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1 **MOTOR UNIT DISCHARGE RATE AND THE ESTIMATED SYNAPTIC INPUT TO**
2 **THE VASTI MUSCLES IS HIGHER IN OPEN COMPARED TO CLOSED KINETIC**
3 **CHAIN EXERCISE**

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23
24

25 **ABSTRACT**

26 **Purpose.** It has been suggested that closed kinetic chain exercises induce a more balanced
27 activation of vastus medialis (VM) and lateralis (VL) muscles compared to open kinetic chain
28 exercise. This study aimed to 1) compare between-vasti motor unit activity and 2) analyze the
29 combined motor unit behavior from both muscles between open and closed kinetic chain
30 exercises.

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

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

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

48

49 **Key Words**

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

51

52 **New and noteworthy**

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.

59 **INTRODUCTION**

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

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

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

91 In this study, we applied state-of-the-art direct measures of vasti motor unit behaviour
92 during submaximal contractions over a wide range of torques (from 10 to 70% of the maximum

93 voluntary torque, MVT) when performing isometric knee extension and leg press exercises.
94 The first aim of this study was to identify possible differences in the contribution between VM
95 and VL across the exercise tasks. Since recent work revealed that the vasti muscles receive a
96 similar amount of synaptic input (19), we hypothesized that these muscles will show similar
97 discharge rates between the exercises. The second aim of the study was to compare the vasti
98 net activation (the combined motor unit activity of both VM and VL) between knee extension
99 and leg press, since single joint exercise are anecdotally adopted to increase muscle activation.

100

101 **METHODS**

102 **Participants**

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

115 **Experimental protocol**

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

122 For the open kinetic chain knee extension exercise, participants were comfortably seated
123 on an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems Inc., Shirley, NY,
124 USA) in an adjustable chair. The trunk was vertical and the hip, knee, and ankle joint angles
125 were 90° in order to keep the thigh in a horizontal position. The rotational axis of the

126 dynamometer was aligned with the right lateral femoral epicondyle while the lower leg was
127 secured to the dynamometer lever arm above the lateral malleolus.

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

147 **Data acquisition**

148 EMG signals were acquired from the VM and VL, biceps femoris (BF) and
149 semitendinosus (ST) muscles during the maximal and submaximal isometric contractions. For
150 VM and VL, surface EMG was recorded in a monopolar montage with two-dimensional
151 adhesive grids (SPES Medica, Salerno, Italy) of 13 × 5 equally spaced electrodes (each of 1
152 mm diameter, with an inter-electrode distance of 8 mm), with one electrode absent from the
153 upper right corner. The electrode grids were positioned as described previously (14, 18). The
154 area of skin where the grids were to be located was firstly slightly abraded with abrasive paste
155 and then cleaned with water. The electrode cavities were filled with conductive paste (SPES
156 Medica, Salerno, Italy) and the electrode grid was positioned over the distal region of the VM
157 and VL muscles. The electrode columns (comprising 13 electrodes) were oriented along the
158 muscle fibers. Signals from the BF and ST were recorded in bipolar mode with Ag–AgCl
159 electrodes (Ambu Neuroline 720, Ballerup, Denmark; conductive area 28 mm², interelectrode

160 distance 2 cm) and were positioned according to guidelines (1). Reference electrodes were
161 positioned around the right wrist and ankle. The location of the EMG electrodes was marked
162 on the participant's skin using a permanent ink marker, allowing similar electrode placement
163 across the experimental sessions.

164 Torque and EMG signals were sampled at 2048 Hz and converted to digital data by a
165 16-bit analog-to-digital converter (Quattrocento, 400-channel EMG amplifier, OT
166 Bioelettronica, Torino, Italy, 3dB, bandwidth 10-500 Hz). EMG signals were amplified by a
167 factor of 150 and were bandpass-filtered (bidirectional, 4th order, zero lag Butterworth,
168 bandwidth 10-500 Hz). All data were stored on a computer hard disk and analyzed with Matlab
169 (v. 2018b, The Mathworks Inc., Natick, Massachusetts, USA). Finally, before decomposition,
170 the 64-monopolar EMG channels were re-referenced offline to form 59 bipolar derivations, as
171 the differences between adjacent electrodes in the column direction.

172 **Signal processing**

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

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

186 **Motor unit decomposition and analysis.** The EMG signals recorded during the
187 submaximal isometric contractions (from 10% to 70% MVT) were decomposed offline with a
188 method that has been extensively validated (25). The signals were decomposed throughout the
189 entire duration of the submaximal contractions, and the discharge times of the identified motor
190 units were converted in binary spike trains (18). The accuracy of the decomposition was tested
191 with the silhouette measure, which was set to 0.90. The mean discharge rate and the discharge
192 rate variability (CoV of the interspike interval [CoV_{isi}] see below for details) were calculated
193 during the stable plateau region of the torque signal. Recruitment thresholds for each motor unit

194 were defined as the torque (%MVT) at the times when the motor unit began discharging action
195 potentials. Discharge rate at recruitment was calculated from the first six motor unit discharges.
196 Discharges that were separated from the next by <33.3 or >250 ms (30 and 4 pps, respectively)
197 (18) were corrected and edited manually by an experienced operator using a custom algorithm.

198 **Motor unit tracking.**

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

208 **Estimates of synaptic input.** The amount of synaptic input received by the vasti
209 muscles was investigated with a method previously suggested by Martinez-Valdes et al. (27).
210 Here, the total synaptic input received by the vasti muscles (which is reflected by changes in
211 motor unit firing properties) represents the sum of all sources of input to motor neurons, such
212 an increase in descending drive from supra-spinal centers (26), as well as afferent Ia input (23),
213 among others. A difference in synaptic input received by the motor neuron pools of the two
214 muscles can be estimated by the difference in the relative rate of increase in discharge rate
215 between motor units in the two muscles. Hence, the discharge rate of motor units with the same
216 recruitment thresholds (i.e., with a difference in threshold 0.5% MVC) in the two muscles was
217 used as a measure to compare the synaptic inputs received by the pools of motor neurons. This
218 measure corresponds to the increase in discharge rate from recruitment to the target torque
219 relative to the increase in torque from the recruitment threshold [target torque (10, 30, 50, and
220 70% MVC) minus recruitment threshold torque].

221

222 **Statistical analysis**

223 Statistical analysis was performed in R (ver 3.5.2, R Development Core Team, 2009).
224 To analyse motor units behaviour, we performed a multilevel mixed linear regression analysis
225 through the package *lme4* Version 1.1.19 (2). Linear mixed effects models are particularly
226 suitable in this experimental design since: 1) they allow the whole sample of extracted motor
227 units to be analyzed and not just the mean observations for each subject and condition. This

228 allows a better evaluation of data variations than conventional ANOVA statistics; 2) they
229 account for the non-independence of observations (e.g. observations from the same subjects)
230 with correlated error. This is particularly useful in such a repeated-measure study because it has
231 been demonstrated that motor unit discharge data is correlated within a subject even across
232 testing days (32), 3) they separately treat the effects caused by the experimental manipulation
233 (fixed effects) and those that were not (random effects).

234

235 **Torque**

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

240

241 **EMG amplitude**

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

245

246 **Motor unit rate coding**

247 Mean discharge rate of motor units was analysed with a linear mixed effects model,
248 using the within-subject fixed effects muscle, exercise, and mean torque, as test variables, and
249 the discharge rate at recruitment and recruitment threshold, as control variables. In such a way
250 it is possible to characterize the discharge rate during the stable part of the contraction (i.e. at \approx
251 10, 30, 50, and 70% MVT) controlling for the discharge rate at recruitment and motor unit
252 recruitment threshold. We considered the random intercept over participants and the random
253 slope of exercise, muscle, and torque over participants as random factors. Each likelihood ratio
254 tests showed that random slope models (subject-specific slopes for the fixed effects exercise,
255 muscle, and torque) significantly improved the model, so we constructed random slope models.
256 Statistical significance of fixed effects was determined using type III Wald F tests with
257 Kenward–Roger degrees of freedom and the ANOVA function from R's *car* package (ver.
258 3.0.3).

259

260 $discharge\ rate \sim muscle \times exercise \times torque \times (exercise + muscle + torque | subject) +$
261 $discharge\ rate\ at\ recruitment + recruitment\ threshold$

262

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

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

275

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

277 Linear regression was used to characterize the association for each motor unit between
278 the differences in discharge rate at the target torque (mean discharge rate at \approx 10, 30, 50, and
279 70% MVT) and at recruitment and between the torque achieved during the stable part of the
280 contraction (i.e. \approx 10, 30, 50, and 70% MVT) and motor unit recruitment threshold. The slopes
281 of these linear regressions were compared between the two muscles by analysis of covariance
282 as done previously (19).

283

284 **RESULTS**

285 **Torque**

286 The torque exerted during the MVT was lower in the knee extension exercise (188 ± 35
287 Nm) compared to the leg press (263 ± 88 Nm, $P = 0.007$). The amount of torque fluctuations was
288 similar between the two tasks. Indeed, the coefficient of variation of torque was not different
289 ($P = 0.259$) between the knee extension exercise ($M = 3.2\%$, $SE = 0.2\%$) and leg press ($M =$
290 2.9% , $SE = 0.2\%$) and across torque levels ($P = 0.358$).

291

292 **Normalized EMG amplitude**

293 A representative example of the EMG signals recorded from the VL is reported in Figure
294 1, left panel. The estimates of normalized ARV for VM and VL are reported in Figure 2. As
295 expected, normalized ARV increased with increasing torque ($F = 3817.3$, $P < 0.0001$). In
296 general, the knee extension exercise was associated with greater normalized ARV at high torque
297 levels, without any difference between muscles. Indeed, there was an exercise \times torque
298 interaction ($F = 82.1$, $P < 0.0001$), indicating that the knee extension exercise induced greater
299 overall vasti activation (i.e. combining VM and VL ARV) than the leg press exercise at 50 (M
300 = 0.11, SE = 0.01, $P = 0.0003$) and 70% MVT (M = 0.17, SE = 0.20, $P < 0.0001$) but not at
301 lower torque levels. However, no differences between muscles were found ($F = 1.8$, $P = 0.179$).

302

303 --- Figure 2 about here ---

304

305 The level of antagonist activation was not different between exercise tasks ($F = 0.3$, P
306 = 0.573). However, the level of antagonist activation increased at increasing torque and on
307 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
308 (SE = 1.4 μV), at 10, 30, 50, and 70% of MVT, respectively.

309

310 **Motor unit population data**

311 The total number of decomposed motor units across the different torque levels and
312 sessions was between 1059 and 1172, for the VM and VL, respectively. Thus, for each subject
313 and torque level, an average of 10 ± 3 and 11 ± 4 motor units were extracted for VM and VL,
314 respectively. A representative example of the results of motor unit decomposition is reported
315 in Fig. 1 (mid and right panel).

316

317 **Recruitment threshold.** The recruitment threshold descriptive statistics are reported in
318 Table 1. Recruitment threshold increased with increasing torque ($F = 14046$, $P < 0.0001$). At
319 high torque levels the recruitment threshold was higher for knee extension compared to the leg
320 press: this difference was more pronounced in VM than in VL. This was indicated by the muscle
321 \times exercise \times torque interaction ($F = 4.6$, $P < 0.031$). Post hoc tests showed that for the VM,
322 higher recruitment thresholds were recorded during knee extension compared to the leg press
323 at 50% (knee extension – leg press: M = 4.6 %, SE = 0.7%, $P < 0.0001$) and 70% (knee
324 extension – leg press: M = 7.5 %, SE = 0.7 %, $P < 0.0001$). Likewise, the knee extension
325 exercise was associated with higher VL recruitment thresholds compared to the leg press, but
326 the magnitude of difference was smaller both at 50% (knee extension – leg press: M = 3.3 %,

327 SE = 0.5 %, P < 0.0011) and 70% (knee extension – leg press: M = 5.2 %, SE = 0.7 %, P <
328 0.0001).

329

330 --- Table 1 about here ---

331

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

345

346 --- Figure 3 about here ---

347

348 **COV of interspike interval.** The COV_{isi} increased with torque (F = 221.1, P < 0.0001):
349 being 12.1%, SE = 0.5%; 13.4%, SE = 0.5%; 14.5%, SE = 0.5%; 15.7%, SE = 0.5%; for 10,
350 30, 50, and 70% of MVT, respectively. No other difference for muscle or exercise type emerged
351 (all P values > 0.18).

352

353 **Tracked motor unit data**

354 The number of tracked motor units across testing sessions was between 165 and 101 for
355 VM and VL, respectively. Thus, for each subject and condition an average of 3.1±1.0 and
356 1.9±0.7 motor units were tracked for VM and VL, respectively. The cross-correlation values
357 from the projecting vectors of the tracked motor units was 0.84±0.04 and 0.80±0.04 for VM
358 and VL respectively. The results of tracked motor units confirmed the results from the group
359 level analysis. When controlling for discharge rate at recruitment and recruitment threshold, the

360 mean motor unit discharge rate increased with increasing torque ($F = 951.9, P < 0.0001$).
361 Similar to the group level findings, when controlling for recruitment threshold and discharge
362 rate at recruitment, the motor unit discharge rates were higher during the knee extension
363 exercise compared to the leg press at torque levels $\geq 50\%$ of MVT as indicated by the exercise
364 \times torque interaction ($F = 272.9, P < 0.0001$). Since there was no difference between muscles (F
365 $= 0.4, P = 0.50$), the post hoc tests are reported on the merged data from VM and VL. When
366 controlling for recruitment threshold and discharge rate at recruitment, the knee extension
367 exercise showed higher motor unit discharge rates compared to the leg press at 50% ($M = 1.1$
368 pps, $SE = 0.3$ pps, $P = 0.0318$) and 70% ($M = 1.7$ pps, $SE = 0.3$ pps, $P = 0.0007$) of MVT. The
369 control variables recruitment threshold ($F = 571.4, P < 0.0001$) and discharge rate at recruitment
370 ($F = 204.9, P < 0.0001$) significantly affected the discharge rates of the tracked motor units.

371 **COV of interspike interval.** The COV_{isi} of the tracked motor units increased with
372 torque ($F = 30.7, P < 0.0001$) and on average was 12.5%, $SE = 0.7\%$; 13.6%, $SE = 0.5\%$; 13.8%,
373 $SE = 0.5\%$; 14.8%, $SE = 0.8\%$; for 10, 30, 50, and 70% of MVT, respectively. No other
374 difference for muscle or exercise emerged (all P values > 0.11).

375

376 **Estimate of synaptic input**

377 **Comparison between muscles.** For each subject and exercise, an average of 5, 6, 6,
378 and 3 motor units were matched (by recruitment threshold) between VM and VL at 10, 30, 50,
379 and 70% of MVT, respectively. The linear regressions between the increase in discharge rate
380 from recruitment to the target torque relative to the increase in torque from the recruitment
381 threshold are reported in Figure 4. At 10% MVT (Figure 4A and 4E) both muscles showed a
382 regression non-different from constant value (both muscles and exercises $P > 0.123$). For all
383 other contraction levels (except for leg press at 70% MVT, VM: $P = 0.834$, VL: $P = 0.481$, see
384 Figure 4H) both vasti muscles showed a regression line which was different from the constant
385 value (all P values < 0.021 , see Figure 4B, 4C, 4D, 4F and 4G). However, the intercept (all P
386 values > 0.291) and slope (all P values > 0.302) were not different between muscles for either
387 exercise at any of the contraction levels.

388 **Comparison between exercises.** At 10% MVT, both exercises showed a regression
389 non-different from constant value (both muscles and exercises $P > 0.329$, see Figure 5A). For
390 all other contraction levels (except for the leg press exercise at 70% MVT, $P = 0.530$, see Figure
391 5B, 5C and 5D), both exercises showed regression line different from constant value (all P

392 values < 0.012). Nonetheless, the intercept was different only at 30% ($P = 0.016$, see Figure
393 5B); the slope was steeper in knee extension than leg press at 50% ($P = 0.023$, Figure 5C) and
394 70% ($P = 0.038$, Figure 5D) of MVT.

395 **DISCUSSION**

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

407

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

409 Previously, the ratio between the activation (i.e. the EMG amplitude) of the VM and VL
410 has been used to assess differences in the contribution of each muscle in different exercises
411 (28). This approach has led to conflicting results (28), with some studies showing greater
412 relative activation of VM compared to VL during closed kinetic chain exercises (e.g. squat and
413 lunge) compared to open kinetic chain exercises (e.g. knee extension) (11, 31) but with others
414 showing no difference (29, 30). While the protocols adopted in these studies may differ from
415 each other for some aspects (namely, subject position, knee angle, etc.), we suggest that these
416 conflicting results are mainly due to limitations of classic bipolar surface EMG methods.
417 Indeed, bipolar surface EMG can be unreliable and influenced by many factors including
418 electrode positioning, thereby reducing the accuracy of amplitude estimates to effectively infer
419 changes in synaptic input (22). Bipolar recordings may under- or over-estimate EMG amplitude
420 because of the uneven distribution of action potentials within the muscle volume (8). In contrast,
421 the HDEMG used in this study provides a superior representation of muscle activation
422 compared to bipolar EMG since the greater number of EMG channels (59 bipolar EMG
423 channels) provides a more representative estimate of muscle activity, increasing the reliability
424 and sensitivity of EMG amplitude parameters. Using this approach, we found very little

425 difference in VM and VL behaviour between the two exercise types (Figure 2). These findings
426 suggest that the activation of the VM and VL did not differ between the two exercises.
427 Nevertheless, analysis of EMG amplitude between the VM and VL cannot be used to infer the
428 synaptic input received by the two muscles (19). For these reasons, the analysis of motor unit
429 firing properties is fundamental to investigate the synaptic input received by muscles.

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

449

450 **Knee extension vs. leg press**

451 The two tasks investigated in this study constitute the isometric version of two popular
452 exercises in clinical and sport settings. They are intrinsically different from many points of
453 view. The knee extension task is a single-joint exercise involving a relatively small amount of
454 muscle mass (mainly the knee extensors) while the leg press is a multi-joint exercise involving
455 more muscles, such as the hip extensors. From the standpoint of torque-vector direction, in the
456 knee extension exercise the torque is directed perpendicularly to the tibia, while in leg press the
457 torque is directed parallel to the tibia. For this reason, the leg press tends to produce lower shear
458 forces and higher compression forces at the knee. Finally, the knee extension is considered an

459 open kinetic chain exercise, while the leg press is a closed kinetic chain exercise. Anecdotally,
460 single-joint/open kinetic chain exercises are thought to induce higher muscle activation
461 compared to multi-joint/closed kinetic chain exercises (21). While it seems reasonable that
462 targeting a specific muscle with a single-joint exercise may result in higher activation, the
463 available literature on this topic is conflicting. While some studies have reported higher vasti
464 EMG amplitude during single-joint compared to multi-joint tasks (11, 29) others studies
465 reported no difference (30, 31). As mentioned above, the most likely cause of such conflicting
466 results are the methodological drawbacks of interference EMG analysis.

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

474 When controlling for discharge rate at recruitment and recruitment threshold, the
475 average motor unit discharge rate was greater in knee extension exercise than the leg press at
476 50 and 70% of MVT (Figure 3). The possibility to track the motor units between the two
477 sessions allowed us to monitor the behaviour of individual motor units across the two exercises.
478 This analysis confirmed that motor unit discharge rate was higher in knee extension than the
479 leg press at 50 and 70% of MVT. The same finding come from the analysis of the synaptic input
480 (Figure 5): the regression lines between delta discharge rate and delta torque showed
481 significantly steeper slope in the knee extension exercise compared to the leg press at 50 and
482 70% MVT. Together, these findings suggested that the synaptic input received by the motor
483 unit pool was greater in the knee extension exercise. A reduction in net synaptic input in the leg
484 press exercise could be attributed to a decrease in excitatory input and/or an increase in
485 inhibitory input to motoneurons (13). On the one hand, a greater antagonist activation may
486 induce an inhibition of agonist muscles, but this seems not to be the case since the activity of
487 the hamstrings did not differ between tasks. However, it is difficult to exclude potential
488 inhibition on the sole basis of the EMG amplitude of the antagonist muscles. In any case, multi-
489 joint exercise implies a larger muscle mass acting to accomplish the task and therefore the load
490 is shared between knee extensors and hip extensors which may reduce the demand on the knee
491 extensors. On the other hand, the higher synaptic input to vasti muscles may be explained by
492 the fact that the torque-vector for knee extension may be more favourable to the activation of

493 the vasti muscles compared to that of the leg press (4). Indeed, the muscle contributions in
494 multi-joint tasks are directionally tuned and combined to produce the movement in the desired
495 direction (24). Thus, in a leg press the activation of the vasti may be modulated in favour of the
496 hip extensors. The observed difference between the exercises emerged at the higher torque
497 levels only which suggests that an increased synaptic input mostly affected high threshold
498 motor units. This confirms the necessity to investigate the motor unit rate coding across the
499 whole range of submaximal contractions since some changes may not be observed for the lower
500 threshold motor units (Martinez-Valdes 2017).

501

502 **Limitations**

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

521

522 **Conclusions**

523 The synaptic input received by VM and VL was similar and their relative contribution
524 was not affected by exercise type. Hence, attempting to change the contribution of either the
525 VM or VL via exercise selection does not seem to be a viable strategy. However, open kinetic
526 chain knee extension was associated with overall greater synaptic input to vasti muscles. This

527 finding suggests a single-joint knee extension is more suitable than a multi-joint leg press
528 exercise to increase the activation of the vasti muscles.

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Table 1 – Descriptive statistics of motor unit recruitment threshold, expressed as % of maximal voluntary torque (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)

Captions

Figure 1. The left panel displays representative examples of raw EMG signals (5 columns and 12 lines) recorded from the vastus lateralis at 70% of maximal voluntary contraction (MVT). In the middle panel, the instantaneous discharges of 13 motor units are reported as vertical lines. The torque signal is reported as the black line. In the right panel, the 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 vastus medialis (VM), vastus lateralis (VL).

Figure 3. Estimates (with 95% confidence intervals) of motor unit discharge rates are reported for vastus medialis (VM) and 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 (dark blue) and leg press (light 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.

FIGURE 1

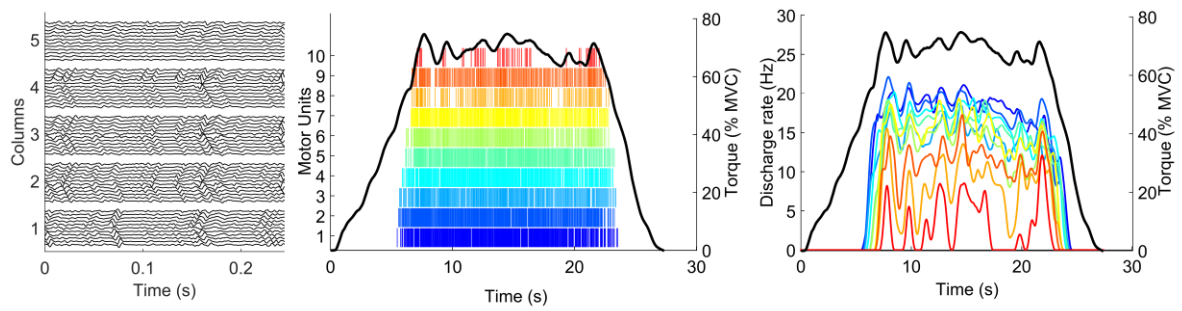


FIGURE 2

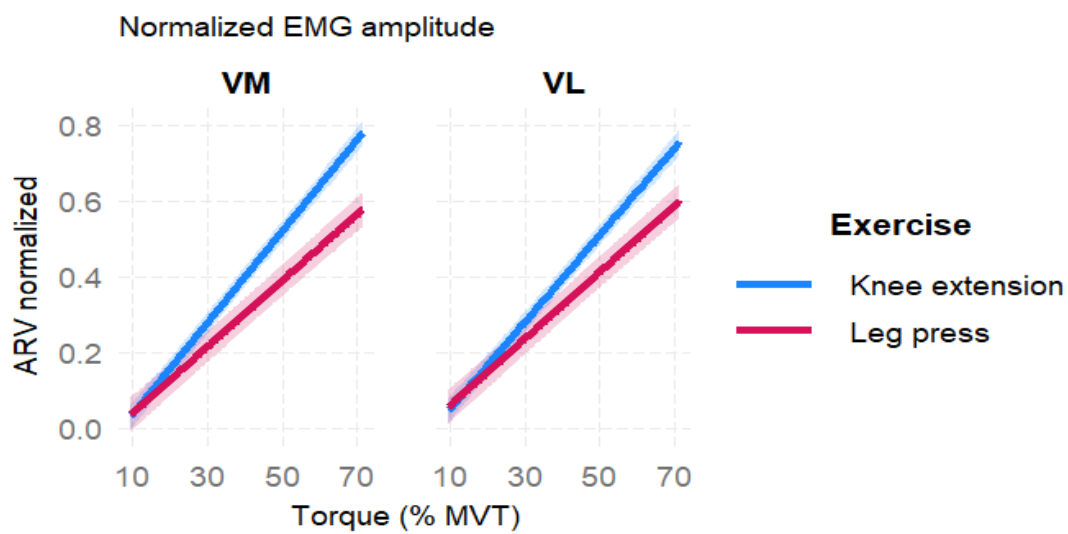


FIGURE 3

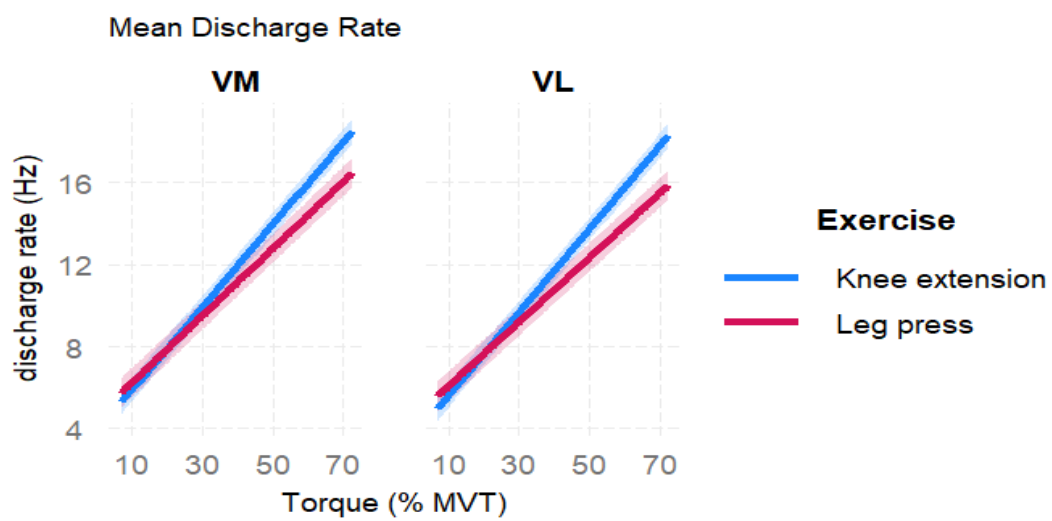


FIGURE 4

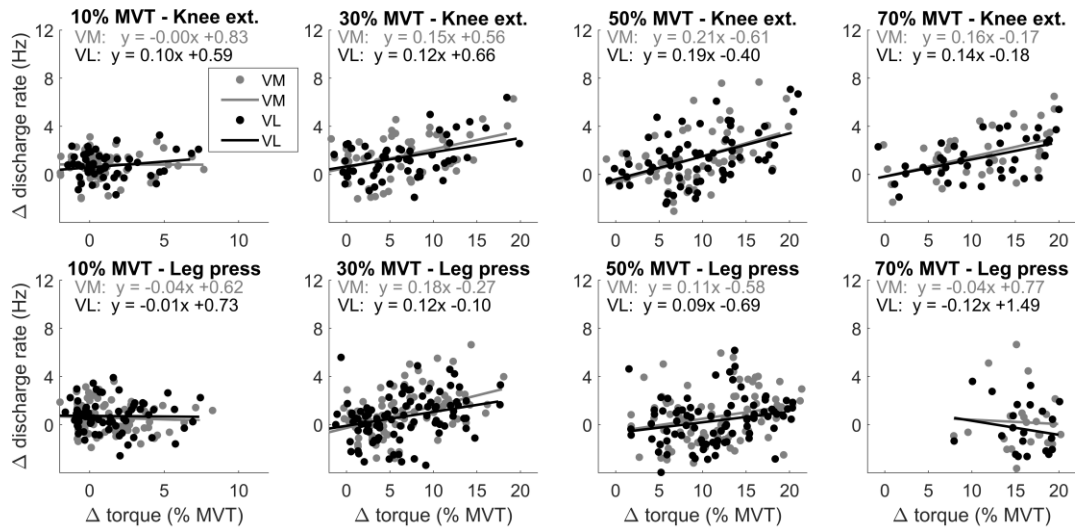


FIGURE 5

