

Early motor unit conduction velocity changes to HIIT versus continuous training

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1 **EARLY MOTOR UNIT CONDUCTION VELOCITY CHANGES TO HIIT VERSUS**
2 **CONTINUOUS TRAINING**

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25 **ABSTRACT**

26 **Purpose:** Moderate-intensity continuous training (MICT) and high-intensity interval
27 training (HIIT) are associated with different adjustments in motor output. Changes in motor
28 unit (MU) peripheral properties may contribute to these adjustments, but this is yet to be
29 elucidated. This study evaluated early changes in MU conduction velocity (MUCV) and
30 MU action potential (MUAP) amplitude following two weeks of either HIIT or MICT.

31 **Methods:** Sixteen men were assigned to either an MICT or HIIT group (n=8 each), and
32 participated in six training sessions over 14 days. HIIT: 8-12×60-s intervals at 100% peak
33 power output. MICT: 90-120min continuous cycling at ~65% VO₂peak. Pre and post
34 intervention, participants performed maximal voluntary contractions (MVC) and
35 submaximal (10, 30, 50 and 70% of MVC) isometric knee extensions while high-density
36 electromyography (HDEMG) was recorded from the vastus medialis (VM) and vastus
37 lateralis (VL) muscles. The HDEMG was decomposed into individual MUs by convolutive
38 blind-source separation and tracked pre-and post-intervention. **Results:** Both training
39 interventions induced changes in MUCV, but these changes depended on the type of
40 training (p<0.001). The HIIT group showed higher values of MUCV following training at
41 all torque levels (p<0.05), MICT only displayed changes in MUCV at low torque levels
42 (10-30% MVC, p<0.002). There were no changes in MUAP amplitude for either group
43 (p=0.2). **Conclusions:** Two weeks of HIIT or MICT elicit differential changes in MUCV,
44 likely due to the contrasting load and volume used in such training regimes. This new
45 knowledge on the neuromuscular adaptations to training has implications for exercise
46 prescription.

47

48

49 **Key words**

50 Motor unit; conduction velocity; amplitude; action potential; high-intensity interval
51 training; endurance training

52

53 **INTRODUCTION**

54 Physical inactivity is a major health concern since it can lead to the development of
55 several metabolic, musculoskeletal and cardiorespiratory diseases (1). Moderate-intensity
56 continuous training (MICT) is regarded as one of the best forms of training to prevent
57 illnesses related to physical inactivity (e.g., diabetes). However, not many people engage in
58 such training typically because it requires a large volume of exercise to be performed in
59 order to induce any significant physiological adaptation (1). In an attempt to reduce the
60 time commitment required to exercise, high-intensity interval training (HIIT) was
61 introduced. This type of exercise consists of short and high-intensity bursts of physical
62 activity (i.e., intensities above the lactate threshold or >90% of heart rate) interspersed by a
63 period of active or passive rest (2).

64 Despite differences in load, volume and time-commitment, several studies reported
65 similar changes in aerobic metabolism, cardiorespiratory fitness and performance following
66 either MICT or HIIT (2-6). Nevertheless, recent research revealed that HIIT and MICT
67 training induce different neuromuscular adaptations. Two weeks of HIIT was shown to
68 increase peak knee extension torque, which was associated with increased vasti muscle
69 activation and motor unit discharge rates at high torque levels [50 and 70% of the
70 maximum voluntary contraction torque (MVC)] while MICT training did not influence
71 peak torque, the level of vasti muscle activity or motor unit discharge rates (7).

72 Both neural and structural factors are the main determinants for an increase in
73 muscle force production following strength (resistance) training (8-10). However, changes
74 in muscle morphology usually take several weeks to influence muscle force (9, 11), and
75 consequently, early changes in muscle strength are usually attributed to neural adaptations
76 (8-11). Neural adjustments associated with increased muscle strength can be due to both
77 central (from the neuromuscular junction to the brain cortex) and peripheral adaptations
78 (from the neuromuscular junction to the muscle cell) (12). Evaluating adaptations in motor
79 unit properties provides direct insight into both central and peripheral adaptations. For
80 instance, central adaptations in motor unit behavior may include changes in motor unit
81 discharge rate, discharge rate variability and/or motor unit recruitment (8), whereas
82 peripheral adaptations are related to changes in the velocity of propagation of motor unit
83 action potentials (MUAP) across the muscle fibers (muscle fiber conduction velocity,
84 MFCV) as well as changes in MUAP morphology (13, 14). MFCV can be quantified by a
85 group of surface (i.e., array of at least 4 electrodes placed parallel to the muscle fibers) or
86 intramuscular (one monopolar needle and one surface electrode serving as an anode) EMG
87 electrodes by dividing the distance between the electrodes and the time of propagation of
88 the MUAP for that distance (15, 16). Most studies analysing MFCV have calculated
89 conduction velocity directly from the interferential EMG, obtaining an “average value” of
90 MFCV from the many active muscle unit’s (group of fibers innervated by the motoneuron)
91 fascicles during a contraction. More recent studies have been able to quantify MFCV from
92 single muscle fibers providing detailed minimum, maximum and average values of MFCV
93 for type I and type II fibers separately (16), however, as this method isolates muscle fibers
94 from their motoneurons (fibers are electrically stimulated), it does not provide information
95 about motor unit peripheral properties. The development of new techniques of surface

96 EMG decomposition, allows conduction velocity to be calculated from the MUAPs of each
97 muscle unit fascicles (17, 18), providing accurate values of motor unit conduction velocity
98 (MUCV) during voluntary contractions. With this method it is now possible to distinguish
99 differences between diverse populations of single motor units (i.e., low threshold and high
100 threshold motor units), unlike methods analysing MFCV from the interferential EMG.

101 In one of the few training studies where MUCV was quantified, MUCV increased
102 after 6 weeks of END and resistance training in low threshold motor units (10 and 30%
103 MVC) (14). Another study using global conduction velocity measurements (MFCV) also
104 found a significant increase in MFCV after 6 weeks of concentric and eccentric resistance
105 training (19). More recently, Methenitis et al. showed that MFCV of resistance-trained
106 individuals was greater than that of endurance athletes, demonstrating that MFCV-related
107 adaptations are training-specific (16). Potential mechanisms for an increase in conduction
108 velocity after training protocols enhancing strength can include an increase in motor
109 unit/muscle fiber recruitment, increase in muscle fiber size, increase in proportion of type II
110 fibers (particularly type IIx which have the highest conduction velocities) and changes in
111 the polarization state of the sarcolemma (i.e. enhanced sodium-potassium pump activity)
112 (14, 16, 19, 20). According to the size principle (21), high intensity contractions induce
113 greater recruitment of motor units compared to low intensity contractions, and therefore
114 activate higher threshold motoneurons, which usually innervate muscle fibers of larger
115 diameter with high conduction velocities (8). It is possible that HIIT activated a larger
116 group of motor units (from low to high threshold), influencing the muscle fiber membrane
117 properties of the muscle units (MUCV) to a greater extent than MICT. This however, has
118 never been investigated. Previous studies suggested that changes in MUAP amplitude can
119 be related to changes in muscle fiber size and morphology (22). Since recent advances in

120 high-density surface EMG (HDEMG) techniques allow motor units to be tracked
121 longitudinally (18), we investigated whether HIIT or END induced changes in MUAP
122 amplitude from a sample of identified motor units was related to changes in MUAP size.
123 Furthermore, we assessed whether changes in MUCV influence MUAP amplitude.

124 Therefore, the aim of this study was to assess early adjustments of motor unit
125 peripheral properties (MUCV) and MUAP amplitude [MUAP root mean square
126 (MURMS)] following 2-weeks of HIIT or MICT using motor unit decomposition and
127 tracking from HDEMG (18). Since it is possible to relate neural and muscular properties
128 with the decomposition of large populations of motor units (20), here we assess MUCV and
129 its association between the recruitment threshold of motor units following a training
130 intervention. It was hypothesized that HIIT and MICT would induce different changes in
131 MUCV behavior which would reflect the differing changes in motor output. Moreover, we
132 hypothesized that tracked motor units would not show any change in MUAP amplitude,
133 confirming that early changes in MUCV are not due to changes in muscle morphology, but
134 due to changes in the muscle fiber membrane.

135

136 **METHODS**

137 In the present study we focused on examining changes in peripheral motor unit
138 properties (MUCV) and motor unit action potential amplitude, following HIIT and
139 END. The participants analyzed here were the same as our previous publication which
140 focused on investigating changes in central motor unit properties (discharge rate,
141 discharge rate variability and recruitment threshold) following these diverse training
142 interventions (7). Therefore, eighteen healthy, recreationally active men (mean (SD) age:

143 29 (3) years, height: 178 (6) cm, mass: 79 (9) kg) took part in the study. All participants
144 practiced some form of exercise at least two to three times per week (e.g. basketball,
145 running, etc.). None of the subjects were engaged in regular training for a sports club and
146 did not compete professionally. Moreover none had previous experience with HIIT or
147 MICT. Exclusion criteria included any neuromuscular and/or musculoskeletal disorder as
148 well as any current or previous history of knee pain and age < 18 or > 35 years. Participants
149 were asked to avoid any strenuous activity 24 h prior to the measurements. The 18
150 participants were randomized into two groups (using <http://www.randomization.com>).
151 Therefore, nine subjects were assigned to the HIIT group and the other nine to the MICT
152 group. The ethics committee of the Universität Potsdam approved the study (approval
153 number 26/2015), in accordance with the declaration of Helsinki (2004). All participants
154 gave written, informed consent.

155

156 **Experimental protocol**

157 The experimental protocol consisted of baseline measurements (i.e., isometric knee
158 extension torque, EMG recordings, peak oxygen uptake (VO_{2peak}) determination), a 2-
159 week intervention of END or HIIT and post-training measurements as presented previously
160 (7).

161 *Baseline measurements* (Torque and EMG measurements). All participants' knee extension
162 torque was measured in an isokinetic dynamometer (CON-TREX MJ, PHYSIOMED,
163 Regensdorf, Switzerland). All isometric knee extensions were exerted with the knee flexed
164 to 90°. Following placement of the surface EMG electrodes (see below), the participants
165 performed three maximal MVCs of knee extension each over a period of 5 s, followed by
166 submaximal isometric knee extensions at 10, 30, 50 and 70% MVC in a randomized order.

167 Contractions at 10-30% were sustained for 20 s, while the contractions at 50 and 70%
168 MVC lasted 15 and 10 s respectively. In each trial, the subjects received visual feedback of
169 the torque applied by the leg to the dynamometer. Further details about the procedures can
170 be found in (7).

171 Then, 24 h after these measurements, all participants performed an incremental test
172 to exhaustion on an electronically braked cycle ergometer (Lode Excalibur Sport V2.0,
173 Groningen, the Netherlands) to determine the VO_{2peak} and the peak power output as
174 presented previously (7). Briefly, the test consisted in a 3-min warm-up at 30 W, followed
175 by a workload increase of 6 W every 12 s until volitional exhaustion. Revolutions per
176 minute were kept between 80 and 90 for both the incremental exercise test as well as for the
177 training sessions (for HIIT and MICT).

178 *Training Protocols.* Two training protocols that have shown similar improvements
179 in cardio-respiratory fitness (VO_{2peak}) and aerobic capacity, despite differences in total
180 training volume and intensity were used (3, 5). Each training protocol started 72 h after the
181 incremental test and consisted of six training sessions performed over 14 days. Sessions
182 were programmed on Mondays, Wednesdays, and Fridays. All training sessions were
183 supervised by an investigator of the study (E. M-V). MICT consisted of 90-120 min of
184 continuous cycling at 65% of VO_{2peak} as described previously (3, 6). Exercise duration
185 increased from 90 min during sessions 1 and 2 to 105 min during sessions 3 and 4, and
186 finally to 120 min during sessions 5 and 6. The HIIT training consisted of 60-s bouts of
187 high-intensity cycling at 100% peak power output as described elsewhere (5). Each of the
188 bouts was interspersed by 75 s of cycling at 30 W for recovery. The subjects completed 8
189 high-intensity intervals during sessions 1 and 2, 10 intervals during sessions 3 and 4, and 12
190 intervals on the final two sessions. 3 min of warm-up (30 W) were performed each session

191 prior to training. The rating of perceived exertion (RPE) and heart rate (heart rate monitor,
192 Polar RS800, Kempele, Finland) were monitored continuously during each training session.
193 The average training intensity for the MICT and HIIT groups were 164.5 ± 19.5 W and
194 334.8 ± 57.9 W, respectively. The maximum RPE averaged across training sessions was
195 13.8 ± 2.6 and 19.2 ± 0.6 , for the MICT and HIIT groups respectively ($p < 0.0001$). Finally,
196 maximum heart rate during training was 156.6 ± 7.0 bpm for the MICT group and $182.6 \pm$
197 11.4 bpm for the HIIT group ($p < 0.0001$).

198 *Post-training measurements.* Post-training measurements were performed 72 h after
199 the training ended and were identical to the pre-training procedures (torque, EMG
200 recordings and incremental test).

201

202 **Data Acquisition**

203 EMG signals were acquired from the vastus medialis (VM) and vastus lateralis (VL)
204 muscles during submaximal isometric contractions. The signals were recorded in
205 monopolar derivation with a two-dimensional (2D) multi-channel adhesive electrode grid
206 (SPES Medica, Salerno, Italy) of 13×5 equally spaced electrodes (1 mm diameter, inter-
207 electrode distance of 8 mm), with one electrode absent from the upper right corner. The
208 electrode grids were positioned as described in previous studies (7, 18, 23). The skin was
209 prepared (shaving, abrasion and water) and the electrode cavities of the grids were filled
210 with conductive paste (SPES Medica, Salerno, Italy). The grids were finally positioned
211 between the proximal and distal tendons of the VL and VM muscles with the electrode
212 columns (13 electrodes) oriented along the muscle fibers. Reference electrodes were placed
213 over the malleoli and patella of the dominant leg. A surgical pen was used to mark the
214 location of the electrodes on the skin of the participants, and the participants were

215 instructed to re-mark the electrode locations daily. Additionally, the position of the
216 electrodes was further reported on a transparent sheet by using anatomical landmarks to
217 ensure similar electrode placement for the post-training measures.

218 Torque and EMG signals were sampled at 2048 Hz, converted to digital data by a
219 12-bit analogue to digital converter (EMG-USB 2, 256-channel EMG amplifier, OT
220 Bioelettronica, Torino, Italy, 3dB, bandwidth 10-500 Hz). EMG signals were amplified by
221 a factor of 2000, 1000, 500 and 500 for the 10, 30, 50 and 70% MVC contractions,
222 respectively. Data were stored on a computer hard disk and analyzed in Matlab offline (The
223 Mathworks Inc., Natick, Massachusetts, USA). Finally, before decomposition, the 64-
224 monopolar EMG channels were re-referenced offline to form 59 bi-polar channels using the
225 difference between the adjacent electrodes in the direction of the muscle fibers.

226 **Signal analysis**

227 *Motor unit analysis.* The EMG signals recorded during the submaximal isometric
228 contractions (from 10 to 70% MVC) were decomposed offline with an extensively
229 validated method (24), which has high reliability and sensitivity to monitor changes in
230 motor unit behavior and properties following training interventions (18, 23). The
231 decomposition accuracy was estimated with the silhouette measure (SIL) and was set at
232 0.90 (24). Therefore, only motor units which had a $SIL > 0.90$ were included in the analysis.
233 Multichannel motor unit action potential (MUAP) waveforms from double differential
234 EMG signals were obtained by spike triggered averaging the identified discharge patterns
235 (25). A window of 15ms (duration of the MUAP) was used for the average of the surface
236 HDEMG signals (17, 20). The first 50 discharges of each identified motor unit (starting
237 from the first action potential) were used for the conduction velocity average. This number
238 of firings minimize the effects of inter-spike interval variations on the estimated conduction

239 velocity (17, 20). A custom MATLAB (Mathworks, Natic, MA) script was used to visually
240 display the MUAPs. A minimum of three to a maximum of nine double-differential
241 channels were manually selected for the estimation of the motor unit root mean square
242 (MURMS) amplitude and conduction velocity (MUCV) of each individual motor unit.
243 Manual selection was chosen because it provided the most accurate approach to identify the
244 channels for MUCV and MURMS estimation (17, 18, 20). Channels that had the clearest
245 propagation of the MUAP, with the highest amplitude in the columns of the grid and a
246 cross correlation coefficient between channels ≥ 0.9 , were selected for further analysis.
247 For each motor unit, the recruitment threshold (the torque at which each motor unit started
248 firing action potentials, expressed as %MVC or Nm torque), MUCV, and MURMS were
249 calculated.

250 *Motor unit tracking.* A recently reported method was used to track motor units pre
251 and post intervention (18). This method is an extension of the convolutive blind source
252 separation technique described by Negro et al. (24) and extracted motor units with MUAP
253 shapes maximally similar across sessions. After the full blind HDEMGM decomposition was
254 performed on the pre-intervention session, a semi-blind separation procedure was applied
255 on the post-training session, focusing on finding only the sources that had MUAP profiles
256 similar to the ones extracted from the pre-intervention session. The normalized cross-
257 correlation between the MUAP profiles was used as a measure of similarity. For each
258 motor unit identified on the baseline session, a semi-blind algorithm was applied on the
259 post-intervention trial until a motor unit with normalized cross-correlation >0.8 was found.
260 The algorithm maximized the probability to find the matched motor units across trials
261 separated by several days. For the tracked motor units, the same channels that were selected
262 for computing MUCV and MURMS on the pre-intervention session were used on the post-

263 intervention session, to maximize the repeatability of the results. Figure 1 depicts the
264 MUCV/MURMS calculation (Figure 1a) and tracking procedure (Figure 1b). Figure 1a:
265 Vastus medialis motor unit spike trains (50 motor unit firings) obtained from a motor unit
266 which was recruited at 50% MVC were used to trigger HDEMG signals (64 channels).
267 Three monopolar EMG signals from the lower left bottom of the grid are presented as a
268 graphical example (Figure 1a, upper right). Double-differential spike triggered averaged
269 (STA) MUAPs of the motor unit muscle unit (fibers which are innervated by the
270 motoneuron) show propagation of MUAPs from proximal to distal (dashed arrows). The
271 innervation zone can be seen on the 8th row of the electrode grid. Channels inside the circle
272 were chosen for MUCV and MURMS calculation. Figure 1b: representative example of the
273 motor unit tracking procedure for VM motor units from one participant in the HIIT group
274 (Figure 1b left) and another participant in the MICT group (Figure 1b right) during a
275 contraction at 70% MVC (recruitment thresholds of these units was ~40% MVC). MUAPs
276 from tracked motor units' pre and post intervention were matched by cross-correlation
277 (cross-correlation coefficient, CCC) to confirm a correct tracking. The same seven double
278 differential EMG channels were used to calculate MURMS and MUCV for the HIIT motor
279 unit (MUAPs inside rectangle Figure 1b left) and six double differential channels were used
280 to calculate MURMS and MUCV for the MICT motor units (MUAPs inside rectangle
281 Figure 1b right). Since MUCV and MURMS have been previously used as parameters to
282 infer motor unit recruitment (17, 26), we analyzed both the full population of identified
283 motor units (sample of motor units including both matched and unmatched across sessions),
284 to check if any change in MUCV and MURMS was due to modifications in motor unit
285 recruitment or intrinsic changes in motor unit peripheral properties, or both. For this
286 purpose, we also compared the recruitment thresholds from all the identified motor units (in

287 % MVC torque) as well as the tracked motor units (in Nm torque), to account for the
288 potential effect of progressive motor unit recruitment on motor unit peripheral properties.

289 *Statistical Analysis*

290 Before comparisons, all variables were tested for normality using the Shapiro-Wilk
291 test. The assumption of sphericity was checked by Mauchley's test and, if violated, the
292 Greenhouse-Geisser correction was made to the degrees of freedom. Statistical significance
293 was set at $p < 0.05$. Results are expressed as mean and standard deviation (\pm) unless stated
294 otherwise.

295 The effects of HIIT and MICT on cardiorespiratory fitness, peak power output and
296 peak torque were analyzed with two-way repeated measures analysis of variance (ANOVA)
297 with factors, group (MICT and HIIT) and time (pre and post).

298 The effects of the two training programs on MUCV and MURMS were firstly
299 assessed with linear regression by comparing the slopes and intercepts of all the identified
300 motor units (full population, pre and post intervention), from all subjects, at all torque
301 levels (recruitment thresholds from 0 to 70% MVC) with analysis of covariance
302 (ANCOVA) (27). The recruitment thresholds (%MVC) of all the identified motor units
303 was averaged for each subject at each torque level and compared pre and post intervention,
304 with a four-way repeated measures ANOVA with factors group, time, torque (10, 30, 50
305 and 70% MVC) and muscle (VM and VL) in order to check if MUCV and MURMS results
306 were influenced by the identification of different populations of MUs pre and post
307 intervention.

308 Additionally, tracked motor unit results [MUCV, MURMS and recruitment
309 threshold (Nm) were averaged for each of the subjects and compared at all target torque
310 levels (10, 30, 50 and 70% MVC) with a four-way repeated measures ANOVA with factors

311 group, time, muscle and torque level. Pairwise comparisons were made with the Student-
312 Newman-Keuls post hoc test when ANOVA was significant. The partial eta-squared (η^2)
313 and observed power for ANOVA was used to examine the effect size of changes in all the
314 aforementioned parameters after the training intervention. A η^2 less than 0.06 was
315 classified as “small”, 0.07-0.14 as “moderate”, and greater than 0.14 as “large” (7).

316 Finally, a post hoc power analysis was employed to determine the actual power of
317 MUCV results (G*Power ver. 3.1.9; Frank Faul, Universitaet Kiel, Germany). According to
318 study design [two groups (HIIT vs MICT) x two measurements (PRE and POST) x four
319 torque levels (10, 30, 50 and 70% MC)], the number of participants, and the average of
320 MUCV on each training group, an effect size of 0.75 was calculated, obtaining an actual
321 power of 1.0 for the difference between groups.

322

323 **RESULTS**

324 One subject from the MICT group and one subject from the HIIT group could not
325 complete the full training protocol and were excluded from the analysis. Results are
326 therefore presented for 8 participants in the MICT group (age: 29 ± 2 years, height: 177 ± 6
327 cm, mass: 77 ± 8 kg) and 8 participants in the HIIT group (age: 29 ± 3 years, height: $177 \pm$
328 7) cm, mass: 79 ± 7 kg). There were no differences between groups for anthropometrics (P
329 > 0.51) as well as in any of the outcome variables at baseline ($P > 0.35$ for all variables).

330 *Cardiorespiratory fitness and Motor output*

331 VO_{2peak} increased similarly following either HIIT or MICT ($6.8 \pm 3.9\%$ and $5.0 \pm$
332 7.3% increase respectively) (7) (time effect: $p=0.001$, $\eta^2=0.54$, observed power= 0.97).
333 Likewise, peak power output increased similarly for HIIT and MICT ($7.0 \pm 3.1\%$ and $6.2 \pm$
334 2.8% increase respectively) (time effect: $p<0.0001$, $\eta^2= 0.87$, observed power= 1.0).

335 Despite this, there was a significant time-group interaction for peak torque ($P=0.01$, $\eta^2=$
336 0.38 , observed power = 0.79) as peak torque only increased in the HIIT group ($6.7\% \pm$
337 2.6% increase, $p=0.01$).

338 *Motor unit decomposition and tracking*

339 A total of 2688 and 2463 motor units with a $SIL > .90$ [average 0.91 ± 0.01] were
340 identified for the VM and VL, respectively. This number considers all 16 subjects and the
341 motor units decomposed from both sessions (pre and post) at all target torque levels.
342 Specific details about the number of identified and tracked motor units across sessions,
343 trainings (HIIT or MICT) and participants (average number of identified and tracked motor
344 units per participant) can be found in **Table 1**.

345 *Motor Unit Conduction Velocity*

346 The MUCV of all identified motor units increased significantly at low torque levels
347 during both interventions; however, it only increased significantly for the HIIT group at the
348 highest torque levels. **Figure 2a** shows the regression lines of MUCV from the full pool of
349 identified motor units for VM and VL muscles in the HIIT group before and after the
350 intervention. **Figure 2b** shows the regression lines of MUCV from the full pool of
351 identified motor units for VM and VL muscles in the MICT group before and after the
352 intervention. The rate of change in MUCV (slope) was significantly correlated with
353 recruitment threshold in all conditions and muscles ($p < 0.0001$ in all cases) with R^2 values
354 ranging from 0.27 to 0.47 (average 0.40).

355 Pre and post intervention MUCV behavior from the full pool of identified motor
356 units differed between groups as revealed by differences in linear regression analysis. In the
357 HIIT group, the y-intercepts of MUCV for both the VM and VL muscles were significantly
358 different after the intervention, with VM MUCV intercepts increasing from 4.15 m/s to

359 4.32 m/s (4.0% increase, $p < 0.0001$, **Figure 2a left**) and VL MUCV intercepts increasing
360 from 4.17 m/s to 4.27 m/s (2.3% increase, $p < 0.0001$, **Figure 2a right**). Moreover, there
361 were no changes in the rate of change of MUCV for any of the muscles following the HIIT
362 intervention ($p = 0.87$ for VM and $p = 0.97$ for VL), showing that MUCV increased
363 systematically at all the investigated torque levels.

364 These results contrast with those observed for the MICT group where despite an
365 initial increase of the intercept in both the VM and VL (by 6.0 and 4.6%, respectively),
366 MICT participants showed a significant reduction in the rate of change in MUCV after the
367 intervention as MUCV values at the higher torques (from 40 to 70% MVC) decreased or
368 remained similar to baseline. This reduction in MUCV ranged from 0.019 to 0.011
369 m/s*%MVC (42.1% decrease, $p < 0.0001$, **Figure 2b left**) and 0.018 to 0.014 m/s*%MVC
370 (38.9% decrease, $p = 0.001$, **Figure 2b right**) for VM and VL, respectively. These findings
371 can be confirmed with the results of the individual regressions where most of the
372 participants on the HIIT group increased their intercept without changing their slopes,
373 while on the MICT group most of the participants decreased their slopes (See Table,
374 Supplemental Digital Content 1, Participant specific pre and post intervention MUCV
375 linear regression analysis).

376 Similarly, the tracked motor units showed an increased MUCV at the lowest torque
377 levels for both groups, but only increased significantly at the highest torques in the HIIT
378 group. **Figure 3** shows the MUCV values recorded from the tracked motor units of the VM
379 and VL contracting at 10, 30, 50 and 70% MVC for both training groups. The results
380 revealed that there was a significant interaction between torque, time and group ($p = 0.001$,
381 $\eta^2 = 0.36$, observed power = 0.96). Therefore, the HIIT and MICT groups showed distinct
382 MUCV torque-related adjustments. HIIT led to a significant increase in MUCV at all

383 torque levels in both the VM (MUCV increased by 5.6, 5.0, 4.1 and 4.2% at 10, 30, 50 and
384 70% MVC, respectively, $p < 0.03$) and VL (MUCV increased by 4.6, 3.1, 4.8 and 2.8% at
385 10, 30, 50 and 70% MVC, respectively, $p < 0.04$). In contrast, the MICT group only showed
386 a significant increase in MUCV at 10 and 30% MVC for VM (4.7 and 4.6% increase,
387 respectively, $p < 0.001$) and VL (4.3 and 4.7% increase, respectively, $p < 0.001$).

388 *MUAP amplitude*

389 The MURMS of all identified motor units increased in both muscles for the HIIT
390 group, but not for MICT. **Figure 4a** shows the regression lines of MURMS results from the
391 full pool of identified motor units for both VM and VL for the HIIT group and **Figure 4b**
392 for the MICT group. All regression lines increased significantly pre and post intervention in
393 both training groups and for both muscles ($p < 0.0001$ in all cases) and R^2 values ranged
394 from 0.37 to 0.45 (average 0.41). HIIT showed significantly higher intercepts, changing
395 from 7.9 μV to 19.2 μV for the VM (58.9% increase, $p = 0.01$, **Figure 4a left**) and 15.8 μV
396 to 19.8 μV for the VL (20.2% increase, $p = 0.01$, **Figure 4a right**), respectively. In contrast,
397 the MICT group showed a significant decrease of the intercepts from 35.1 μV to 20.6 μV
398 for the VM (41.3% decrease, $p = 0.01$), with the results for VL showing no change of the
399 intercepts (pre: 23.8 μV vs. post: 23.3 μV , $p > 0.11$). These differences in slopes and
400 intercepts can be explained with individual regression results where just two participants
401 increased their intercepts for VM in the HIIT group and two participants decreased their
402 intercepts for VM in the MICT group. Similar results were found for VL (See Table,
403 Supplemental Digital Content 2, Participant specific pre and post intervention MURMS
404 linear regression analysis).

405 In contrast, the tracked motor units MURMS did not show any change following the
406 training intervention in both groups. **Figure 5** shows MURMS results from tracked motor

407 units. The VM muscle had higher MURMS values compared to the VL (muscle effect:
408 $p=0.004$, $\eta^2=0.51$, observed power=0.90), at all force levels in both groups. However,
409 there were no changes in MURMS from the tracked MUs after the intervention for either
410 group.

411 *Recruitment threshold*

412 The recruitment thresholds from the full pool of identified motor units was similar
413 pre and post intervention in both training groups for VM [HIIT (mean and range) = pre:
414 26.1 (0.01-69.5) % vs. post: 25.7 (1.0-69.8) %, and MICT= pre: 27.0 (0.16-67.2) % vs.
415 post: 27.6 (0.6-66.4) %] and VL [(HIIT (mean and range) = pre: 23.7 (0.2-70.6) % vs. post:
416 24.9 (0.02-67.2) % and MICT= pre: 27.8 (0.4-70.6) % vs. post: 26.6 (0.5-70.9) %),
417 interaction: time-group-torque, $p=0.17$, $\eta^2=0.019$. The recruitment thresholds from the
418 tracked motor units were also similar in HIIT and MICT for VM [HIIT (mean and range) =
419 pre: 63.0 (9.1-147.0) Nm vs. post: 65.5 (9.3-142.0) Nm and MICT = pre: 65.6 (8.3 – 155.7)
420 Nm vs. post: 65.6 (9.1-163.0) Nm] and VL [HIIT (mean and range) = pre: 66.1 (8.4- 158.4)
421 Nm vs. post: 65.5 (8.5-153.0) Nm and MICT = pre: 69.7 (8.0 – 183.9) Nm vs. post: 67.5
422 (7.9 – 183.7) Nm] and did not change after the intervention (time-group-torque interaction:,
423 $p=0.16$, $\eta^2= 0.16$).

424

425 **DISCUSSION**

426 Two weeks of either HIIT or MICT elicited distinct early adjustments in MUCV
427 recorded from the knee extensor muscles (VM and VL) with no changes in MURMS.
428 MUCV adaptations between trainings were dependent on the level of voluntary torque,
429 since HIIT induced an increase in MUCV at all torque levels, while END induced an
430 increase in MUCV only at the lowest torque levels (10 and 30% MVC). These findings

431 provide novel evidence that HIIT and MICT induce specific adaptations in motor unit
432 peripheral properties, probably due to the divergent nature of both training paradigms.

433 *Motor unit conduction velocity*

434 MICT mainly increased the conduction velocity for the low threshold motor units
435 (10 and 30% MVC) while HIIT increased the MUCV in both low and high threshold motor
436 units (10% to 70% MVC). These results were consistent when analyzing both the full
437 population of motor units as well as the tracked motor units. For the full pool of motor
438 units, when comparing the regression lines pre and post intervention, the HIIT group
439 displayed a significant increase in the initial values of MUCV, for both VM and VL (Fig.
440 2a). Albeit MUCV increased systematically with voluntary force, the rate of change in
441 MUCV was similar pre and post intervention. Similar results were observed in the tracked
442 motor units (Fig. 3), where increases in MUCV were seen at all torque levels. In contrast to
443 these results, the MICT group showed a significant increase in MUCV for low-threshold
444 motor units (Figs. 2b and 3), however, this was not observed for motor units recruited at
445 higher torques. These findings can be due to differences in load intensity and exercise
446 volume between the training protocols, which might have induced a predominant
447 recruitment of different populations of motor units. Due to the high intensity nature of
448 HIIT, it is likely that the HIIT protocol was associated with recruitment of most motor units
449 (including high threshold) (28, 29), while the MICT protocol, which was performed for
450 longer periods at a lower intensity, likely involved lower and middle threshold units, which
451 are typically associated to muscle fibers that have greater aerobic capacity (e.g. most type I
452 and some IIa fibers) (28, 29). This observation can be supported by both the RPEs and
453 maximum heart rate between protocols, as HIIT was performed until or very close to
454 maximal exertion (max RPE: 19-20, max heart rate 183 bpm), likely demanding high vasti-

455 muscle activation. On the contrary, the participants performing the MICT protocol only
456 reached moderate levels of exertion (max RPE: 13-14, max heart rate 157 bpm), possibly
457 requiring lower activation of the knee extensors to complete the training sessions.

458 Previous research has also provided evidence showing that the adaptation of high-
459 threshold motor units is load intensity dependent. For instance, Piitulainen et al. (31)
460 reported that discharge rate of high threshold (50 and 75% MVC) motor units of the biceps
461 brachii increased after maximal eccentric exercise, without any observable change in the
462 discharge rates of low threshold motor units. Moreover, Kamen and Knight (32) also
463 observed increased VL discharge rates at 100% MVC but not at 10% or 50% MVC
464 following 6 wk of maximal knee extension isometric training. Since the activation of high
465 threshold motor units is important to achieve an increase in muscle strength (8), is apparent
466 that the high loads utilized for the HIIT group were able to activate most of the pool of
467 motor units (from low to high threshold) and thus the participants were able to increase
468 their peak torque. Indeed, we previously observed that vasti motor unit discharge rates
469 changed differently following HIIT and MICT, with only the HIIT group displaying higher
470 discharge rate and HDEMG amplitude at high torque levels (50 and 70% MVC) (7).
471 Increases in motor unit discharge rate and recruitment (number of active motor units) have
472 been considered as one of the main neural mechanisms to increase muscle force/torque (8).
473 However, it is important to mention that other neural mechanisms such as increased reflex-
474 activity and/or reduction of intracortical inhibition (10), might have also played a role in the
475 increased peak torque after HIIT. Regarding the changes in peripheral motor unit properties
476 observed in the present study, it would be tempting to suggest that increases in MUCV
477 (faster propagation of MUAPS) might also be responsible for changes in muscle
478 force/torque, however, this association has not been found in previous studies (30).

479 Consequently is not strange to find increases in MUCV for training protocols which not
480 induce an increase in muscle strength. For instance, the observed increase in conduction
481 velocity at 10 and 30% MVC has also been observed previously between MICT and
482 resistance training (13, 14), suggesting that the electrophysiological properties of the
483 muscle membrane are likely to vary similarly among low threshold motor units, even in
484 such divergent protocols. Nevertheless, only HIIT showed an increase in MUCV among
485 high threshold motor units (50 and 70% MVC). A potential explanation for these
486 differences is a differential adaptation in ionic channels (Na^+ and K^+) and/or Na^+ - K^+
487 pump activity in the muscle fibers of low and high threshold motor units. Ionic channels are
488 responsible for the propagation of action potentials while the Na^+ - K^+ pump is responsible
489 to restore and maintain the resting membrane potential. Previous research has shown that
490 conduction velocity is highly sensitive to increased concentration of extracellular K^+ ,
491 which reduces MUAP propagation velocity (31, 32). Enhanced activity of the Na^+ - K^+
492 pump is crucial to reduce the extracellular concentration of K^+ . Indeed, stimulation of the
493 Na^+ - K^+ -ATPase enzyme with adrenaline (catecholamine) increases the conduction
494 velocity of muscle fibers with high extracellular levels of K^+ (31). Moreover, Rongen et al.
495 reported that conduction velocity is influenced by inhibition of the Na^+ - K^+ -ATPase with
496 Ouabain (33). Taken together, the changes in MUCV observed in the present study could at
497 least be partly due to specific Na^+ - K^+ -ATPase adaptations. Various authors reported
498 enhanced Na^+ - K^+ -ATPase activity after training. For instance, Green et al. (34)
499 documented changes in Na^+ - K^+ -ATPase by using a similar MICT protocol to the one
500 employed in the current study. Since Na^+ - K^+ -ATPase activity is also enhanced by
501 increased aerobic capacity, it is very likely that the observed changes in low-threshold
502 MUCV after MICT are due to changes in muscle fiber membrane properties. However,

503 such activity was also enhanced in high threshold motor units following HIIT. A previous
504 study comparing prolonged endurance exercise and high-intensity resistance training
505 showed similar up-regulation in Na⁺ -K⁺ -ATPase concentration between these two
506 training regimes, despite of their large differences in training load and volume (35). This
507 suggests that differences in MUCV for high threshold motor units between HIIT and MICT
508 cannot be due to different adaptations in Na⁺ -K⁺ -ATPase/ Na⁺ -K⁺ pump activity. In one
509 of the few studies where MUCV from high-threshold motor units was quantified,
510 Piitulainen et al. (36) was able to show specific changes in MUCV for high threshold motor
511 units after a session of maximal eccentric exercise. The authors suggested that these high-
512 intensity contractions were able to stimulate fast twitch fibers (which are usually found in
513 high threshold muscle units) to a greater extent than slow twitch fibers (which are usually
514 found in low-threshold muscle units), implying that MUCV can be related to the type of
515 muscle fibers recruited during the exercise. Accordingly, Methenitis et al. (16) recently
516 reported differences in MFCV between endurance, strength and power athletes, with the
517 latter group showing the highest values of MFCV, and the endurance group showing the
518 lowest values. Therefore, it is likely that the HIIT group induced a higher recruitment of
519 type II fibers which are known to have higher conduction velocities (16). In the same study,
520 the authors also showed that conduction velocity can be influenced by changes in muscle
521 fiber size and the % distribution of fibers (e.g. higher proportion of type Ix fibers will lead
522 to larger conduction velocities). It could be possible that differential changes in muscle
523 fiber size between HIIT and MICT protocols might have been responsible for the observed
524 differences in MUCV for high threshold motor units. However, it is very unlikely for these
525 protocols to induce any change in muscle fiber size or change in the proportion of fibers as
526 most studies examining fiber hypertrophy usually report significant changes after a

527 minimum of 6 weeks of resistance training (9). Another potential factor related to
528 differences in MUCV at high torques could be discharge rate. Conduction velocity is
529 indeed influenced by discharge rate (37). Therefore, the higher discharge rates observed for
530 high threshold motor units might have induced an increased MUCV at higher torques for
531 the HIIT group only. Nevertheless, the exact mechanisms by which MUCV might have
532 increased for high threshold motor units in the HIIT group need to be investigated further.

533 *MUAP amplitude*

534 The size of the MUAPs from the tracked motor units did not change after either
535 intervention. This finding is expected since the tracking algorithm uses the MUAP profiles
536 to find the same motor units longitudinally (18). Some factors that might influence MUAPs
537 size are changes in muscle architecture and morphology. Since these training protocols
538 were too short to induce such changes, it is very unlikely to observe changes in MUAP
539 amplitude, even when changes in conduction velocity might have influenced the MUAP
540 shapes to some extent (18). However, and despite these observations, we found changes in
541 MURMS when analyzing the full population of motor units following HIIT and END
542 training (Figs. 4a and 4b). The HIIT group showed a systematic increase in MURMS (at all
543 torque levels) in both vasti muscles, while the MICT group either decreased MURMS
544 systematically (VM) or it remained unchanged (VL). Previous studies suggested that motor
545 unit amplitude (commonly reported as peak-to-peak amplitude) could be used as a
546 parameter to infer motor unit recruitment (38) and/or hypertrophy (22). This observation is
547 related to the high level of correlation between surface EMG amplitude and muscle force
548 (17). Therefore, authors assumed that increases in surface EMG amplitude were related to
549 an increase in the MUAP size. Accordingly, we found a linear increase in MURMS, which
550 was also observed previously in other muscles with parallel/fusiform fibers (36, 39).

551 However, and similar to the results for MUCV, the increase in MURMS observed after
552 HIIT cannot be related to an increase in motor unit recruitment since the recruitment
553 thresholds of the identified units previously and after both trainings were maintained
554 throughout the intervention. One possible explanation for the increase in MURMS can be
555 related to the net increase in surface EMG previously observed for HIIT (7). Two weeks of
556 HIIT increased the surface EMG amplitude (7), likely influencing the identification of
557 motor units of larger MUAPs. Indeed, HDEMG motor unit decomposition algorithms
558 identify the largest motor units, leaving the smallest ones as background noise (24, 40).
559 Therefore, it is probable that, due to the increase in surface EMG after the HIIT
560 intervention, the decomposition algorithm identified some groups of motor units with larger
561 MUAPs but similar recruitment thresholds, influencing the results of the regression slopes
562 for the full identified pool of motor units. In strong support of this explanation, recent
563 research has shown that MURMS does not always relate to muscle force, since deeper
564 motor units having a higher recruitment threshold might show smaller MUAPs (17).
565 Moreover, amplitude estimates (from both surface EMG and motor units) can be influenced
566 by the volume conductor effect of muscles (39) and discharge rate (15), thus increases in
567 MUAP amplitude are not always related to the identification of larger, high-threshold
568 motor units, but rather the identification of different motor units (of similar recruitment
569 thresholds) that were not detected by the recording electrodes prior the intervention.
570 However, all these limitations can be avoided by tracking motor units, since this would
571 minimize the effect that different populations of motor units have on MUAP amplitude
572 parameters.

573 *Limitations and methodological considerations*

574 Due to limitations of both HDEMG and intramuscular EMG decomposition, it is not
575 possible to identify the full population of active motor units during a contraction, and
576 therefore, obtaining a large sample of motor units is crucial to make inferences about
577 changes in motor unit behavior (18, 23). HDEMG-based motor unit decomposition
578 methods allow a larger sample of motor units to be identified compared to previous
579 intramuscular methods, and also allow single motor units to be tracked longitudinally (18).
580 However, these HDEMG decomposition techniques only include information from
581 superficial motor units and are only able to identify the most superficial fascicles of the
582 muscle units. A combination of both HDEMG and intramuscular methods such as that
583 described by Methenitis et al. (16) could provide a better understanding of how MUCV is
584 distributed across different muscle regions, as present methods estimating MFCV or
585 MUCV with HDEMG systems assume that fascicles belonging to a specific muscle unit are
586 uniformly distributed (i.e. motor unit superficial fascicles will have the same properties as
587 the deep fascicles).

588 In this study we utilized two training protocols which, despite large differences in total
589 work, induce similar adaptations in aerobic metabolism and endurance performance. This
590 diversity, however, elicited different neuromuscular adaptations in both the central (7) and
591 peripheral motor unit properties as shown in the present study. It would be relevant to
592 understand whether these differences are maintained if the HIIT and MICT protocols were
593 matched in terms of total work or energy expenditure, as differences in total training
594 volume and intensity might bias results favoring one training over the other (e.g., larger
595 adaptations for high-threshold motor units after HIIT). However, since in work-energy
596 matched protocols the average intensity and total training time is equal, it is likely that they
597 will induce similar changes in neuromuscular function, but this is yet to be elucidated.

598 Another relevant consideration is the baseline training status of the participants. In the
599 present study, we enrolled individuals which were not experienced in either MICT or HIIT,
600 therefore, we cannot discard the possibility that the early adaptations presented herein
601 occurred because the novice participants had not been exposed to such training previously,
602 and were therefore, likely to show greater and more rapid changes in neuromuscular
603 function compared to people regularly participating in such exercise. Longer intervention
604 studies with trained individuals should be conducted to observe if the adaptations presented
605 herein would be present and maintained. Due to the lack of studies comparing the
606 neuromuscular adaptations of “endurance” training protocols [e.g. MICT vs. HIIT or HIIT
607 vs. Sprint interval training (SIT)], differences in MUCV between trainings were mainly
608 discussed based on previous studies focusing on the neuromuscular adaptations of
609 resistance training [e.g. “endurance” vs. resistance training (14)]. It is important to mention
610 that we do not suggest that HIIT has the same metabolic-physiological demands as
611 resistance training, but these adaptations help to explain the neural mechanisms behind
612 differences in strength between protocols. Further research is needed to study the main
613 neuromuscular mechanisms responsible for changes in muscle strength between different
614 endurance training protocols, as the physiological mechanisms leading to increases in
615 muscle strength might differ between endurance and resistance training. Finally, it would
616 have been interesting to add histological and molecular analyses in the present study, in
617 order to analyze the specific mechanisms responsible for the observed differences in
618 MUCV. Therefore, future studies should aim to understand the cellular/molecular
619 mechanisms behind these electrophysiological adaptations.

620 *Conclusion*

621 This study revealed that just two weeks of HIIT or MICT is sufficient to induce
622 different adjustments in motor unit peripheral properties. HIIT increases MUCV from low
623 to high threshold motor units (from 10 up to 70% MVC) whilst MICT only increased
624 MUCV in low threshold motor units (10 and 30% MVC). These changes were not
625 accompanied by changes in MURMS or recruitment threshold, implying that the observed
626 motor unit adaptations were due to intrinsic changes in the muscle membrane properties.
627 These findings are likely related to the divergent nature of both training protocols,
628 suggesting that changes in MUCV are dependent on the load, volume and intensity of the
629 training regime and this has important implications for exercise prescription.

630

631

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638 manipulation.

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757 **Figure Legends**

758

759 **Figure 1.** Motor unit (MU) identification, MU conduction velocity (MUCV) and MU root
760 mean square amplitude (MURMS) calculation, and MU tracking. A). Vastus medialis
761 motor unit spike trains (50 motor unit firings) obtained from a MU which was recruited at
762 50% MVC (70% MVC target torque) were used to trigger HDEMGS signals (64 channels).
763 Three monopolar EMG signals from the lower left bottom of the grid are presented as a
764 graphical example (Figure 1A, upper right). Double-differential spike triggered averaged
765 (STA) MU action potentials (MUAPs) of the MU muscle unit (fibers which are innervated
766 by the motoneuron) show propagation of MUAPs from proximal to distal (dashed arrows).
767 The innervation zone can be seen on the 8th row of the electrode grid. Channels inside the
768 circle were chosen for MUCV and MURMS calculation. B) Representative example of
769 MURMS and MUCV calculation procedure applied to tracked motor units can be observed
770 for vastus medialis (VM) MUs from one participant in the HIIT group (Figure 1B, left) and
771 another participant in the END group (Figure 1B, right) during a contraction at 70% MVC.
772 MUAPS from tracked MU's pre (blue MUAPS) and post (red MUAPS) intervention were
773 matched by cross-correlation to confirm a correct tracking (Figure 1B, below). The cross-
774 correlation coefficient (CCC) is displayed above the matched MUAPS. The same seven
775 double differential EMG channels were used to calculate MURMS and MUCV for the HIIT
776 MU (MUAPs inside rectangle (Figure 1B, left) and six double differential channels were
777 used to calculate MURMS and MUCV for the END MUs (MUAPs inside rectangle, Figure
778 1B, right). MUCV, MURMS and recruitment threshold (% of the maximum voluntary
779 contraction, MVC) values are displayed below the MUAPs of each identified motor unit.

780

781 **Figure 2.** Motor unit conduction velocity (MUCV) regression lines [MUCV vs. recruitment
782 threshold in percent of the maximum voluntary contraction torque (MVC)] from the full
783 pool of identified motor units (MU) before (PRE, blue dots) and after (POST, red dots) two
784 weeks of high-intensity interval training (HIIT, figure 2A) and moderate-intensity
785 continuous training (MICT, figure 2B) in vastus medialis (VM, left) and vastus lateralis
786 (VL, right). PRE intervention regression line is shown in black, while POST intervention
787 regression line is shown in red. Regression equations, Pearson's correlation coefficient, p-
788 value and coefficient of determination (R^2) is displayed on the bottom right corner of each
789 graph.

790

791 **Figure 3.** Motor unit conduction velocity (MUCV) results from tracked motor units at 10,
792 30, 50 and 70% maximum voluntary contraction (MVC) target torque before and after two
793 weeks of high-intensity interval training (HIIT, black dots) and moderate-intensity
794 continuous training (MICT, white dots) in vastus medialis (VM, left) and vastus lateralis
795 (VL, right). Bars represent the mean, lines represent individual values. Significant
796 differences by pairwise comparisons, * $P < 0.01$, # $P < 0.05$.

797

798 **Figure 4.** Motor unit root mean square (MURMS) regression lines [MURMS vs.
799 recruitment threshold in percent of the maximum voluntary contraction torque (MVC)]
800 from the full pool of identified motor units (MU) before (PRE, blue dots) and after (POST,
801 red dots) two weeks of high-intensity interval training (HIIT, figure 4A) and moderate-
802 intensity continuous training (MICT, figure 4B) in vastus medialis (VM, left) and vastus
803 lateralis (VL, right). PRE intervention regression line is shown in black, while POST
804 intervention regression line is shown in red. Regression equations, Pearson's correlation

805 coefficient, p-value and coefficient of determination (R^2) is displayed on the upper left
806 corner of each graph.

807

808 **Figure 5.** Motor unit root mean square (MURMS) results from tracked motor units at 10,
809 30, 50 and 70% maximum voluntary contraction (MVC) target torque before and after two
810 weeks of high-intensity interval training (HIIT, black dots) and moderate-intensity
811 continuous training (MICT, white dots) in vastus medialis (VM, left) and vastus lateralis
812 (VL, right). Bars represent the mean, lines represent individual values.

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