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Original

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(Article begins on next page)

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Title: Does the activity of ankle plantar flexors differ between limbs while healthy, young subjects stand at ease?

Article Type: Short Communication (max 2000 words)

Keywords: postural control, electromyography, center of pressure, triceps surae, standing.

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Abstract: Inferences on the active contribution of plantar flexors to the stabilisation of human standing posture have been drawn from surface electromyograms (EMGs). Surface EMGs were however often detected unilaterally, presuming the myoelectric activity from muscles in a single leg reflects the pattern of muscle activation in both legs. In this study we question whether surface EMGs detected from plantar flexor muscles in both legs provide equal estimates of the duration of activity. Arrays of surface electrodes were used to collect EMGs from gastrocnemius and soleus muscles while twelve, young male participants stood at ease for 60 s. Muscles in each leg were deemed active whenever the Root Mean Square amplitude of EMGs (40ms epochs) detected by any channel in the arrays exceeded the noise level, defined from EMGs detected during rest. The Chi-Square statistics revealed significant differences in the relative number of active periods for both muscles in 10 out of 12 participants tested, ranging from 2% to 65% ($\chi^2 > 17.90$; $P < 0.01$). Pearson correlation analysis indicated side differences in the duration of gastrocnemius though not soleus activity were associated with the centre of pressure mean, lateral position ($R = 0.60$; $P = 0.035$). These results suggest therefore that surface EMGs may provide different estimates of the timing of plantar flexors' activity if collected unilaterally during standing and that asymmetric activation may be not necessarily associated with weight distribution between limbs. Depending on the body side from which EMGs are collected, the active contribution of plantar flexors to standing stabilization may be either under- or over-valued.

Dear Editor-in-chief

We respectfully submit to your attention the revised manuscript: “Does the activity of ankle plantar flexors differ between limbs while healthy, young subjects stand at ease?” This manuscript was submitted as a Short Communication and it contains new interpretations and relevant experimental information on how young, healthy individuals activate their plantar flexors during standing. Our manuscript has four figures and the word count is currently 2000.

In the revised manuscript we addressed the main concerns raised by reviewers. More specifically, the reviewers suggested some minor revisions to clarify our study. A list of points of how we have responded to the reviewers’ suggestions was uploaded with the revised manuscript. We appreciate the comments raised by both reviewers and believe they assisted us in producing a stronger manuscript.

Other information: this manuscript is authored by FV dos Anjos, M Gazzoni and TM Vieira. The material within has not been and will not be submitted for publication elsewhere except as an abstract or as part of academic thesis. All the authors contributed substantially to all of the following areas indicated: i) the conception and design of the study, or acquisition of data, or analysis and interpretation of data; ii) drafting the article or revising it critically for important intellectual content; iii) final approval of the version to be submitted.

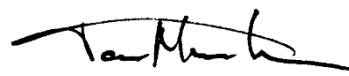
We look forward to your acknowledgment and we would like to thank the reviewers for their thoughtful comments about the manuscript. We thank you very much for your attention.



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RESPONSE TO REVIEWERS' COMMENTS

Manuscript: Does the activity of ankle plantar flexors differ between limbs while healthy, young subjects stand at ease?

Date: 13/09/2018

REVIEWER #1: The authors addressed all my concerns.

REVIEWER #2:

The authors have submitted a revised short communication on the comparison of calf muscle activation between left and right sides during standing. I think this study is interesting and relevant. Many biomechanics studies assume symmetry particularly in healthy individuals. I think this study highlights that this is not necessarily the case. I think further work could be done for this manuscript.

We appreciate the reviewer for the comment. We are pleased to know our message has been well received and we believe the comments from both reviewers assisted us in producing a strong manuscript.

1) The authors only evaluate the task of standing with eyes open, this does not challenge the subjects balance, which may explain why the within-subject variation is large as small sways elicit comparatively large changes in muscle activation. Also this explains why the authors see a modest correlation to the CoP. Perhaps additional task to challenge the subjects balance could have been used, such as standing eyes closed, balance on single leg ect.

We thank the reviewer for the comment. Although we strongly value the importance of exploring the effect of additional standing tasks on muscle activity, inferences on the optimal neuromuscular mechanisms of posture control have been often drawn from the calf muscles' EMGs while subjects stand with eyes open. Thus, our focus was on side differences in the plantar flexors' activity during this reference, standing condition.

We would like to add that we are currently unable to identify the contribution of different sources of variability within subjects. We agree that a more demanding task would possibly lead to greater consistency of plantar flexors' activation within subjects. Nevertheless, given we are unsure on how much more demanding the standing task should be, we feel it would be too speculative to advance that more demanding variants of standing could reduce the variability within subjects. It should be noted though that this variability further aggravates the issue we raise in the manuscript; i.e., unilateral sampling may provide unrepresentative EMGs from the calf muscles during standing.

2) The authors have investigated the differences in activation timing during standing. However, the level of activation is not considered in the analysis and warrants identification in the discussion section as a limitation of the study.

We thank the reviewer for this suggestion. We slightly amended discussion to accommodate the reviewer suggestion (Lines 208-218).

“First, we would like to mention we assessed asymmetries in the timing of activity, although we acknowledge the importance of quantifying the degree of muscle activity during standing. By averaging the amplitude of EMGs across the whole standing duration, a biased indication on the degree of activity would be provided; low amplitude may not indicate low activation but e.g. longer inactive than active periods (Dos Anjos et al., 2017). Moreover, the timing of muscle activity has provided substantial contribution to our understanding of the human, postural control (Di Giulio et al., 2009; Laughton et al., 2003). Finally, it should be noted we were able to account for spatial differences in the timing of activity within plantar flexors with arrays of electrodes (Fig. 2; see also Dos Anjos et al., 2017), providing representative estimations of side differences in the timing of activity.”

3) The result of activity identified in different regions of the muscle is particularly interesting, however is not explored in any detail in this manuscript.

We thank the reviewer for raising this point. In agreement with the reviewer suggestion, we added a brief statement on the importance of sampling EMGs with multiple electrodes from plantar flexors (Lines 208-218).

1 **Title**

2 Does the activity of ankle plantar flexors differ between limbs while healthy, young
3 subjects stand at ease?

4

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19 **Notes**

20 - We are submitting this work as a Short communication.

21 - Word count (Introduction through Acknowledgments): 2.000.

22

23

24

25

26

27 **Abstract**

28 Inferences on the active contribution of plantar flexors to the stabilisation of human
29 standing posture have been drawn from surface electromyograms (EMGs). Surface
30 EMGs were however often detected unilaterally, presuming the myoelectric activity from
31 muscles in a single leg reflects the pattern of muscle activation in both legs. In this study
32 we question whether surface EMGs detected from plantar flexor muscles in both legs
33 provide equal estimates of the duration of activity. Arrays of surface electrodes were
34 used to collect EMGs from gastrocnemius and soleus muscles while twelve, young male
35 participants stood at ease for 60 s. Muscles in each leg were deemed active whenever
36 the Root Mean Square amplitude of EMGs (40ms epochs) detected by any channel in
37 the arrays exceeded the noise level, defined from EMGs detected during rest. The Chi-
38 Square statistics revealed significant differences in the relative number of active periods
39 for both muscles in 10 out of 12 participants tested, ranging from 2% to 65% ($\chi^2 > 17.90$;
40 $P < 0.01$). Pearson correlation analysis indicated side differences in the duration of
41 gastrocnemius though not soleus activity were associated with the centre of pressure
42 mean, lateral position ($R = 0.60$; $P = 0.035$). These results suggest therefore that surface
43 EMGs may provide different estimates of the timing of plantar flexors' activity if collected
44 unilaterally during standing and that asymmetric activation may be not necessarily
45 associated with weight distribution between limbs. Depending on the body side from
46 which EMGs are collected, the active contribution of plantar flexors to standing
47 stabilization may be either under- or over-valued.

48

49 **Keywords:** postural control, electromyography, center of pressure, triceps surae,
50 standing.

51

52 **1.Introduction**

53 Insights into the neuromuscular mechanisms underpinning the control of human
54 standing posture have been gained from surface electromyograms (EMGs; Di Giulio et
55 al., 2009; Heroux et al., 2014; Gatev et al., 1999). While this evidence substantiates the
56 potential relevance of surface electromyography, inferences on the neuromuscular
57 determinants of posture control have been often drawn from EMGs collected from calf
58 muscles in either left or right leg. Controversial results suggest however EMGs collected
59 unilaterally may provide a biased indication on the postural activation of plantar flexors.
60 For example, although Masani et al. (2013) reported the left and right plantar flexors are
61 activated equally during standing, others detected EMGs with different amplitudes
62 between legs (Liang et al., 2016; Mochizuki et al., 2007). These controversies may lie in
63 the local, postural activation of ankle extensors (Hodson-Tole et al., 2013; Vieira et al.,
64 2011). It seems therefore relevant to ask whether inferences on the activation of plantar
65 flexors may be drawn from EMGs collected unilaterally during standing.

66

67 In this study we specifically ask: are the left and right plantar flexors activated for equal
68 durations during standing? Instances of muscle activation were estimated from multiple
69 surface EMGs, providing a more representative view of activity of the whole muscle (Dos
70 Anjos et al., 2017). To our knowledge this is the first study to systematically evaluate the
71 bilateral representation of plantar flexors' myoelectric activity during standing.

72

73 **2.Methods**

74 *2.1. Participants*

75 Twelve male volunteers (range: 24-34years; 60-90kg; 1.70-1.87m) were recruited after
76 providing written, informed consent. Experimental procedures conformed with the
77 *Declaration of Helsinki* and were approved by the Local Ethics Committee.

78

79 *2.2. Experimental protocol*

80 Two different tasks were applied. First, subjects relaxed their muscles completely while
81 in supine position. Surface EMGs collected at this condition were considered to set the
82 background, noise level. Second, subjects stood barefoot on a force plate for 60 s, with
83 eyes open, arms alongside the body and feet in a comfortable position (Fig. 1). Feet
84 contours were drawn to ensure participants would keep the same stance throughout
85 experiments. Subjects were engaged in active conversation to suppress any voluntary
86 control of calf muscles' activity during standing (Loram and Lakie, 2002a). The second
87 task was applied three times, with 2 min intervals in-between.

88

89 *2.3. Data acquisition*

90 Single-differential EMGs were collected from the soleus and medial gastrocnemius
91 muscles of both legs with linear arrays electrodes. Ground reactions forces were
92 sampled synchronously with EMGs. See Appendix for description on electrodes'
93 positioning (Fig 1A) and centre of pressure (CoP) computation.

94

95 *2.4. Assessment of muscle activity*

96 Initially, EMGs were visually inspected for the identification of channels with contact
97 problems or power line interference; 13 out of 432 channels were discarded. Specifically
98 for gastrocnemius, the distal channels sampling from the same muscle fibres were
99 excluded (cf. Fig. 1 in Hodson-Tole et al., 2013). EMGs were then band-pass filtered

100 (15–350Hz cut-off; 4th order Butterworth bidirectional filter) and the Root Mean Square
101 (RMS) amplitude was computed over 40 ms epochs (Laughton et al., 2003), providing a
102 total of 1,500 RMS values per channel.

103

104 The duration of muscle activity was estimated from RMS values. First, the background
105 level was set as the mean plus three standard deviations calculated over 3s of rest (40
106 ms epochs; Laughton et al., 2003), ensuring minimal, if any, false positives (Di Fabio
107 1987). As multiple EMGs were collected from each muscle, the background level was
108 defined separately for each channel. Muscles active-inactive states were therefore
109 assigned for each channel and were processed through the logical disjunction (“Or”) to
110 provide a series of *active* instances; whenever the RMS amplitude of any channel in a
111 given array exceeded the background level, the corresponding muscle was deemed
112 *active* (cf. Fig. 2 in Dos Anjos et al., 2017). Finally, the duration of muscle activity for
113 each leg was computed by counting the relative number of *active* periods during the
114 whole standing test. The duration of muscle activity was averaged across the three
115 standing tasks for statistical analysis.

116

117 *2.5. Statistical Analysis*

118 The Chi-square (χ^2 ; Dawson-Saunders and Trapp, 1994) test was applied separately for
119 each muscle and subject to test for whether the proportion of *active* periods between
120 limbs and muscles was similar during standing. Within-subjects variability was assessed
121 with the coefficient of variation (CoV) of the absolute right-left differences in the
122 proportion of active periods. After ensuring the data Gaussian distribution (Shapiro-
123 Wilk’s test, $P>0.23$), Pearson correlation was applied to verify whether asymmetries in
124 the duration of activity (i.e., right/left ratio of the number of *active* periods) were
125 associated with the CoP mean lateral position.

126

127 **3.Results**

128 *3.1.Side differences in plantar flexors' activity*

129 Activation periods obtained from multiple surface EMGs were not the same. As shown
130 for a representative participant (Fig. 2A), gastrocnemius EMGs with relatively high
131 amplitude were detected distally. Close inspection of these EMGs further indicates that
132 action potentials of different motor units were detected from different regions, resulting in
133 the identification of different periods of activity across channels. Our procedure for
134 estimating the duration of activity was insensitive however to regional differences in
135 EMG amplitude; regardless of where action potentials were detected they were
136 considered to estimate periods of activity (cf. grey rectangles in Fig. 2B right panel).

137

138 Side differences in the duration of calf muscles' activity were observed for 10 out of 12
139 participants (Fig. 3). The absolute right-left difference in the duration of activity ranged
140 from 3.7% to 65.3% for gastrocnemius (Fig. 3A; $\chi^2 > 33.35$; $P < 0.01$) and from 2.0% to
141 37.2% for soleus (Fig. 3B; $\chi^2 > 17.90$; $P < 0.01$). Differences in the duration of activity were
142 not observed consistently for the same side and muscle; two and four participants
143 activated respectively more frequently the right gastrocnemius and soleus muscles
144 (circles and squares in Fig. 3). Although participants #5 and #6 activated the left and
145 right gastrocnemius for a similar duration (~50%; Fig. 3A), both muscles were
146 concurrently active during less than 30% of the time (grey rectangles in Fig. 3).
147 Regardless of the leg considered, soleus was generally active for a longer duration than
148 gastrocnemius ($\chi^2 > 4.19$ and $P < 0.04$ for 20 legs), except for participants #2 and #11 (left
149 leg) and subjects #4 and #5 (right leg). Within-subjects variability was on average
150 49.7% (20.6%–80.9%; CoV median and inter-quartile interval) for gastrocnemius and

151 48.0% (21.1%–84.7%) for soleus, indicating relatively high and somewhat moderate
152 repeatability of asymmetries in the duration of activity across trials.

153

154 *3.2. Correlation between CoP lateral position and asymmetric activity*

155 Associations between side-differences in *active* periods and CoP lateral position were
156 muscle dependent. Subjects whose CoP was on average located closer to the right leg
157 activated more frequently their right gastrocnemius (Fig. 4A). For soleus, no significant
158 correlation between asymmetric activity and CoP lateral position was observed (Fig. 4B).

159

160 **4. Discussion**

161 ***Were plantar flexors active for similar durations between legs?***

162 Individual results indicate gastrocnemius and soleus were active for different durations
163 between legs. We analysed subjects separately because there was no reason to
164 choose a grouping criterion. Our hypothesis that plantar flexors in both limbs would be
165 activated for different durations during standing was based on side-differences in the
166 amplitude of EMGs (Liang et al., 2016; Mochizuki et al., 2007). Even though subjects
167 may alternate weight distribution between limbs (Blaszczyk et al., 2000; Haddad et al.,
168 2011), we are not aware of any evidence suggesting subjects should activate
169 consistently muscles in either leg. Indeed, our results show some subjects activated for
170 longer durations the right plantar flexors whereas others showed the opposite (Fig. 3).
171 Similarly, given the low and variable intrinsic ankle stiffness across subjects (Loram and
172 Lakie, 2002b), there was no reason to expect different subjects would load equally
173 muscle in either side and that such loading would persist across trials. If standing is the
174 results of periodic, active compensations to unpredictable falls (Loram et al., 2005;
175 Bottaro et al., 2005), with minimization of muscle activity being the primary goal of the
176 postural control system (Kiemel et al., 2011), between and within-subjects variability is

177 not surprising. This possibly explains both the: i) variable side differences in the duration
178 of activity between subjects, ranging from 4% to 65% for gastrocnemius and from 2% to
179 40% for soleus; ii) large (~50%) CoV values across trials. Regardless of these inter and
180 intra-individual differences in the duration of activity, asymmetries in the duration of
181 gastrocnemius and soleus activity were generally observed (Fig. 3). Current results
182 seem therefore to support the notion that muscles in both limbs were elicited for different
183 durations during standing.

184

185 Side differences in the duration of activity differed between muscles (Fig. 3). Concerning
186 gastrocnemius, the duration of activity was associated with CoP lateral position; subjects
187 standing closer to the right leg activated for longer duration their right gastrocnemius
188 (Fig. 4). This observation is consistent with the gastrocnemius contribution to ankle
189 inversion torque (Lee and Piazza, 2008; Vieira et al., 2013). Similar reports for soleus
190 were not found, possibly because its line of action is directed more closely to the midline
191 of the foot than that of gastrocnemius (Lee and Piazza, 2008). Asymmetries in the
192 timing of gastrocnemius' activity though not of soleus were partly explained (36%; Fig. 4)
193 by lateral differences in CoP position, which may be associated with the uneven weight
194 distribution between limbs (Genthon et al., 2008). Corroborating this differential muscle
195 response, previous study observed the medial gastrocnemius responds to surface
196 translations directed over a larger, oblique range than soleus (cf. Fig. 3 in Henry et al.,
197 1998). When drawing considerations on differences between muscles from current
198 results, it should be noted we sample EMGs from a small, medial soleus region (Fig. 1).
199 As discussed by Agur et al. (2003), EMGs collected medially may reflect a predominant,
200 plantar flexion action. Regardless of the actual, predominant action of the soleus region
201 sampled here, asymmetries were observed (Fig. 3). Factors other than the uneven
202 loading of both limbs may thus explain side differences in the duration of plantar flexors'

203 activity. While the identification of these sources urges further investigation, current
204 results suggest inferences on muscle activation during standing may not proceed from
205 EMGs collected unilaterally.

206

207 ***What are the implications of asymmetric activation of plantar flexors?***

208 First, we would like to mention we assessed asymmetries in the timing of activity,
209 although we acknowledge the importance of quantifying the degree of muscle activity
210 during standing. By averaging the amplitude of EMGs across the whole standing
211 duration, a biased indication on the degree of activity would be provided; low amplitude
212 may not indicate low activation but e.g. longer inactive than active periods (Dos Anjos et
213 al., 2017). Moreover, the timing of muscle activity has provided substantial contribution
214 to our understanding of the human, postural control (Di Giulio et al., 2009; Laughton et
215 al., 2003). Finally, it should be noted we were able to account for spatial differences in
216 the timing of activity within plantar flexors with arrays of electrodes (Fig. 2; see also Dos
217 Anjos et al., 2017), providing representative estimations of side differences in the timing
218 of activity.

219

220 Our results (Fig. 3) indicate that surface EMGs detected bilaterally do not provide equal
221 estimates of the duration of plantar flexors' activity. Although these results are not in
222 contrast with the view that humans sway as an inverted pendulum, the inverted
223 pendulum assumption does not seem to justify stating ankle muscles in both legs are
224 activated similarly during standing (Fig. 3). According to current results, the active
225 participation of plantar flexors to the correction of bodily sways may be either under- or
226 over-valued, depending on the body side from which EMGs are detected. Drawing
227 inferences on the neural mechanisms governing the activation of plantar flexors during
228 standing may therefore require the detection of EMGs from both legs.

229 **Conflict of interest statement**

230 There were no known conflicts of interest associated with this work.

231

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238

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306 sagittal plane? Human Movement Science 32, 753–767.

307

308

309

310 **Figure Captions**

311 **Figure 1. Electrodes and feet positioning.**

312 *A*, shows the position of electrode arrays over the medial gastrocnemius and soleus
313 muscles in both legs. A schematic illustration of feet positioning on the force-plate is
314 shown in *B*. Foot length was calculated as the distance between the tip of the third
315 metatarsal head and the calcaneus bone. The distance between the centers of the
316 length of each foot was considered to define the lateral distance between feet and thus
317 the anterior-posterior (AP_{axis}) and medio-lateral (ML_{axis}) axes.

318

319 **Figure 2. Raw surface EMGs and active periods of plantar flexors.**

320 *A*, example of single-differential EMGs recorded by channels 3, 5 and 9 from the left and
321 the right medial gastrocnemius of a single, representative participant. *B*, shows an
322 expanded view (500 ms; dashed area) of the raw EMGs shown in *A*. Grey rectangles
323 indicate the *active* periods identified separately per channel and for all channels (grey
324 rectangles shown below EMGs; cf. Methods). Note different channels detected different
325 action potentials and therefore provided different *active* periods for the right
326 gastrocnemius. Percentages denote the relative number of *active* periods (i.e., duration
327 of muscle activity) throughout the whole (60 s) standing test.

328

329 **Figure 3. Asymmetries in the duration of plantar flexors' activity.**

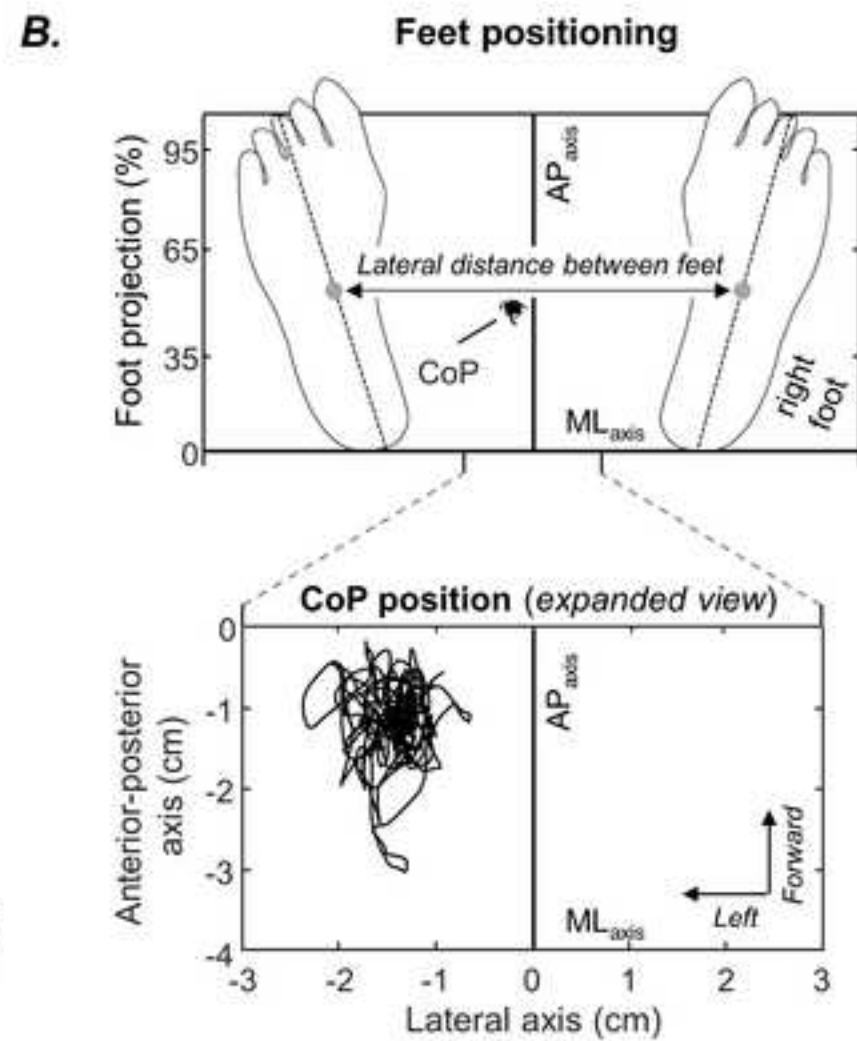
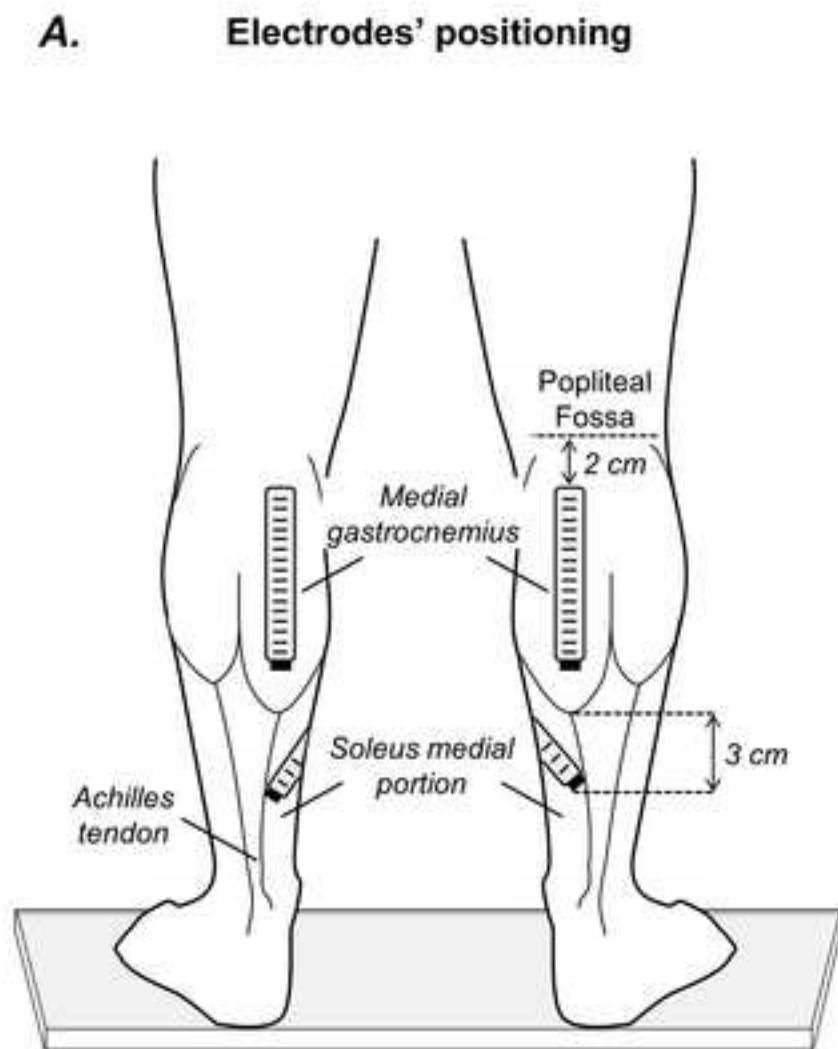
330 The relative duration of activity of the left (circles) and right (squares) medial
331 gastrocnemius (*A*) and soleus (*B*) muscles is shown for the 12 participants tested.
332 Vertical, grey rectangles indicate the relative amount of the standing duration within
333 which muscles in both legs were active concurrently. Asterisks indicate significant
334 differences in the duration of activity between the legs ($P < 0.05$).

335

336 **Figure 4. Side differences in the duration of muscle activity and centre of pressure**
337 **position.**

338 Scatter plots are shown, with the ratio (right/left) of the duration of medial gastrocnemius
339 (A) and soleus (B) activity plotted in the y axis and the centre of pressure (CoP) position
340 in the frontal plane plotted in the x axis. CoP position was normalised with respect to the
341 lateral distance between feet (cf. Fig. 1). Regression (dashed) lines were drawn for
342 clarity.

Figure 1
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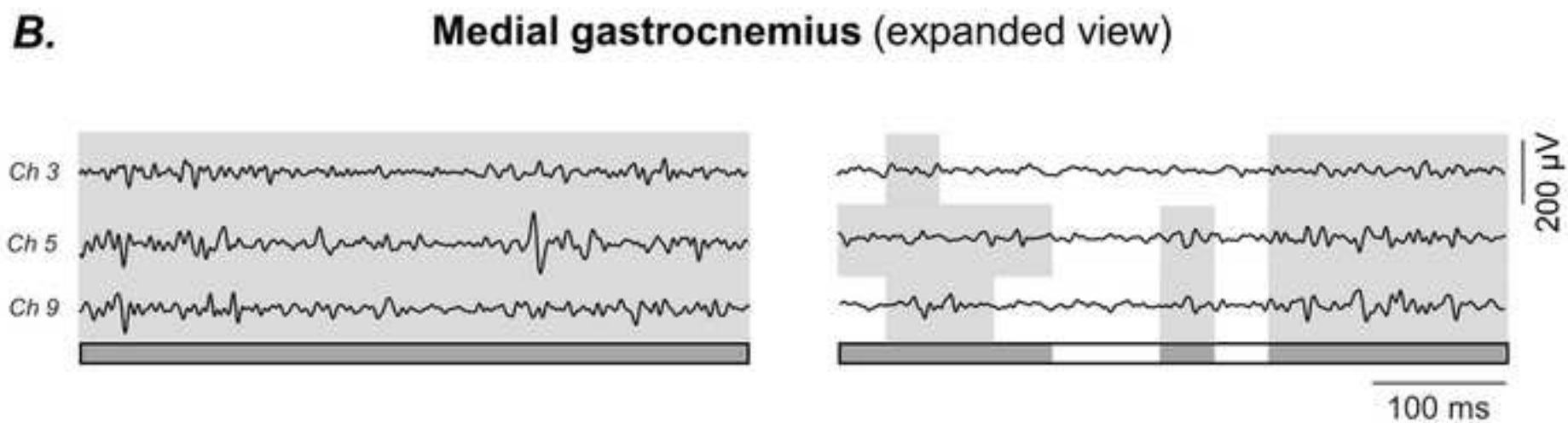
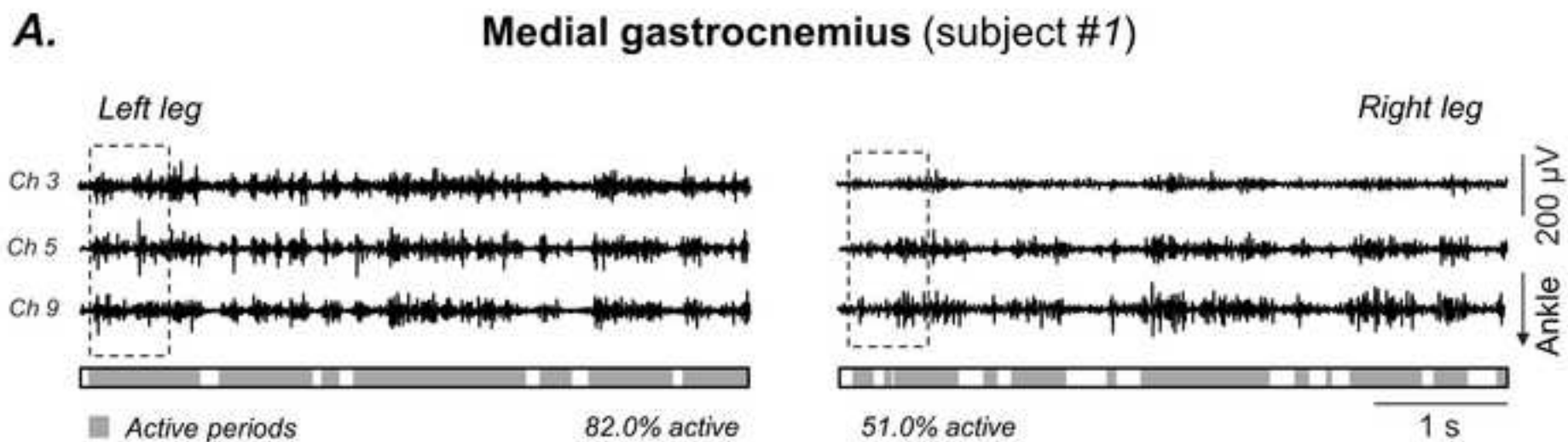
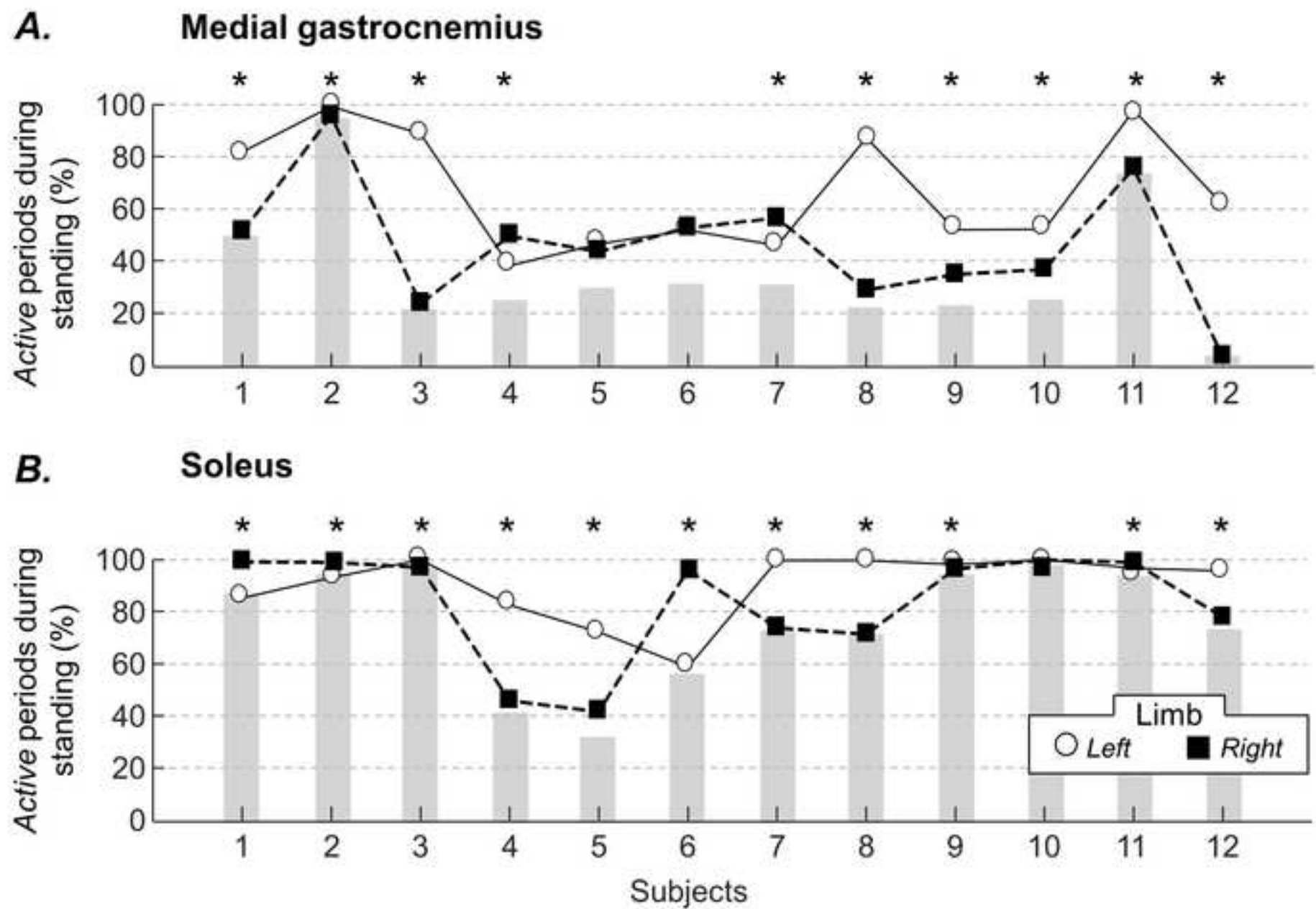
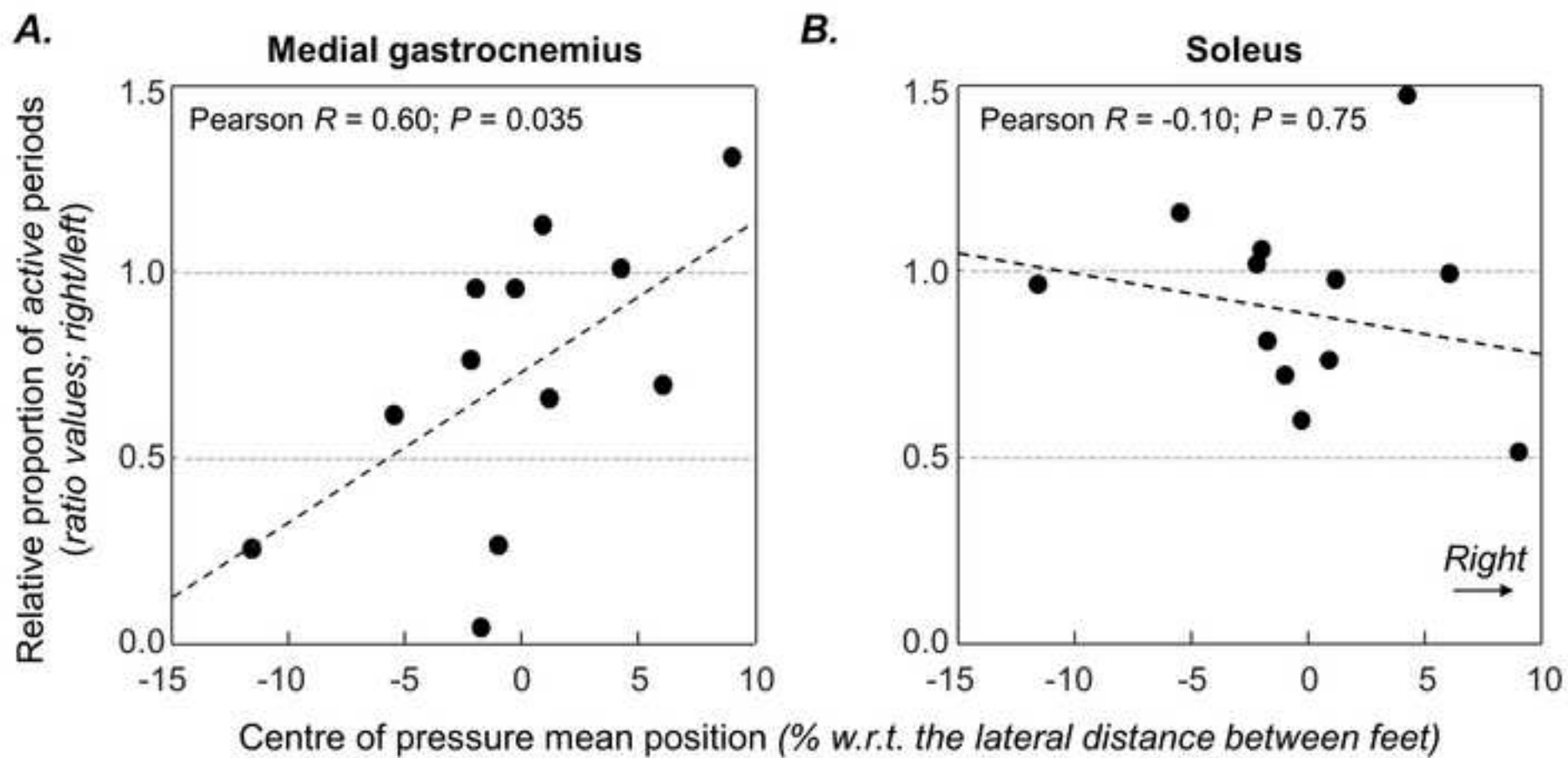


Figure 3
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Supplementary Material

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***Conflict of Interest Statement**

The authors declare that there were no known conflicts of interest associated with this work. The funders were not involved in the study design, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.