer and knee extensor MVCs and
565 submaximal contractions were repeated. Representative torque traces of an MVC from
566 a single subject before and after the IC and Sham conditions are shown in panels B and
567 C, respectively. Note the increase in MVC magnitude for the IC condition.

568 **Figure 2.** Individual knee extensor maximum voluntary contraction (MVC) responses of
569 the paretic leg before and after either Ischemic Conditioning (IC) or Sham treatment.
570 Individuals in the IC group demonstrated an increase in knee extensor MVC following IC
571 (panel A; $p < 0.05$; two-way repeated measures ANOVA), and no difference following
572 Sham treatment (panel B; $p > 0.05$). On average, individuals in the IC group
573 demonstrated a $16.1 \pm 14.5\%$ increase in knee extensor strength following IC (panel C;
574 $p < 0.05$).

575 **Figure 3.** Changes in knee extensor strength following IC as a function of leg
576 impairment. There was a strong correlation between asymmetry in MVC magnitude

577 between the paretic and non-paretic leg and percent change in MVC in response to IC
578 (Panel A). Subjects who showed a greater degree of asymmetry in knee extensor
579 strength between their paretic and non-paretic legs showed a greater improvement in
580 paretic leg strength following IC ($R^2 = 0.55$; $p = 0.014$). Subjects who had the lowest
581 Lower Extremity Fugl Meyer Score (panel B) also had the largest improvements in knee
582 extensor strength following IC (Panel B, $R^2 = 0.61$; $p = 0.008$). There was a moderate
583 correlation between self-selected walking speed and gains in strength following IC
584 whereby subjects who walked the slowest tended to have the largest increases in
585 strength. (Panel C; $R^2 = 0.33$; $p = 0.08$).

586 **Figure 4.** Changes in vastus lateralis EMG measurements that accompanied IC-
587 induced increases in knee extensor torque. (A) The average root mean square of the
588 EMG signal during MVCs was increased following IC ($p = 0.01$; paired t-test). (B) A
589 single subject spatial activation map of the change in the RMS of the EMG across the
590 EMG array, pre to post IC during the MVCs. Coloring reflects the degree of change
591 where red indicates the largest increases and blue indicates decreases in the RMS of
592 the EMG. (C) Modified entropy increased following IC (panel C; $p = 0.02$; paired t-test).
593 This indicates increased homogeneity in the potential distribution across the array. (D)
594 There was an IC-induced decrease in the average coefficient of variation of the EMG
595 signal from each channel in the array ($p=0.02$, t-test).

596 **Figure 5.** Motor unit firing behavior and recruitment during the sub-maximal ramp and
597 hold task. (A) Single subject raster plot of incidences of action potentials superimposed
598 on the torque generated during the ramp and hold pre (left) and post (right) IC. Each
599 row is a separate motor unit matched between time points. (B) Average force

600 recruitment thresholds decreased following IC reflecting increased excitability of the
601 motoneuron pools ($p < 0.01$, paired t-test).

602 **Figure 6.** Resting twitch torque values following maximal electrical stimulation of the
603 knee extensor muscles. There was no difference in the knee extensor resting twitch
604 torque in either the IC or Sham group in response to electrical stimulation of the muscle
605 at a level 20% above the threshold required to elicit a maximal twitch response ($p =$
606 0.60).

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620 **Table 1. Characteristics of all Subjects.**

Characteristic	<i>n</i> =10
Sex	
Male (<i>n</i>)	4
Female (<i>n</i>)	6
Age (yrs)	60±12
Height (cm)	168±11
Weight (kg)	78±16
Body Mass Index (kg/m ²)	27±4
Time Since Stroke (yrs)	16±9
Type of Stroke	
Ischemic (<i>n</i>)	7
Hemorrhagic (<i>n</i>)	3
Affected Side	
Left (<i>n</i>)	6
Right (<i>n</i>)	4
Lower Extremity Fugl-Meyer Score (0-34)	26±6
Physical Activity (MET-h/week)	14±7
Self-Selected Walking Speed (m/s)	0.81±0.35

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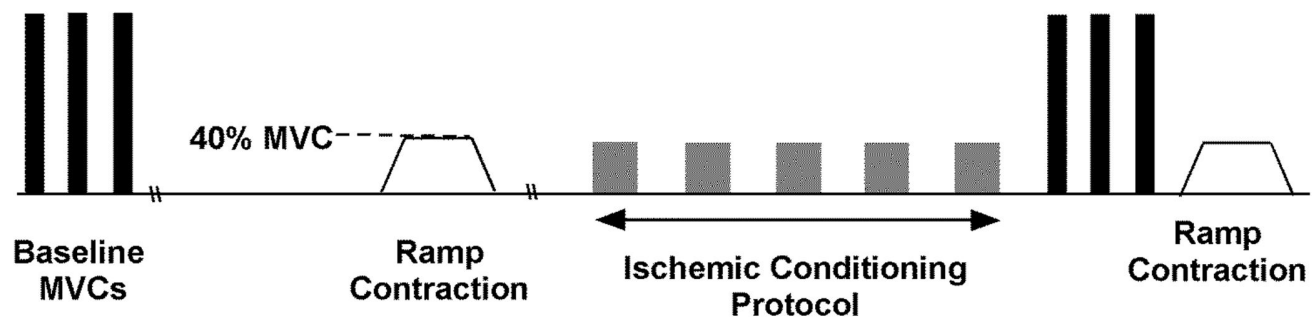
622 All values are expressed as number (*n*) or mean ± SD. MET, Metabolic Equivalent of

623 Task

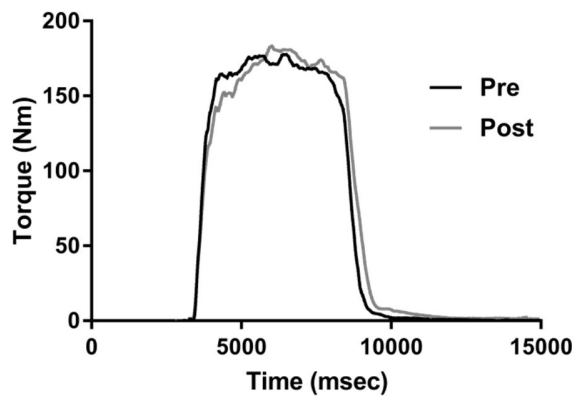
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FIGURE 1

A.



B.



C.

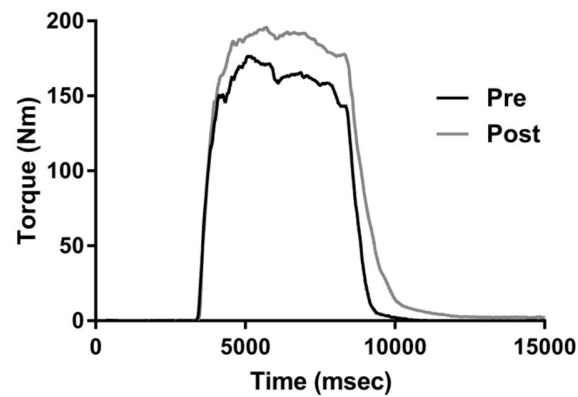


FIGURE 2

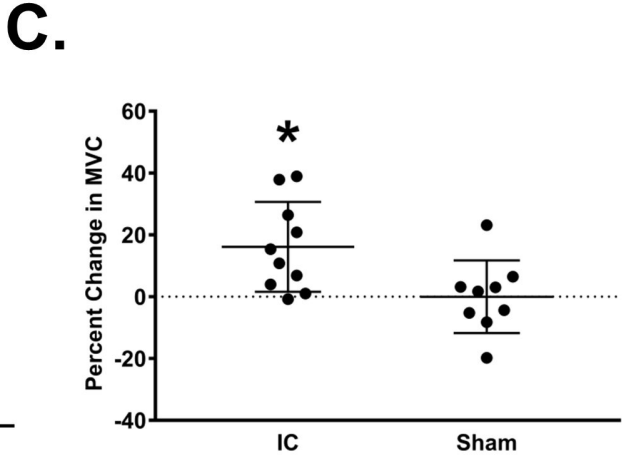
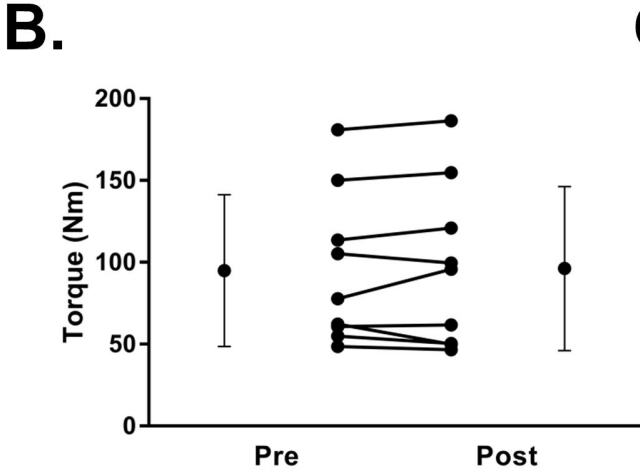
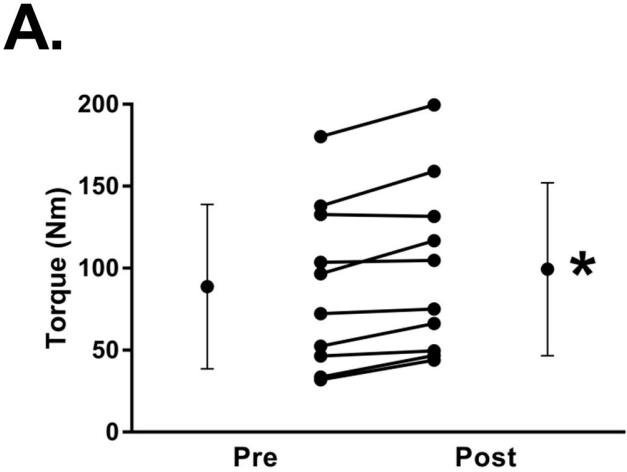
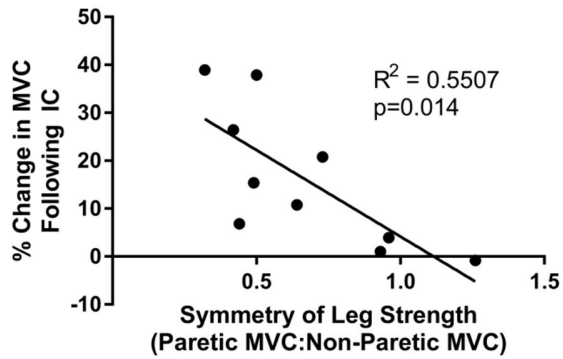
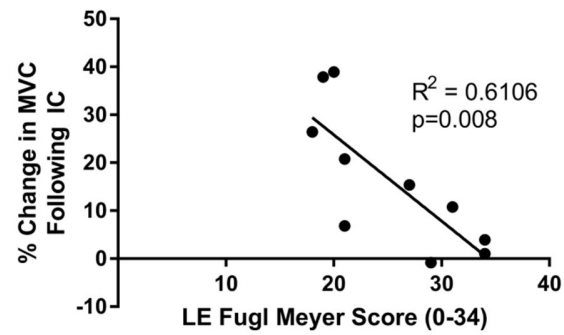


FIGURE 3

A.



B.



C.

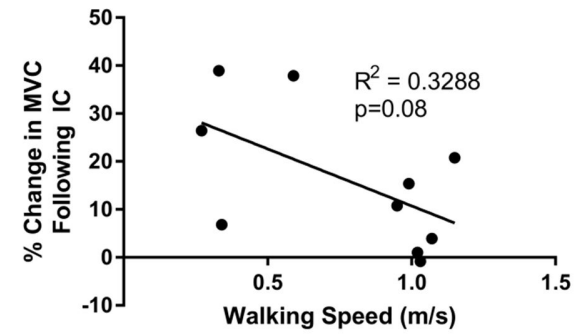


FIGURE 4

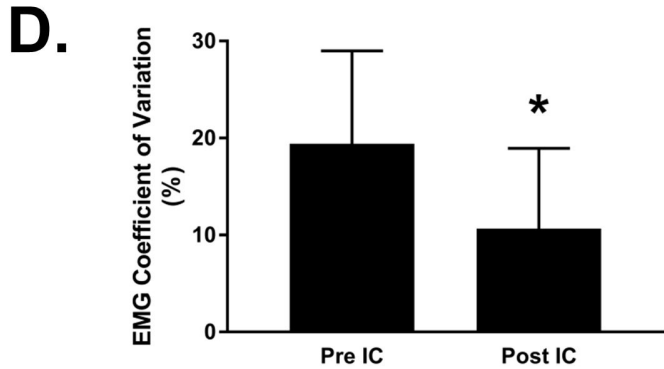
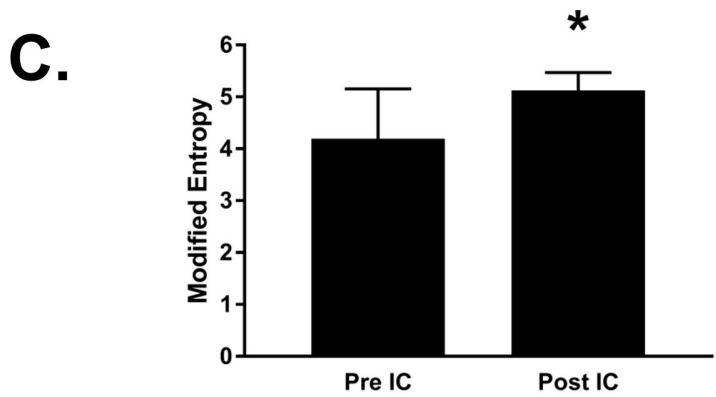
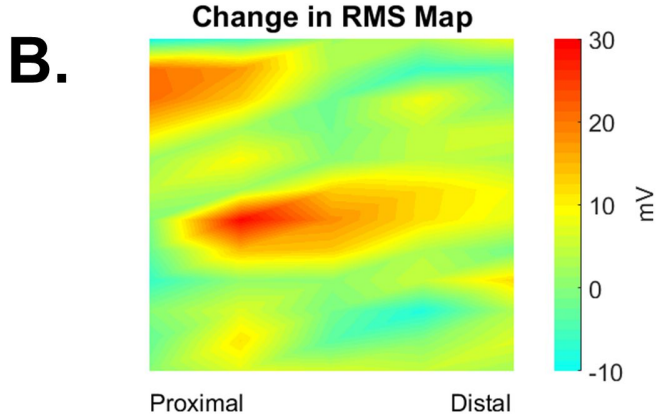
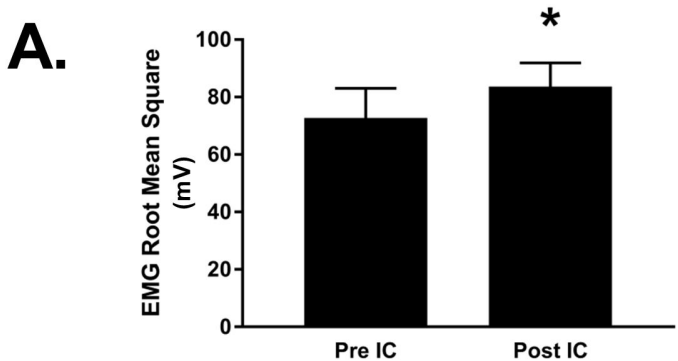
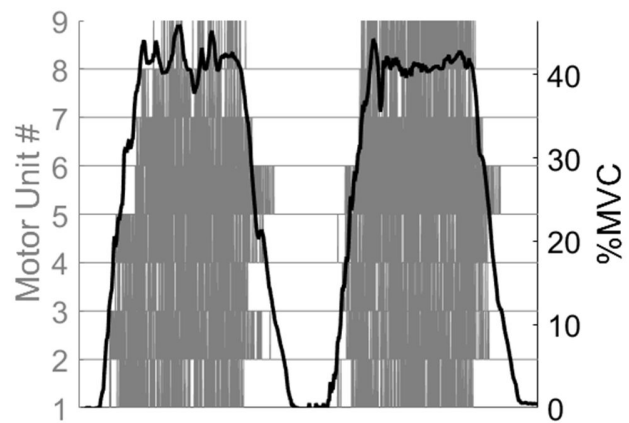


FIGURE 5

A.



B.

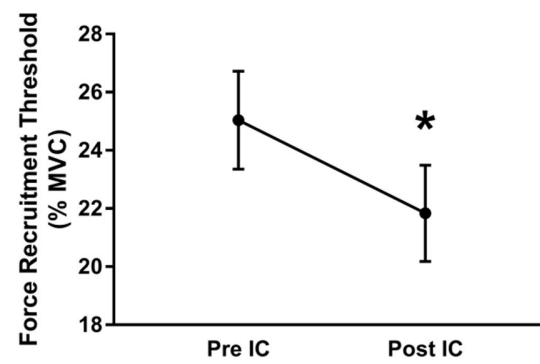


FIGURE 6

