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1 **Motor unit discharge rate and the estimated synaptic input to the vasti muscles is higher**
2 **in open compared to closed kinetic chain exercise**

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23

24 **ABSTRACT**

25 **Purpose.** Conflicting results have been reported on whether closed kinetic chain exercises (such
26 as a leg press) may induce more balanced activation of vastus medialis (VM) and lateralis (VL)
27 muscles compared to open kinetic chain exercise (such as pure knee extension).
28 This study aimed to 1) compare between-vasti motor unit activity and 2) analyze the combined
29 motor unit behavior from both muscles between open and closed kinetic chain exercises.

30 **Methods.** Thirteen participants (four women, mean±SD age: 27±5 years) performed isometric
31 knee extension and leg press at 10, 30, 50, 70% of the maximum voluntary torque. High density
32 surface EMG signals were recorded from the VM and VL and motor unit firings were
33 automatically identified by convolutive blind source separation. We estimated the total synaptic
34 input received by the two muscles by analyzing the difference in discharge rate from
35 recruitment to target torque for motor units matched by recruitment threshold.

36 **Results.** When controlling for recruitment threshold and discharge rate at recruitment, the
37 motor unit discharge rates were higher for knee extension compared to the leg press exercise at
38 50% (estimate = 1.2 pps, standard error (SE) = 0.3 pps, P = 0.0138) and 70% (estimate = 2.0
39 pps, SE = 0.3 pps, P = 0.0001) of maximal torque. However, no difference between the vasti
40 muscles were detected in both exercises. The estimates of synaptic input to the muscles
41 confirmed these results.

42 **Conclusion.** The estimated synaptic input received by VM and VL was similar within and
43 across exercises. However, both muscles had higher firing rates and estimated synaptic input at
44 the highest torque levels during knee extension. Taken together, the results show that knee-
45 extension is more suitable than leg-press exercise at increasing the concurrent activation of the
46 vasti muscles.

47

48 **Key Words**

49 motor unit; discharge rate; single-joint; multi-joint; kinetic chain

50

51 **New and noteworthy**

52

53 There is a significant debate on whether open kinetic chain, single-joint knee extension
54 exercise can influence the individual and combined activity of the vasti muscles compared to
55 closed kinetic chain, multi-joint leg press exercise. Here we show that attempting to change the
56 contribution of either the VM or VL via different forms of exercise, does not seem to be a viable

57 strategy. However, the adoption of open kinetic chain knee extension induces greater discharge
58 rate and estimated synaptic input to both vasti muscles compared to the leg press.

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60

61 **INTRODUCTION**

62 An imbalance in the activation of vastus medialis (VM) and vastus lateralis (VL) has
63 been associated with the development of patellofemoral pain syndrome (15, 27); one cause of
64 anterior knee pain (33). The possibility that an exercise could allow one synergistic muscle to
65 be preferentially activated with respect to another, has therefore been of longstanding clinical
66 interest.

67 In the selection of an exercise regime, a distinction between the so-called open kinetic
68 chain and closed kinetic chain exercises has been made. Nevertheless, it is difficult to identify
69 pure “open” or “closed” kinetic chain exercises. Open kinetic chain exercises, such as knee
70 extension, are usually considered to be single-joint movements that are performed in non-
71 weight bearing with a free distal extremity (21). In contrast, closed kinetic chain exercises, such
72 as the leg press, are multi-joint movements performed in weight bearing or simulated weight
73 bearing with a fixed distal extremity (21). Beyond the biomechanical differences between the
74 two exercises, previous studies have reported that the muscles of the quadriceps femoris are not
75 homogeneously activated during such exercises (4). To date, surface electromyography (EMG)
76 has been used to evaluate differences in quadriceps femoris activation between these exercise
77 tasks. Earlier studies suggested a more balanced activation (31), defined as a ratio between the
78 EMG amplitude of VM and VL close to 1, in a leg press exercise compared to open kinetic
79 chain knee extension. For instance, Irish and colleagues (11) showed that the ratio between the
80 activation of VM with respect to VL was greater during closed kinetic chain (e.g. squat and
81 lunge) than in open kinetic chain exercises (e.g. knee extension). Conversely, Spairani et al.
82 (29) did not find any difference between knee extension and leg press in the relative activation
83 of VM and VL.

84 Recent work has confirmed that high-density EMG (HDEMG) can be decomposed to
85 identify and assess a large number of motor units over a wide range of torques (5, 18, 25),
86 providing more direct evidence on the strategies used by the central nervous system to control
87 muscle force/torque (13) and overcome the limitations of global surface EMG measurements
88 (19). Indeed, when the firings of a large number of motor units are recorded, it is possible to
89 extract reliable information about the synaptic organization of motor commands to the
90 motoneurons (7). However, to date there have been no studies directly evaluating differences

91 in the synaptic input received by the vasti muscles between open versus closed kinetic chain
92 knee exercises.

93 In this study, we applied state-of-the-art direct measures of vasti motor unit behaviour
94 during submaximal contractions over a wide range of torques (from 10 to 70% of the maximum
95 voluntary torque, MVT) when performing isometric knee extension and leg press exercises.
96 The first aim of this study was to identify possible differences in the contribution between VM
97 and VL across the exercise tasks. Since recent work revealed that the vasti muscles receive a
98 similar amount of synaptic input (19), we hypothesized that these muscles will show similar
99 discharge rates between the exercises. The second aim of the study was to compare the vasti
100 net activation (the combined motor unit activity of both VM and VL) between knee extension
101 and leg press, since single joint exercise are anecdotally adopted to increase muscle activation.

102

103 **METHODS**

104 **Participants**

105 Thirteen healthy and physically active participants (four women) (mean±SD age: 27±5
106 years, height: 174±9 cm, body weight: 69±9 kg) took part in the study. All participants were
107 right leg dominant (determined by asking which leg they would use to naturally kick a ball).
108 Exclusion criteria included any neuromuscular disorders, current or previous history of knee
109 pain which warranted treatment from a health care practitioner and age > 18 or < 35 years.
110 Participants were asked to avoid any strenuous activity 24 h prior to the measurements. Data
111 were collected between April and July 2017 and at a laboratory within the Centre of Precision
112 Rehabilitation for Spinal Pain (CPR Spine). The study was conducted according to the
113 Declaration of Helsinki (2004) and the ethics committee of the School of Sport, Exercise and
114 Rehabilitation Sciences (University of Birmingham) approved the study (approval code
115 CM09/03/17-1). All participants gave their written, informed consent. The study is reported
116 according to the STROBE guidelines.

117 **Experimental protocol**

118 Participants attended the laboratory on two occasions, separated by 48 hours, at the same
119 time of the day. Experimental procedures were the same on the two occasions, with the only
120 difference being the exercise type performed (knee extension versus leg press) which were
121 assigned in a randomised balanced order. All measurements were conducted on the right lower
122 limb. In both sessions, the setup was arranged so that participants could see the feedback of the
123 exerted torque on a monitor mounted 1.5 m in front of their eyes.

124 For the open kinetic chain knee extension exercise, participants were comfortably seated
125 on an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems Inc., Shirley, NY,
126 USA) in an adjustable chair. The trunk was vertical and the hip, knee, and ankle joint angles
127 were 90° in order to keep the thigh in a horizontal position. The rotational axis of the
128 dynamometer was aligned with the right lateral femoral epicondyle while the lower leg was
129 secured to the dynamometer lever arm above the lateral malleolus.

130 For the leg press exercise, participants were in supine with their hip, knee, and ankle
131 joint angles in 90° in order to keep the tibia in a horizontal position. The rearfoot was fixed on
132 the lever of the dynamometer through a custom-built board. They were requested to push in a
133 horizontal direction against the board. At the beginning of each session, the subjects performed
134 three maximum voluntary contractions each over a period of 5 s, with 2 min of rest between
135 trials. The highest MVT was used as a reference for the definition of the submaximal torque
136 levels. In each of the experimental sessions, the submaximal torques were expressed as a
137 percent of the MVT measured during the same session. Five minutes of rest was provided after
138 the MVT measurement. Then, following a few familiarization trials at low torque levels, the
139 participants performed two sets of submaximal isometric knee extension contractions at 10, 30,
140 50 and 70% MVT in a randomized order. The randomization order of these contractions was
141 kept constant for each subject in the two sessions to minimize the possible influence of
142 cumulative fatigue on the results. The contractions at 10-30% were sustained for 30 s, while the
143 contractions at 50 and 70% MVT were maintained for 15 s. In each trial, the subjects were
144 instructed to keep the torque exertion as stable as possible during the hold-phase. To this aim,
145 they received visual feedback of the torque exerted, which was displayed as a trapezoidal path,
146 with hold-phase durations as specified above. The rate of change of torque in ramp phases was
147 kept constant in all contractions (10% of the MVT per second), thus the ascending and
148 descending ramps lasted 1 s for 10%, 3 s for 30%, 5 s for 50%, and 7 s for 70% of MVT.

149 **Data acquisition**

150 EMG signals were acquired from the VM and VL, biceps femoris (BF) and
151 semitendinosus (ST) muscles during the maximal and submaximal isometric contractions. For
152 VM and VL, surface EMG was recorded in a monopolar montage with two-dimensional
153 adhesive grids (SPES Medica, Salerno, Italy) of 13 × 5 equally spaced electrodes (each of 1
154 mm diameter, with an inter-electrode distance of 8 mm), with one electrode absent from the
155 upper right corner. The electrode grids were positioned as described previously (14, 18). The
156 area of skin where the grids were to be located was firstly slightly abraded with abrasive paste
157 and then cleaned with water. The electrode cavities were filled with conductive paste (SPES

158 Medica, Salerno, Italy) and the electrode grid was positioned over the distal region of the VM
159 and VL muscles. The electrode columns (comprising 13 electrodes) were oriented along the
160 muscle fibers. Signals from the BF and ST were recorded in bipolar mode with Ag–AgCl
161 electrodes (Ambu Neuroline 720, Ballerup, Denmark; conductive area 28 mm², interelectrode
162 distance 2 cm) and were positioned according to guidelines (1). Reference electrodes were
163 positioned around the right wrist and ankle. The location of the EMG electrodes was marked
164 on the participant's skin using a permanent ink marker, allowing similar electrode placement
165 across the experimental sessions.

166 Torque and EMG signals were sampled at 2048 Hz and converted to digital data by a
167 16-bit analog-to-digital converter (Quattrocento, 400-channel EMG amplifier, OT
168 Bioelettronica, Torino, Italy, 3dB, bandwidth 10-500 Hz). EMG signals were amplified by a
169 factor of 150 and were bandpass-filtered (bidirectional, 4th order, zero lag Butterworth,
170 bandwidth 10-500 Hz). All data were stored on a computer hard disk and analyzed with Matlab
171 (v. 2018b, The Mathworks Inc., Natick, Massachusetts, USA). Finally, before decomposition,
172 the 64-monopolar EMG channels were re-referenced offline to form 59 bipolar derivations, as
173 the differences between adjacent electrodes in the column direction.

174 **Signal processing**

175 **Torque.** The torque signal was low-pass filtered offline with an averaging moving
176 window of 0.5 s. During the submaximal contractions, the stable torque region was visually
177 identified by an operator blinded to the condition. The standard deviation (SD) and coefficient
178 of variation (CoV) of torque (SD torque/mean torque) were calculated from the stable torque
179 region.

180 **EMG amplitude.** The average rectified value (ARV) was computed over epochs of 1 s
181 and averaged over all HDEMG channels to increase the repeatability between sessions (9, 16).
182 These values were extracted from the first 15 s of stable torque region of the contractions. ARV
183 was normalized for the ARV recorded during the MVT, in order to compensate for peripheral
184 differences between the two muscles (3). Indeed, a number of confounding factors affects the
185 difference in EMG amplitude between the two muscles (6) and therefore normalizing the EMG
186 amplitude relative to that recorded during the MVT may partially overcome this drawback (3).
187 The level of antagonist activation was quantified as the mean ARV values of BF and ST.

188 **Motor unit decomposition and analysis.** The EMG signals recorded during the
189 submaximal isometric contractions (from 10% to 70% MVT) were decomposed offline with a
190 method that has been extensively validated (25). The signals were decomposed throughout the
191 entire duration of the submaximal contractions, and the discharge times of the identified motor

192 units were converted in binary spike trains (18). The accuracy of the decomposition was tested
193 with the silhouette measure, which was set to 0.90. The mean discharge rate and the discharge
194 rate variability (CoV of the interspike interval [CoV_{isi}] see below for details) were calculated
195 during the stable plateau region of the torque signal. Recruitment thresholds for each motor unit
196 were defined as the torque (%MVT) at the times when the motor unit began discharging action
197 potentials. Discharge rate at recruitment was calculated from the first six motor unit discharges.
198 Discharges that were separated from the next by <33.3 or >250 ms (30 and 4 pps, respectively)
199 (18) were corrected and edited manually by an experienced operator using a custom algorithm.

200 **Motor unit tracking.**

201 A motor unit tracking procedure was adopted to increase the robustness of the
202 comparison between the two exercise. Motor units were tracked across the two sessions (knee
203 extension and leg press) with the approach described in Martinez-Valdes et al. (20). Briefly,
204 after the full blind HDEMG decomposition was performed on the data from the first session,
205 we applied a semi-blind procedure on the data from the second session, focusing on motor unit
206 action potential profiles similar to the ones extracted from the first session. The cross-
207 correlation threshold for the two-dimensional spatial representation of motor unit action
208 potentials was set to 0.8. This procedure was successfully applied for the VM and VL for at
209 least 8 out of 13 participants, depending on torque level.

210

211 **Estimates of synaptic input.** The amount of synaptic input received by the vasti
212 muscles was investigated with a method previously suggested by Martinez-Valdes et al. (19).
213 Here, the total synaptic input received by the vasti muscles (which is reflected by changes in
214 motor unit firing properties) represents the sum of all sources of input to motor neurons, such
215 an increase in descending drive from supra-spinal centers (26), as well as afferent Ia input (23),
216 among others. A difference in synaptic input received by the motor neuron pools of the two
217 muscles can be estimated by the difference in the relative rate of increase in discharge rate
218 between motor units in the two muscles. Hence, the discharge rate of motor units with the same
219 recruitment thresholds (i.e., with a difference in threshold 0.5% MVC) in the two muscles was
220 used as a measure to compare the synaptic inputs received by the pools of motor neurons. This
221 measure corresponds to the increase in discharge rate from recruitment to the target torque
222 relative to the increase in torque from the recruitment threshold [target torque (10, 30, 50, and
223 70% MVC) minus recruitment threshold torque].

224

225 **Statistical analysis**

226 Statistical analysis was performed in R (ver 3.5.2, R Development Core Team, 2009).
227 To analyse motor units behaviour, we performed a multilevel mixed linear regression analysis
228 through the package *lme4* Version 1.1.19 (2). Linear mixed effects models are particularly
229 suitable in this experimental design since: 1) they allow the whole sample of extracted motor
230 units to be analyzed and not just the mean observations for each subject and condition. This
231 allows a better evaluation of data variations than conventional ANOVA statistics; 2) they
232 account for the non-independence of observations (e.g. observations from the same subjects)
233 with correlated error. This is particularly useful in such a repeated-measure study because it has
234 been demonstrated that motor unit discharge data is correlated within a subject even across
235 testing days (32), 3) they separately treat the effects caused by the experimental manipulation
236 (fixed effects) and those that were not (random effects).

237

238 **Torque**

239 MVT achieved in the two exercise tasks was compared using a paired student t-test.
240 COV of torque was analyzed with a generalized linear mixed effects model, with the within-
241 subject fixed effects exercise and torque, as test variables and the random slope of exercise and
242 torque over participants as random factors.

243

244 **EMG amplitude**

245 ARV was analyzed with a linear mixed effects model, with the within-subject fixed
246 effects muscle, exercise and torque, as test variables and the random slope of muscle and
247 exercise over participants as random factors.

248

249 **Motor unit rate coding**

250 Mean discharge rate of motor units was analysed with a linear mixed effects model,
251 using the within-subject fixed effects muscle, exercise, and mean torque, as test variables, and
252 the discharge rate at recruitment and recruitment threshold, as control variables. In such a way
253 it is possible to characterize the discharge rate during the stable part of the contraction (i.e. at \approx
254 10, 30, 50, and 70% MVT) controlling for the discharge rate at recruitment and motor unit
255 recruitment threshold. We considered the random intercept over participants and the random
256 slope of exercise, muscle, and torque over participants as random factors. Each likelihood ratio
257 tests showed that random slope models (subject-specific slopes for the fixed effects exercise,
258 muscle, and torque) significantly improved the model, so we constructed random slope models.
259 Statistical significance of fixed effects was determined using type III Wald *F* tests with

260 Kenward–Roger degrees of freedom and the ANOVA function from R’s *car* package (ver.
261 3.0.3).

262

263
$$\text{discharge rate} \sim \text{muscle} \times \text{exercise} \times \text{torque} \times (\text{exercise} + \text{muscle} + \text{torque} | \text{subject}) +$$

264
$$\text{discharge rate at recruitment} + \text{recruitment threshold}$$

265

266 After running the model, the residuals were checked for normality using the Shapiro–
267 Wilk test. When the assumption of normality was violated the residual outliers were removed
268 with the Cook’s distance method (using a distance of 4 times the standard deviations) as
269 previously suggested (32). *Post hoc* pairwise comparisons (with Tukey correction) were
270 performed using least squares contrasts, as employed in R’s *lsmeans* package (ver. 2.30.0). The
271 *post hoc* tests were evaluated at 10, 30, 50, and 70% of the continuous variable torque. The *post*
272 *hoc* results were reported with mean estimate (M) and standard error (SE).

273 Motor unit recruitment threshold, discharge rate at recruitment, and CoV_{isi} were
274 analyzed with a linear mixed effects model, with the within-subject fixed effects muscle,
275 exercise and torque, as test variables and the random slope of muscle and exercise over
276 participants as random factors. We could not include the random slope of torque in these cases
277 because of singular fit violation (i.e. multiple collinearity).

278

279 **Task-related differences in firing rate and estimated synaptic input**

280 Linear regression was used to characterize the association for each motor unit between
281 the differences in discharge rate at the target torque (mean discharge rate at \approx 10, 30, 50, and
282 70% MVT) and at recruitment and between the torque achieved during the stable part of the
283 contraction (i.e. \approx 10, 30, 50, and 70% MVT) and motor unit recruitment threshold. The slopes
284 of these linear regressions were compared between the two muscles by analysis of covariance
285 as done previously (19).

286

287 **RESULTS**

288 **Torque**

289 The torque exerted during the MVT was lower in the knee extension exercise (188 ± 35
290 Nm) compared to the leg press (263 ± 88 Nm, $P = 0.007$). The amount of torque fluctuations was
291 similar between the two tasks. Indeed, the coefficient of variation of torque was not different

292 (P = 0.259) between the knee extension exercise (M = 3.2%, SE = 0.2%) and leg press (M =
293 2.9%, SE = 0.2%) and across torque levels (P = 0.358).

294

295 **Normalized EMG amplitude**

296 A representative example of the EMG signals recorded from the VL is reported in Figure
297 1A. The estimates of normalized ARV for VM and VL are reported in Figure 2A and 2B,
298 respectively. As expected, normalized ARV increased with increasing torque (F = 3817.3, P <
299 0.0001). In general, the knee extension exercise was associated with greater normalized ARV
300 at high torque levels, without any difference between muscles. Indeed, there was an exercise ×
301 torque interaction (F = 82.1, P < 0.0001), indicating that the knee extension exercise induced
302 greater overall vasti activation (i.e. combining VM and VL ARV) than the leg press exercise at
303 50 (M = 0.11, SE = 0.01, P = 0.0003) and 70% MVT (M = 0.17, SE = 0.20, P < 0.0001) but not
304 at lower torque levels. However, no differences between muscles were found (F = 1.8, P =
305 0.179).

306

307 --- Figure 2 about here ---

308

309 The level of antagonist activation was not different between exercise tasks (F = 0.3, P
310 = 0.573). However, the level of antagonist activation increased at increasing torque and on
311 average was 3.8 μV (SE = 1.3 μV), 11.0 μV (SE = 1.1 μV), 18.2 μV (SE = 1.2 μV), 25.4 μV
312 (SE = 1.4 μV), at 10, 30, 50, and 70% of MVT, respectively.

313

314 **Motor unit population data**

315 The total number of decomposed motor units across the different torque levels and
316 sessions was between 1059 and 1172, for the VM and VL, respectively. Thus, for each subject
317 and torque level, an average of 10±3 and 11±4 motor units were extracted for VM and VL,
318 respectively. A representative example of the results of motor unit decomposition is reported
319 in Fig. 1A and 1B.

320

321 **Recruitment threshold.** The recruitment threshold descriptive statistics are reported in
322 Table 1. Recruitment threshold increased with increasing torque (F = 14046, P < 0.0001). At
323 high torque levels the recruitment threshold was higher for knee extension compared to the leg
324 press: this difference was more pronounced in VM than in VL. This was indicated by the muscle
325 × exercise × torque interaction (F = 4.6, P < 0.031). Post hoc tests showed that for the VM,

326 higher recruitment thresholds were recorded during knee extension compared to the leg press
327 at 50% (knee extension – leg press: $M = 4.6\%$, $SE = 0.7\%$, $P < 0.0001$) and 70% (knee
328 extension – leg press: $M = 7.5\%$, $SE = 0.7\%$, $P < 0.0001$). Likewise, the knee extension
329 exercise was associated with higher VL recruitment thresholds compared to the leg press, but
330 the magnitude of difference was smaller both at 50% (knee extension – leg press: $M = 3.3\%$,
331 $SE = 0.5\%$, $P < 0.0011$) and 70% (knee extension – leg press: $M = 5.2\%$, $SE = 0.7\%$, $P <$
332 0.0001).

333

334 --- Table 1 about here ---

335

336 **Motor unit discharge rate.** The estimates of the motor unit discharge rate described by
337 the model are reported in Figure 3A and 3B for VM and VL, respectively. As expected, when
338 controlling for discharge rate at recruitment and recruitment threshold, the mean motor unit
339 discharge rate increased with increasing torque ($F = 567.5$, $P < 0.0001$). In general, motor unit
340 discharge rates were influenced by the exercise type but were not different between muscles.
341 The difference between the two exercises emerged only at high torque levels, as indicated by
342 the exercise \times torque interaction ($F = 272.9$, $P < 0.0001$). Since there was no difference between
343 muscles ($F = 0.4$, $P = 0.50$), the post hoc tests are reported by merging the data from VM and
344 VL. When controlling for recruitment threshold and discharge rate at recruitment, higher motor
345 unit discharge rates were recorded during the knee extension exercise compared to the leg press
346 at 50% ($M = 1.2$ pps, $SE = 0.3$ pps, $P = 0.0138$) and 70% ($M = 2.0$ pps, $SE = 0.3$ pps, $P =$
347 0.0001) of MVT. The control variables of recruitment threshold ($F = 2617.2$, $P < 0.0001$) and
348 discharge rate at recruitment ($F = 871.0$, $P < 0.0001$) significantly affected motor unit discharge
349 rates.

350

351 --- Figure 3 about here ---

352

353 **COV of interspike interval.** The COV_{isi} increased with torque ($F = 221.1$, $P < 0.0001$):
354 being 12.1%, $SE = 0.5\%$; 13.4%, $SE = 0.5\%$; 14.5%, $SE = 0.5\%$; 15.7%, $SE = 0.5\%$; for 10,
355 30, 50, and 70% of MVT, respectively. No other difference for muscle or exercise type emerged
356 (all P values > 0.18).

357

358 **Tracked motor unit data**

359 The number of tracked motor units across testing sessions was between 165 and 101 for
360 VM and VL, respectively. Thus, for each subject and condition an average of 3.1 ± 1.0 and
361 1.9 ± 0.7 motor units were tracked for VM and VL, respectively. The cross-correlation values
362 from the projecting vectors of the tracked motor units was 0.84 ± 0.04 and 0.80 ± 0.04 for VM
363 and VL respectively. The results of tracked motor units confirmed the results from the group
364 level analysis. When controlling for discharge rate at recruitment and recruitment threshold, the
365 mean motor unit discharge rate increased with increasing torque ($F = 951.9, P < 0.0001$).
366 Similar to the group level findings, when controlling for recruitment threshold and discharge
367 rate at recruitment, the motor unit discharge rates were higher during the knee extension
368 exercise compared to the leg press at torque levels $\geq 50\%$ of MVT as indicated by the exercise
369 \times torque interaction ($F = 272.9, P < 0.0001$). Since there was no difference between muscles (F
370 $= 0.4, P = 0.50$), the post hoc tests are reported on the merged data from VM and VL. When
371 controlling for recruitment threshold and discharge rate at recruitment, the knee extension
372 exercise showed higher motor unit discharge rates compared to the leg press at 50% ($M = 1.1$
373 pps, $SE = 0.3$ pps, $P = 0.0318$) and 70% ($M = 1.7$ pps, $SE = 0.3$ pps, $P = 0.0007$) of MVT. The
374 control variables recruitment threshold ($F = 571.4, P < 0.0001$) and discharge rate at recruitment
375 ($F = 204.9, P < 0.0001$) significantly affected the discharge rates of the tracked motor units.

376 **COV of interspike interval.** The COV_{isi} of the tracked motor units increased with
377 torque ($F = 30.7, P < 0.0001$) and on average was 12.5%, $SE = 0.7\%$; 13.6%, $SE = 0.5\%$; 13.8%,
378 $SE = 0.5\%$; 14.8%, $SE = 0.8\%$; for 10, 30, 50, and 70% of MVT, respectively. No other
379 difference for muscle or exercise emerged (all P values > 0.11).

380

381 **Estimate of synaptic input**

382 **Comparison between muscles.** For each subject and exercise, an average of 5, 6, 6,
383 and 3 motor units were matched (by recruitment threshold) between VM and VL at 10, 30, 50,
384 and 70% of MVT, respectively. The linear regressions between the increase in discharge rate
385 from recruitment to the target torque relative to the increase in torque from the recruitment
386 threshold are reported in Figure 4. At 10% MVT (Figure 4A and 4E) both muscles showed a
387 regression non-different from constant value (both muscles and exercises $P > 0.123$). For all
388 other contraction levels (except for leg press at 70% MVT, VM: $P = 0.834$, VL: $P = 0.481$, see
389 Figure 4H) both vasti muscles showed a regression line which was different from the constant
390 value (all P values < 0.021 , see Figure 4B, 4C, 4D, 4F and 4G). However, the intercept (all P

391 values > 0.291) and slope (all P values > 0.302) were not different between muscles for either
392 exercise at any of the contraction levels.

393 **Comparison between exercises.** At 10% MVT, both exercises showed a regression
394 non-different from constant value (both muscles and exercises $P > 0.329$, see Figure 5A). For
395 all other contraction levels (except for the leg press exercise at 70% MVT, $P = 0.530$, see Figure
396 5B, 5C and 5D), both exercises showed regression line different from constant value (all P
397 values < 0.012). Nonetheless, the intercept was different only at 30% ($P = 0.016$, see Figure
398 5B); the slope was steeper in knee extension than leg press at 50% ($P = 0.023$, Figure 5C) and
399 70% ($P = 0.038$, Figure 5D) of MVT.

400 **DISCUSSION**

401 This study uniquely compared knee extensor motor unit rate coding between open
402 kinetic chain knee extension and closed kinetic chain leg press exercise using HDEMG. When
403 controlling for recruitment threshold and discharge rate at recruitment, mean motor unit firing
404 rates at target torque were similar between VM and VL in both exercise types suggesting that
405 the amount of synaptic input received by the two muscles was similar and their relative
406 contribution did not differ with exercise type. These findings refute the value of using the leg
407 press exercise over open kinetic chain knee extension exercises for the selective activation of
408 the VM. When comparing the overall vasti activation, the motor unit discharge rates were
409 higher during the knee extension exercise compared to the leg press exercise when performed
410 at 50% and 70% of MVT. Collectively these findings indicate that the synaptic input to the vasti
411 muscles was higher during the knee extension exercise compared to the leg press.

412

413 **Differences between the vastus medialis and lateralis**

414 Previously, the ratio between the activation (i.e. the EMG amplitude) of the VM and VL
415 has been used to assess differences in the contribution of each muscle in different exercises
416 (28). This approach has led to conflicting results (28), with some studies showing greater
417 relative activation of VM compared to VL during closed kinetic chain exercises (e.g. squat and
418 lunge) compared to open kinetic chain exercises (e.g. knee extension) (11, 31) but with others
419 showing no difference (29, 30). While the protocols adopted in these studies may differ from
420 each other for some aspects (namely, subject position, knee angle, etc.), we suggest that these
421 conflicting results are mainly due to limitations of classic bipolar surface EMG methods.
422 Indeed, bipolar surface EMG can be unreliable and influenced by many factors including
423 electrode positioning, thereby reducing the accuracy of amplitude estimates to effectively infer

424 changes in synaptic input (22). Bipolar recordings may under- or over-estimate EMG amplitude
425 because of the uneven distribution of action potentials within the muscle volume (8). In contrast,
426 the HDEMG used in this study provides a superior representation of muscle activation
427 compared to bipolar EMG since the greater number of EMG channels (59 bipolar EMG
428 channels) provides a more representative estimate of muscle activity, increasing the reliability
429 and sensitivity of EMG amplitude parameters. Using this approach, we found very little
430 difference in VM and VL behaviour between the two exercise types (Figure 2). These findings
431 suggest that the activation of the VM and VL did not differ between the two exercises.
432 Nevertheless, analysis of EMG amplitude between the VM and VL cannot be used to infer the
433 synaptic input received by the two muscles (19). For these reasons, the analysis of motor unit
434 firing properties is fundamental to investigate the synaptic input received by muscles.

435 The motor unit discharge rate at a given torque depends on discharge rate at recruitment
436 and recruitment threshold (10). Hence, the mere analysis of motor unit firing rates, without
437 taking into account these variables, does not provide a suitable estimate of the input received
438 by the motoneurons. Conversely, controlling for the discharge rate at recruitment and
439 recruitment threshold provides a robust estimate of the synaptic input received by the motor
440 neuron pools since discharge rates indicate the nonlinear transformation of synaptic input into
441 motor neuron outputs (13). When controlling for recruitment threshold and discharge rate at
442 recruitment, the discharge rate of VM and VL motor units were similar for both exercise types,
443 see Figure 3. This suggests that the net excitatory synaptic input to the pool of motor neurons of
444 the vasti was similar. This was furthermore confirmed by the analysis of regression between
445 delta discharge rate and delta torque which was previously adopted as a way to estimate
446 synaptic input (19). In addition, this analysis, which is based on the same assumptions, clearly
447 showed no difference between the synaptic input received by VM and VL at all torque levels
448 in both exercises (Figure 4). These results are in line with the recent finding that the vasti
449 muscles share most of their synaptic input (14, 19). Taken together, these findings strongly
450 suggest that the vasti muscles were controlled in a similar way by the central nervous system
451 in leg extension (open kinetic chain) and leg press (closed kinetic chain) tasks. Thus, attempting
452 to selectively activate either the VM or VL via different knee extension exercises does not seem
453 to be a viable strategy in rehabilitation settings.

454

455 **Knee extension vs. leg press**

456

457 The two tasks investigated in this study constitute the isometric version of two popular
458 exercises in clinical and sport settings. They are intrinsically different from many points of
459 view. The knee extension task is a single-joint exercise involving a relatively small amount of
460 muscle mass (mainly the knee extensors) while the leg press is a multi-joint exercise involving
461 more muscles, such as the hip extensors. From the standpoint of torque-vector direction, in the
462 knee extension exercise the torque is directed perpendicularly to the tibia, while in leg press the
463 torque is directed parallel to the tibia. For this reason, the leg press tends to produce lower shear
464 forces and higher compression forces at the knee. Finally, the knee extension is considered an
465 open kinetic chain exercise, while the leg press is a closed kinetic chain exercise. Anecdotally,
466 single-joint/open kinetic chain exercises are thought to induce higher muscle activation
467 compared to multi-joint/closed kinetic chain exercises (21). While it seems reasonable that
468 targeting a specific muscle with a single-joint exercise may result in higher activation, the
469 available literature on this topic is conflicting. While some studies have reported higher vasti
470 EMG amplitude during single-joint compared to multi-joint tasks (11, 29) others studies
471 reported no difference (30, 31). As mentioned above, the most likely cause of such conflicting
472 results are the methodological drawbacks of interference EMG analysis.

473 Since the level of hamstring muscle activity was not different between the two exercises,
474 the greater vasti activation in the pure knee extension task cannot be explained by higher
475 coactivation of antagonist muscles. However, in the leg press the load is shared between knee
476 extensors and hip extensors muscles, hence the greater involvement of hip extensors at the
477 expense of knee extensors cannot be excluded. In any case, the addition of motor unit
478 decomposition in this study allowed us to directly clarify the amount of synaptic input delivered
479 to the vasti muscles.

480 When controlling for discharge rate at recruitment and recruitment threshold, the
481 average motor unit discharge rate was greater in knee extension exercise than the leg press at
482 50 and 70% of MVT (Figure 3). The possibility to track the motor units between the two
483 sessions allowed us to monitor the behaviour of individual motor units across the two exercises.
484 This analysis confirmed that motor unit discharge rate was higher in knee extension than the
485 leg press at 50 and 70% of MVT. The same finding come from the analysis of the synaptic input
486 (Figure 5): the regression lines between delta discharge rate and delta torque showed
487 significantly steeper slope in the knee extension exercise compared to the leg press at 50 and
488 70% MVT. Together, these findings suggested that the synaptic input received by the motor
489 unit pool was greater in the knee extension exercise. A reduction in net synaptic input in the leg
490 press exercise could be attributed to a decrease in excitatory input and/or an increase in

491 inhibitory input to motoneurons (13). On the one hand, a greater antagonist activation may
492 induce an inhibition of agonist muscles, but this seems not to be the case since the activity of
493 the hamstrings did not differ between tasks. However, it is difficult to exclude potential
494 inhibition on the sole basis of the EMG amplitude of the antagonist muscles. In any case, multi-
495 joint exercise implies a larger muscle mass acting to accomplish the task and therefore the load
496 is shared between knee extensors and hip extensors which may reduce the demand on the knee
497 extensors. On the other hand, the higher synaptic input to vasti muscles may be explained by
498 the fact that the torque-vector for knee extension may be more favourable to the activation of
499 the vasti muscles compared to that of the leg press (4). Indeed, the muscle contributions in
500 multi-joint tasks are directionally tuned and combined to produce the movement in the desired
501 direction (24). Thus, in a leg press the activation of the vasti may be modulated in favour of the
502 hip extensors. The observed difference between the exercises emerged at the higher torque
503 levels only which suggests that an increased synaptic input mostly affected high threshold
504 motor units. This confirms the necessity to investigate the motor unit rate coding across the
505 whole range of submaximal contractions since some changes may not be observed for the lower
506 threshold motor units (Martinez-Valdes 2017).

507

508 **Limitations**

509 The current findings should be considered in light of some limitations. First, the relative
510 intensity between the two exercises was controlled by normalizing the requested torque by
511 MVT. However, there remains a possible inter-exercise difference in the torque produced by
512 the vasti due to different torque-vector directions. Second, due to small shifts in skin
513 displacement between the two sessions, the tracking of motor units across sessions was not
514 possible in some subjects at high torque levels (50 and 70% of MVT). However, in the subset
515 of conditions where the tracking was possible, the tracking confirmed the observed results from
516 the full motor unit pool. Because of the limitations of surface EMG, the present results could
517 be influenced by the more superficial motor units which seem to be associated with fast-twitch
518 type II fibers (12). These units tend to have larger action potentials (17, 19) and are therefore
519 easier to identify by the decomposition algorithm in comparison to deeper motor units (25).
520 Furthermore, while all participants were physically active and they were familiar with exercises
521 typically adopted in the gym, they may not be accustomed with both exercises at the same
522 extent. This may potentially lead to MVT underestimation with less practiced exercise or with
523 the more complex exercise, in this case the leg press. Finally, in this study we adopted isometric
524 contractions because currently the motor unit decomposition algorithms are best suited for this

525 specific condition. For this reason, the applicability of the present findings to dynamic
526 conditions should be considered with caution.

527

528 **Conclusions**

529 The synaptic input received by VM and VL was similar and their relative contribution
530 was not affected by exercise type. Hence, attempting to change the contribution of either the
531 VM or VL via exercise selection does not seem to be a viable strategy. However, open kinetic
532 chain knee extension was associated with overall greater synaptic input to vasti muscles. This
533 finding suggests a single-joint knee extension is more suitable than a multi-joint leg press
534 exercise to increase the activation of the vasti muscles.

535

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Table 1 – Descriptive statistics of motor units recruitment threshold, expressed as % of MVT, for each muscle and exercise. Data are reported as mean±SD (range)

Contraction level (% MVT)	Knee extension		Leg press	
	Vastus Medialis	Vastus Lateralis	Vastus Medialis	Vastus Lateralis
10%	8.3±2.7 (1.42 – 13.6)	8.57±3.10 (0.2 – 15.4)	8.4±2.5 (2.4 – 13.9)	8.3±3.1 (1.5 – 14.0)
30%	23.6±6.3 (6.3 – 34.8)	22.9±6.7 (4.4 – 37.4)	23.1±5.8 (6.3 – 35.0)	22.4±5.7 (4.11 – 35.5)
50%	34.4±7.6 (20.3 – 53.2)	36.2±8.9 (14.0 – 52.5)	34.6±7.9 (11.6 – 50.0)	34.9±7.8 (8.7 – 49.2)
70%	53.8±10.2 (27.1 – 72.2)	52.2±10.3 (21.8 – 71.4)	45.0±9.2 (16.9 – 70.4)	44.3±9.5 (18.2 – 75.6)

Legend

MVT, Maximal Voluntary Torque

Captions

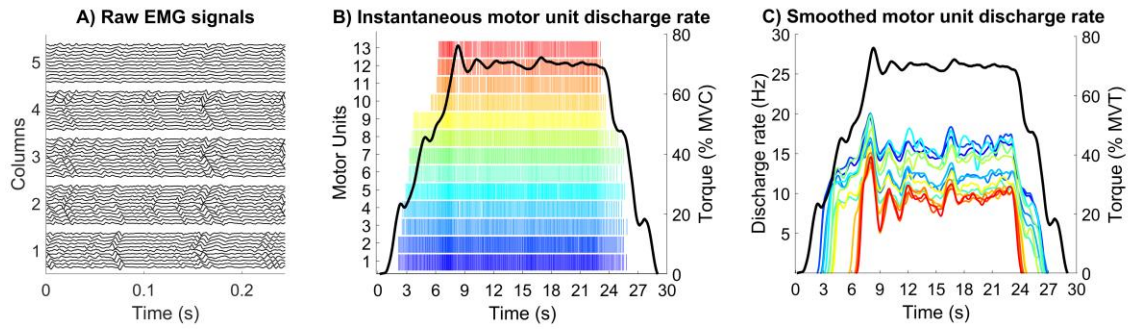


Figure 1 – A) Representative examples of raw electromyographic (EMG) signals (5 columns and 12 lines) recorded from the vastus lateralis at 70% of maximal voluntary contraction (MVT). B) Instantaneous discharges of 13 motor units are reported as vertical lines. The torque signal is reported as the black line. C) Smoothed discharge rates (smoothed with a Hanning window of 1 s) are reported for the same 13 motor units. Note that the late recruited motor units (represented in orange and red) are those with the lower discharge rate in the plateau phase of the contraction. Note also that the shape of the discharge rate profiles of motor units are similar to the shape of torque signal.

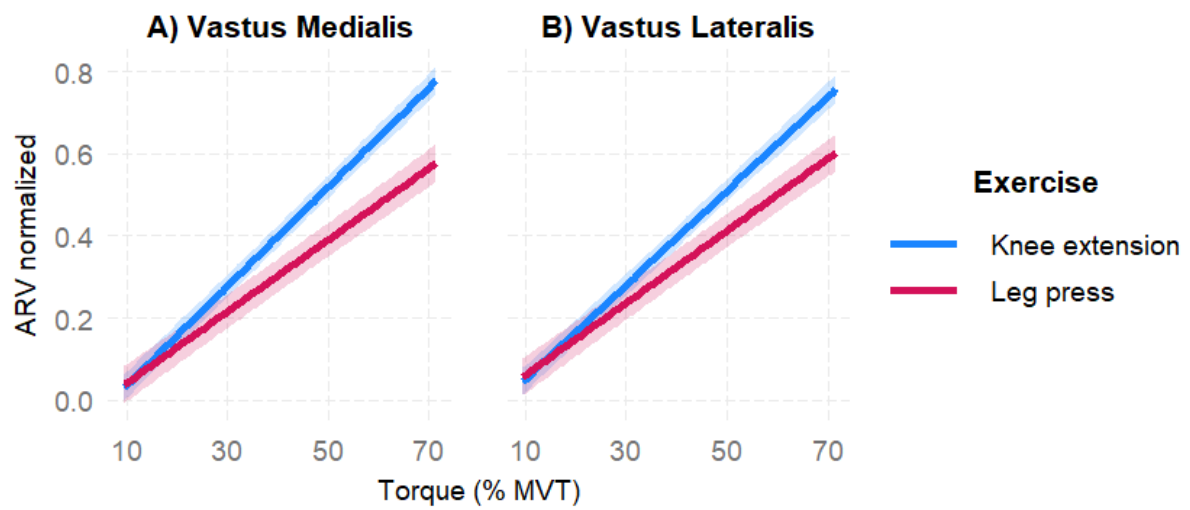


Figure 2. Estimates (with 95% confidence intervals) of EMG amplitude (average rectified value, ARV) normalized for ARV in maximal voluntary contraction across torque levels are reported for A) vastus medialis (VM) and B) vastus lateralis (VL).

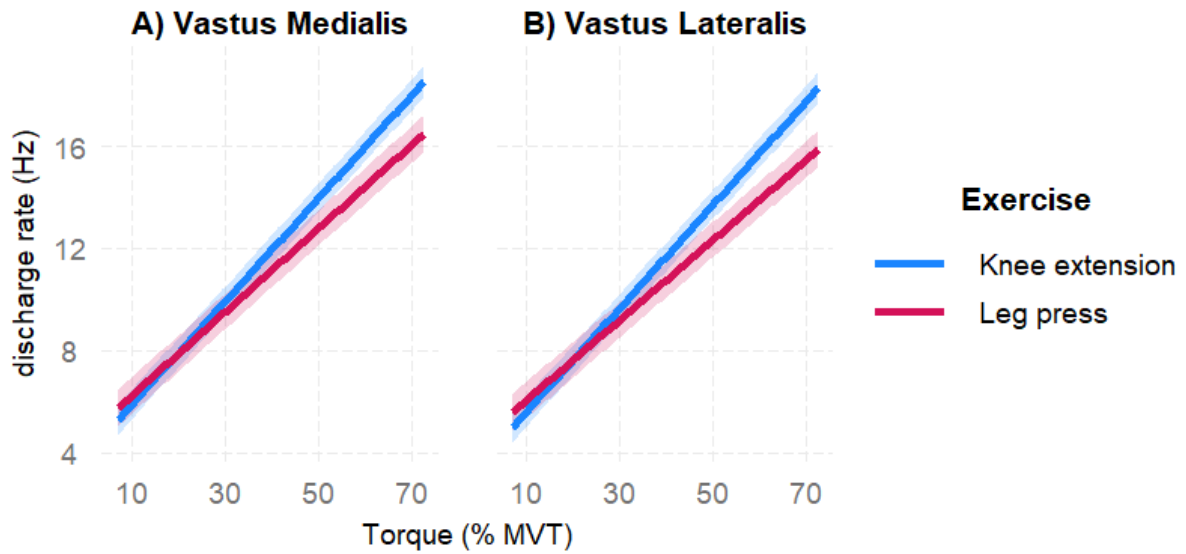


Figure 3. Estimates (with 95% confidence intervals) of motor unit discharge rates are reported for A) vastus medialis (VM) and B) vastus lateralis (VL) muscles. The estimates are calculated from the motor units population (a total of 1059 and 1172 motor units for VM and VL respectively), adjusted for motor unit recruitment threshold and discharge rate at recruitment. The linear mixed model adopted to obtain these estimates included random slope (i.e. subject specific variation) of the factor muscle, torque level and exercise.

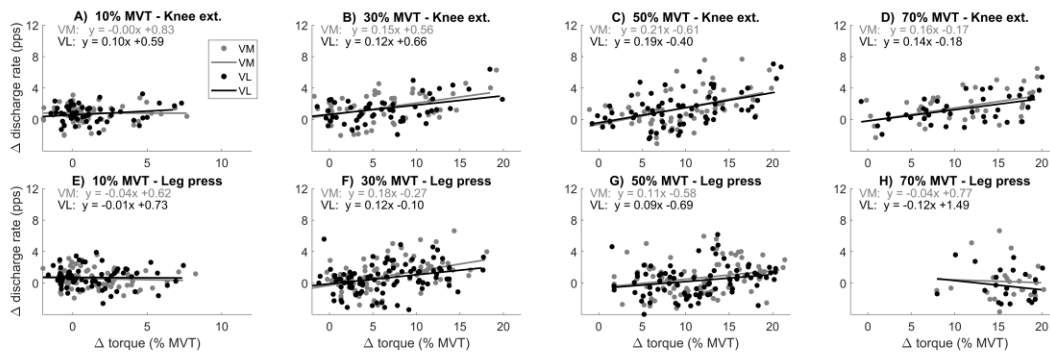


Figure 4. Linear regression analysis of the difference between vastus medialis (VM, in grey) and vastus lateralis (VL, in black) mean discharge rate at target torque and discharge rate at recruitment (y-axis) and the difference between target torque [10, 30, 50, and 70% maximal voluntary torque (MVT)] and motor unit recruitment threshold (x-axis) at 10%, 30%, 50%, and 70% of MVT. The motor units were matched between VM and VL for recruitment threshold. Linear regression equations are shown in the figure. None of the regression lines (slopes and intercepts) differed significantly between muscles.

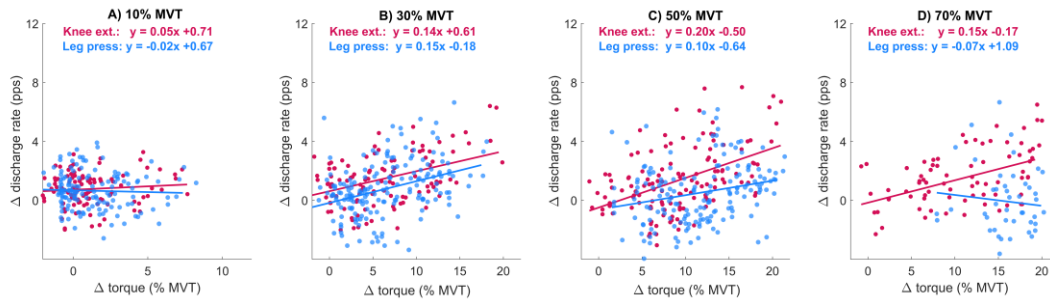


Figure 5. Linear regression analysis of the difference between knee extension (red) and leg press (blue) mean discharge rate at target torque and discharge rate at recruitment (y-axis) and the difference between target torque [10, 30, 50, and 70% maximal voluntary torque (MVT)] and motor unit recruitment threshold (x-axis) at 10%, 30%, 50%, and 70% of MVT. Since there was no difference between muscles, the vastus medialis and lateralis data are merged. Linear regression equations are shown in the figure. The slope of the regression lines was significantly steeper in knee extension than leg press at 50% and 70% of MVT, see results section.