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# 1 **Influence of low muscle activation levels on the ankle torque and** 2 **muscle shear modulus during plantar flexor stretching**

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30

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32

33 **ABSTRACT**

34 During stretching studies, surface electromyography (sEMG) is used to ensure the passive state of the  
35 muscle, for the characterization of passive muscle mechanical properties. Different thresholds (1%, 2%  
36 or 5% of maximal) are indifferently used to set “passive state”. This study aimed to investigate the  
37 effects of a slight activity on the joint and muscle mechanical properties during stretching.

38 The joint torque and muscle shear modulus of the *triceps surae* muscles were measured in fifteen healthy  
39 volunteers during ankle dorsiflexions: i) in a “fully relaxed” state, ii) during active conditions where  
40 participants were asked to produce an sEMG amplitude of 1%, 2% or 5% of their maximal sEMG  
41 amplitude of the *triceps surae*. The 1% condition was the only that did not result in significant  
42 differences in joint torque or shear modulus compared to the relaxed condition. In the 2% condition,  
43 increases in joint torque were found at 80% of the maximal angle in dorsiflexion, and in the shear  
44 modulus of *gastrocnemius medialis* and *gastrocnemius lateralis* at the maximal angle in dorsiflexion.  
45 During the 5% condition, joint torque and the shear modulus of *gastrocnemius medialis* were higher  
46 than during relaxed condition at angles larger than 40% of maximal angle in dorsiflexion. The results  
47 provide new insights on the thresholds that should be considered for the design of stretching studies. A  
48 threshold of 1% seems much more appropriate than a 2% or 5% threshold in healthy participants. Further  
49 studies are required to define similar thresholds for patients.

50

## 51 INTRODUCTION

52 Passive mechanical properties are important components of muscle function (Proske & Morgan, 1999;  
53 Azizi, 2014) and are related to muscle extensibility (Gajdosik, 2001). Several studies have shown the  
54 importance of measuring these properties to understand acute or chronic adaptations after trauma  
55 (Shelbourne *et al.*, 1996), injury (Diong *et al.*, 2012), immobilization (de Boer *et al.*, 2007), or training  
56 (Fouré *et al.*, 2013). In humans, the majority of studies evaluating these properties measure the passive  
57 joint [for review, see Magnusson (1998); Gajdosik (2001)], muscle fascicle length [using B-mode  
58 ultrasound, (Herbert *et al.*, 2002)] or muscle shear modulus using shear wave elastography (Maisetti *et*  
59 *al.*, 2012). To accurately reflect passive mechanical properties, these approaches require the muscle to  
60 be fully inactive during stretch.

61 To ensure that muscles are in a passive state, it is important to monitor muscle activation to detect any  
62 unwanted activity that might influence the passive joint torque, the muscle fascicle length or the muscle  
63 shear modulus. For that purpose, myoelectrical activity is often measured through surface  
64 electromyography (sEMG) (McNair & Portero, 2005). The amplitude of the sEMG signal is then  
65 normalized to measurements during isometric maximal voluntary contractions (MVC) (McNair &  
66 Portero, 2005). Most of previous studies considered muscle to be passive when the sEMG amplitude  
67 remained below a given threshold (Gajdosik, 2001). However, a wide range of thresholds has been used  
68 to qualify such a passive state: 1% (McNair *et al.*, 2001), 2% (Nordez *et al.*, 2010), 5% (Gajdosik *et al.*,  
69 2005), up to 10% of the maximal activation (Halbertsma *et al.*, 1999; Bar-On *et al.*, 2018). As some of  
70 these thresholds were relatively high, it is likely that low activation was present during a supposedly  
71 passive stretch. To our knowledge, only Foo *et al.* (2019) reported that hamstring activity might decrease  
72 the range of motion of the hip joint during a straight leg raise test ( $-0.6^\circ$  for every increase of 1% in  
73 myoelectrical activation). The effects of such low muscle activation on muscle mechanical properties  
74 are still misunderstood. Even though activation as low as 1% of maximal activation are likely to have  
75 minimal influence, it is necessary to determine activation levels that significantly affect the estimation  
76 of muscle mechanical properties.

77 The present study was designed to quantify the effect of low muscle activation levels on the mechanical

78 properties of ankle joint and *triceps surae* (TS) muscles. Ankle joint was chosen here because ankle  
79 joint torque and TS mechanical properties are often examined in stretching studies. To this end, joint  
80 torque and shear modulus of the three heads of TS were measured while the ankle was dorsiflexed by  
81 an ergometer. Participants were asked to produce low muscle activation using a visual feedback of  
82 sEMG amplitude (detailed below in protocol; at 1%, 2% and 5 % of maximal sEMG amplitude) or to  
83 stay fully passive [named “relaxed” condition] where participants were asked to stay as relaxed as  
84 possible during stretching.

## 85 **METHODS**

### 86 **Participants**

87 A sample size calculation for the study was based on analyse of variance (ANOVA repeated measures,  
88 within-between interactions) for testing the association at 5% level between the conditions, angles, and  
89 muscle shear modulus. Using an effect size of  $\eta_p^2=0.14$  and a power of 0.95, 12 participants would be  
90 required to detect significant difference [Gpower 3.1.9.2; Faul *et al.* (2007)]. Fifteen healthy participants  
91 (male/female: 12/3; age: 24.1±4.3 years; height: 177±7 cm; body mass: 65.5±14.9 kg) volunteered to  
92 participate in this study. The study received Institutional Ethics Committee approval (CPP-MIP-015).  
93 The procedures conformed to the Declaration of Helsinki.

### 94 **Equipment**

#### 95 *Ergometer*

96 An isokinetic dynamometer (Biodex 3 Medical, Shirley, New York, USA) was used. The ankle joint  
97 was carefully aligned with the center of rotation of the ergometer by and experienced user (Bruening *et*  
98 *al.*, 2008). 0° of ankle dorsiflexion was defined as the foot sole is perpendicular to the tibia, and negative  
99 angles relative to plantarflexion (PF). Additional thermoformable customized-device and adhesive non-  
100 elastic taping to ergometer Velcro-straps were realized to avoid foot or heel motion. Ankle angles and  
101 joint torque were recorded using an external 16-bit analog/digital converter (1 kHz, 16-35 PowerLab  
102 ADInstruments Inc., Colorado Springs, USA).

103

104 *Surface electromyography (sEMG)*

105 The myoelectrical activity of the *gastrocnemius medialis* and *gastrocnemius lateralis* (GM and GL,  
106 respectively), the *soleus* (SOL) and the *tibialis anterior* (TA) were recorded using hydrogel adhesive  
107 surface electrodes (Kendall™ 100 foam-series, Covidien, Mansfield, USA). The skin was prepared  
108 according to the SENIAM guidelines (Hermens *et al.*, 2000). Due to overlapping probe locations on the  
109 skin, ultrasound imaging ensured that the electrodes were positioned appropriately: longitudinally with  
110 respect to the muscle fascicle alignment, and away from neighboring muscles (Lacourpaille *et al.*, 2017).  
111 sEMG signals were amplified with a biosignal amplifier (g.BSamp 0201a, Guger Technologies,  
112 Schiedlberg, Austria; gain =1000) and digitized together with mechanical signals using the same  
113 acquisition system (see Ergometer) at a sampling rate of 1 kHz. For each muscle, the sEMG signal was  
114 filtered (band-pass: 20-400 Hz and band-stop: 50 Hz) in order to remove unwanted noise associated  
115 with artifacts. The signals were quantified in terms of root mean square (EMG-RMS) using a moving  
116 average window (300 ms width, LabChart v7.0, Inc., Colorado Springs, USA). The EMG-RMS signals  
117 for each muscle (GM, GL, SOL, and TA) was normalized to the maximal EMG-RMS value recorded  
118 during MVC. The EMG-RMS of TS was provided in real time on a computer screen (Figure 1). The  
119 quality of the sEMG signals was evaluated by calculating i) the baseline noise in % of the maximal value  
120 reached during MVC; ii) a signal-to-noise ratio (SNR, Matlab® function *snr*) (Table 1).

121

122 *Shear wave elastography (SWE)*

123 An ultrasound scanner (Supersonic Imagine, v. 6.1, Aix-en-Provence, France) coupled with a linear  
124 transducer [2–10 MHz for SOL or 4–15 MHz for GM and GL, Supersonic Imagine, Aix-en-Provence,  
125 France] was used in shear wave elastography mode (MSK preset, penetration mode, spatial smoothing  
126 5/9, persistence off, scale: 0-600 kPa). The shear modulus ( $\mu$ ) is related to the shear wave velocity ( $V_s$ )  
127 measured using elastography:

128  $\mu = \rho V_s^2$  (Equation 1)

129 where  $\rho$  the density of the tissue ( $1000 \text{ kg}\cdot\text{m}^{-3}$  for muscle).

130 The shear modulus has been shown to have strong linear relationship with Young's modulus ( $R^2$  between  
131 0.916-0.988) (Eby *et al.*, 2013; Koo *et al.*, 2013) and thus, can be used as an index of changes in muscle

132 stiffness (Hug *et al.*, 2015). The transducer was aligned along the longitudinal axis of the leg (i.e., the  
133 direction of shortening/lengthening) and perpendicular to the skin so that the image plane  
134 perpendicularly intersects the muscle aponeurosis (Le Sant *et al.*, 2017). Previous results from our team  
135 showed very good reliability of SWE for measuring the shear modulus of *triceps surae* muscles during  
136 stretching (Le Sant *et al.*, 2017). A transistor-transistor logic pulse (duration <50 ms) was sent by the  
137 ultrasound scanner at each shear modulus measurement (i.e. each second) that allowed to synchronize  
138 shear modulus measurements with the ankle angle, passive torque and sEMG signals.

### 139 **Protocol**

140 Participants first performed a familiarization session. They laid prone on the ergometer with the ankle  
141 to be tested firmly strapped to the footplate of the device. First, the participants performed 3 isometric  
142 MVCs (separated by a rest of 90 s) in PF and in DF, respectively. Second, the maximal angle in DF  
143 (max DF) was defined. For that purpose, the ankle was moved by the ergometer at 2°/s, and participants  
144 pressed a button when they felt their maximum tolerable stretch limit (onset of pain in the posterior  
145 region of the leg). The highest angle obtained during three trials was kept as their maximal DF. Three  
146 conditioning cycles (from 40° of PF to max DF, 2°/s) were then performed (Nordez *et al.*, 2008). Third,  
147 participants underwent stretching at 2°/s for each condition [1%, 2%, 5% of maximal EMG-RMS(TS)  
148 and “relaxed” conditions]. The peak EMG-RMS(TS) from maximal plantarflexion was used to calculate  
149 submaximal levels. Participants were asked to maintain a given muscle activity level during the ankle  
150 motion, using feedback provided in real time on a screen (Figure 1). The familiarization continued until  
151 satisfactory, i.e. if the averaged muscle activity [EMG-RMS(TS)] corresponded to the targeted level  $\pm$   
152  $\sim 0.5\%$ . All the participants were able to perform the task and completed the second session dedicated to  
153 data collection.

154 The second session was performed between one and seven days after the familiarization session. The  
155 procedures were repeated: MVCs, maximal ROM assessments, and conditioning. Then, the participants  
156 were asked to match a target during ankle dorsiflexions (40° of PF to max DF, 2°/s) at either 1%, 2%,  
157 5% of maximal EMG-RMS(TS) or during the “relaxed” condition (random order). For each condition,  
158 three dorsiflexions were performed such that the shear modulus of each muscle of the TS was measured

159 (random order). The transducer was placed over the mid muscle region for GM and GL, and over the  
160 distal region for SOL, as defined in Le Sant *et al.* (2017). A total of twelve dorsiflexions were realized  
161 (4 conditions x 3 muscles) during which the joint torque, shear modulus of one muscle and muscle  
162 activity levels of four muscles were measured.

### 163 **Data analysis**

164 Data were processed using Matlab® scripts (The MathWorks Inc., Natick, USA) as previously described  
165 in details (Le Sant *et al.*, 2017). Shear modulus values were averaged over the largest region of interest  
166 (ROI) that avoided aponeurosis and artefacts (average surface of 92 mm<sup>2</sup>, 89 mm<sup>2</sup> and 86 mm<sup>2</sup> for the  
167 GM, GL and SOL). Angle and joint torque were filtered (Butterworth low-pass filter, cutoff 10Hz) and  
168 torque corrected for gravity. Maximal EMG-RMS for each muscle corresponded to the peak value  
169 reached during MVC. For each trial, the EMG-RMS of each muscle (moving windows width: 300 ms)  
170 and joint torque were calculated for each shear modulus measurement (ie. at 1 Hz, timing determined  
171 by using the transistor-transistor logic pulse sent by the ultrasound scanner). Because the max DF angle  
172 differed among individuals, the angle was normalized to the maximal ROM (i.e., 0% as 40° PF, and  
173 100% as the max DF). Then, EMG-RMS, passive torque and shear modulus values were calculated  
174 every 5% (i.e., 21 values) using linear interpolation.

### 175 **Statistics**

176 The data were processed using Statistica® (v.10, Statsoft Inc., Tulsa, USA). Data passed the Shapiro-  
177 Wilk normality test. For each condition, the averaged value of the 3 trials was used for joint torque and  
178 RMS-EMG. The changes in the measured EMG-RMS across conditions were assessed using a repeated-  
179 measures ANOVA (4 conditions x 4 angles x 3 muscles). The four conditions were “relaxed”, 1%, 2%,  
180 and 5% of max EMG-RMS(TS). The four angles corresponded to the angle reached by each participant  
181 at 0%, 40%, 80% and 100% (i.e., max DF) of ankle ROM. Another repeated measures ANOVA (4  
182 conditions x 4 angles) was used to assess changes in EMG-RMS(TA). **These analyses were realized to**  
183 **confirm that the conditions were well achieved (ie, targeted level, without any potential influences of**  
184 **TA). In addition, we wanted to assess whether conditions affect in the same way or not the various**  
185 **muscles depending on joint angles.** Two repeated-measures ANOVAs were conducted to assess the



186 impact of the condition on joint torque (4 conditions x 4 angles) and on shear modulus (4 conditions x  
187 4 angles x 3 muscles [GM, GL and SOL]). We hypothesized an increase in joint torque and muscle shear  
188 modulus at a same ankle angle with the increasing level of muscle activity. Partial eta square ( $\eta_p^2$ ) was  
189 reported as effect-size estimation. Small, medium and large effects were considered for  $\eta_p^2 = 0.01$ ,  $\eta_p^2$   
190  $= 0.07$  and  $\eta_p^2 \geq 0.14$ , respectively (Cohen, 1988). To account for hidden multiplicity in exploratory  
191 multiway ANOVA, a Benjamini-Hochberg procedure was used as described in Cramer *et al.* (2016).  
192 The p-values from the ANOVAS were sorted in ranks from smallest to largest (7 ranks for RMS-EMG,  
193 3 ranks for torque, 7 ranks for shear modulus, respectively) and were judged significant if inferior to the  
194 corresponding Benjamini-Hochberg critical value (Benjamini & Hochberg, 1995). Post-hoc analyses  
195 were performed using Bonferroni correction. In order to better describe the small differences between  
196 the “relaxed” and 1% conditions, Bland-Altman plots were generated to study the agreements for joint  
197 torque, shear modulus and muscle activity (Bland & Altman, 1986). The statistical significance was set  
198 at  $p < 0.05$ . Data are reported as mean  $\pm$  standard deviation (SD).

## 199 **RESULTS**

200 The averaged $\pm$ SD angles were  $-40.0 \pm 0.4^\circ$  PF (0%),  $-10.0 \pm 2.6^\circ$  PF (40%),  $20.2 \pm 5.3^\circ$  DF (80%) and  
201  $35.3 \pm 6.6^\circ$  DF (100%), respectively (Figures 1 to 4).

202

### 203 *Effects of condition on muscle activation*

204 The averaged EMG-RMS of the TS were  $0.4 \pm 0.1\%$  (range 0.2%-0.7%),  $1.2 \pm 0.2\%$  (range 0.9%-1.6%),  
205  $2.1 \pm 0.3\%$  (range 1.7%-2.7%) and  $4.8\% \pm 0.7\%$  (range 4.3%-6.7%) of max EMG-RMS(TS) for the  
206 “relaxed”, 1%, 2% and 5% conditions, respectively (Figure 2).

207 As expected, there was a main effect of condition ( $F(3,42)=417.96$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.97$ ) indicating an  
208 increase in EMG-RMS among TS muscles with the increase in activation level target. Main effects of  
209 angle ( $F(3,42)=6.62$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.32$ ), and muscle ( $F(2,28)=6.13$ ,  $p = 0.006$ ,  $\eta_p^2 = 0.30$ ) were also  
210 found. Condition x muscle ( $F(6,84)=5.12$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.27$ ), condition x angle ( $F(9,126)=3.39$ ,  
211  $p < 0.001$ ,  $\eta_p^2 = 0.19$ ), muscle x angle ( $F(6,84)=23.65$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.63$ ), and condition x muscle x  
212 angle ( $F(18,252)=21.12$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.59$ ) interactions were also significant. Post-hoc analyses

213 revealed an increase in EMG-RMS(SOL) at 80% and at max DF during the 2% and 5% conditions (all  
214 p-values <0.001) and a decrease in EMG-RMS(GM) at max DF (p<0.001) and in EMG-RMS(GL)  
215 during the 5% condition (all p-values <0.005). Regarding the TA, there were main effects of condition  
216 ( $F(3,42)=32.62$ ,  $p<0.001$ ,  $\eta_p^2 = 0.70$ ) and angle ( $F(3,42)=4.09$ ,  $p=0.012$ ,  $\eta_p^2 = 0.23$ ), but the interaction  
217 condition x angle was not significant ( $F(9,126)=0.78$ ,  $p=0.64$ ). EMG-RMS(TA) was significantly higher  
218 during 5% condition than during the other conditions (all p-values<0.018). During the 2% condition,  
219 EMG-RMS(TA) was higher at max DF than at 40% and 80% (p<0.001 and p=0.032, respectively).  
220 During the 5% condition, EMG-RMS(TA) was higher at max DF (all p-values<0.008) (Figure 2).  
221 In addition, regardless the muscle, EMG-RMS was not different between the “relaxed” and the 1%  
222 conditions (all p-values>0.11). The mean difference between “relaxed” and 1% EMG-RMS was  
223 0.77±0.11% for the GM, 0.51±0.05% for the GL, 0.97±0.13% for the SOL, and 0.10±0.32% for the TA  
224 (Supplementary File 1).

225

#### 226 *Effects of muscle activation on joint torque*

227 A significant main effect of condition ( $F(3,42)=56.56$ ,  $p<0.001$ ,  $\eta_p^2 = 0.80$ ) and angle ( $F(3,42)=268.97$ ,  
228  $p<0.001$ ,  $\eta_p^2 = 0.94$ ) were found. There was also a significant interaction condition x angle  
229 ( $F(9,126)=26.23$ ,  $p<0.001$ ,  $\eta_p^2 = 0.65$ ). Compared to the “relaxed” condition, joint torque was not  
230 different for the 1% condition (all p-values>0.41). The mean difference between “relaxed” and 1%  
231 torque values was 1.2±1.9N.m (Supplementary File 1). When compared to the relaxed condition, joint  
232 torque was significantly larger in the 5% condition at 40%, 80% and max DF angles, and in the 2%  
233 condition at 80% and max DF angles (all p-values<0.001) (Figure 3). At max DF, joint torque was  
234 44.5±12.0N.m, 46.5±11.8N.m, 50.3±12.4N.m, and 57.7±12.6N.m, for the “relaxed”, 1%, 2%, and 5%  
235 conditions, respectively (Figure 3). When compared to the “relaxed” condition, the peak torque was  
236 increased by 33±24% for the 5% condition, and by 14±10% for the 2% condition.

237

#### 238 *Effects of muscle activation on shear modulus*

239 The main effects of condition ( $F(3,42)=55.01$ ,  $p<0.001$ ,  $\eta_p^2 = 0.80$ ), muscle ( $F(2,28)=67.05$ ,  $p<0.001$ ,

240  $\eta_p^2 = 0.83$ ) and angle ( $F(3,42)=256.90$ ,  $p<0.001$ ,  $\eta_p^2 = 0.95$ ) were found on shear modulus (Figure 4).  
241 There were also significant interactions: condition x muscle ( $F(6,84)=4.96$ ,  $p<0.001$ ,  $\eta_p^2 = 0.26$ ),  
242 condition x angle ( $F(9,126)=13.30$ ,  $p<0.001$ ,  $\eta_p^2 = 0.58$ ), muscle x angle ( $F(6,84)=65.64$ ,  $p<0.001$ ,  $\eta_p^2$   
243  $= 0.82$ ) and condition x muscle x angle ( $F(18,252)=2.62$ ,  $p<0.001$ ,  $\eta_p^2 = 0.16$ ). When the 2% condition  
244 was compared to the relaxed condition, the only significant differences in shear modulus were found for  
245 GM and GL at max DF ( $p<0.001$ ) (Figure 4). When the 5% condition was compared to the relaxed  
246 condition, significant differences were found at 40%, 80% and max DF for GM (all p-values<0.001),  
247 and at 80% and max DF for GL and SOL (all p-values<0.002), respectively. When compared to the  
248 “relaxed” condition, the peak shear modulus was increased by  $26\pm 20\%$ ,  $19\pm 9\%$  and  $49\pm 41\%$  for the 2%  
249 condition, and by  $55\pm 40\%$ ,  $38\pm 13\%$  and  $100\pm 87\%$  for the 5% condition, for GM, GL and SOL,  
250 respectively. No significant differences were found for the shear modulus between “relaxed” and 1%  
251 conditions (all p-values=1.000). The mean difference between the “relaxed” and 1% shear modulus was  
252  $2.9\pm 0.9$ kPa for the GM,  $1.7\pm 0.6$ kPa for the GL, and  $1.4\pm 0.7$ kPa for the SOL (Supplementary File 1).

## 253 **DISCUSSION**

254 This study describes the effects of muscle activation levels that are usually considered ‘negligible’  
255 during passive stretching on both joint torque and muscle shear modulus. The main finding was that  
256 muscle activation higher than 1% affects the joint torque and shear modulus, which increase with ankle  
257 dorsiflexion. In the 5% condition, the effect was evident at low angles (i.e., 40% of maximal DF)  
258 (Figures 3 and 4).

259 Measuring myoelectrical activity during stretching studies is used to confirm the absence of unwanted  
260 muscle activation (McNair & Portero, 2005). This is crucial because muscle activation, albeit small,  
261 may affect muscle mechanical properties. For that purpose, participants are trained to stay “as relaxed  
262 as possible” and their spontaneous myoelectrical activity is measured during joint rotation (McNair &  
263 Portero, 2005). According to these studies, intrinsic stiffness and muscle activation should both  
264 contribute to joint torque in the last degrees of ankle’s ROM towards DF. As a result of the study  
265 conducted by McNair *et al.* (2001), 80% of the max DF is commonly used to limit this unwanted activity.  
266 Beyond this ankle angle, myoelectrical activity was shown to increase significantly (McNair *et al.*,

267 2001). In our study, a significant increase in the EMG-RMS of TS at 80% of max DF was only found  
268 during the 2% and 5% conditions. The results also revealed that it is possible for participants to maintain  
269 a very low level of myoelectrical activity among TS during stretching [ $0.4 \pm 0.1\%$  of max EMG-  
270 RMS(TS), during the “relaxed condition” Figure 2]. We think that the familiarization session was  
271 helpful to obtain low EMG values during the stretching in the relaxed condition.

272 Regarding the shear modulus, the values reached at max DF were the highest for GM when compared  
273 to GL and SOL, whatever the tested condition. This result is in accordance with previous studies that  
274 reported between-muscle differences in shear modulus among plantar flexors under ‘relaxed’ conditions  
275 (Hirata *et al.*, 2015; Hirata *et al.*, 2016; Le Sant *et al.*, 2017; Le Sant *et al.*, 2018). Considering that the  
276 GM cross-sectional area is larger than that of GL (Fukunaga *et al.*, 1992), passive force developed by  
277 the GM muscle during stretch is likely much larger than that produced by the GL muscle. However,  
278 considering a lower shear modulus for the SOL compared to the GM and GL and a higher volume, it is  
279 more difficult to estimate the contribution to the passive torque of the SOL using the methods of this  
280 study.

281 A threshold such as 5% of EMG-RMS(TS) is often used to evaluate elderly patients (Gajdosik *et al.*,  
282 2004, 2005) or people with neurological conditions (Kalkman *et al.*, 2018; Le Sant *et al.*, 2018). Such  
283 an activity level could have significantly influenced the results of these studies (Figures 3 and 4). Our  
284 results indicate that using such a threshold could induce an overestimation of 64% and 33% for the  
285 muscle shear modulus and passive torque, respectively. However, because of structural differences with  
286 young populations (e.g., muscle volume, intra-muscular connective/adipose composition) it is difficult  
287 to estimate how much a 5% of EMG-RMS(TS) might have influenced the shear modulus and joint  
288 torque among elderly/patient populations. In addition, it remains difficult to be sure that patients reached  
289 maximal activity levels during MVC. It should have influenced the activity levels measured during  
290 stretching in previous studies with patients (Kalkman *et al.*, 2018; Le Sant *et al.*, 2018). Thus, further  
291 studies should be conducted in patients to assess the effects of unwanted EMG activity on passive torque  
292 and muscle behaviour during stretching. The use of motor blocks could help isolate the passive  
293 contribution to joint stiffness (Deltombe *et al.*, 2004; Bollens *et al.*, 2011). Few studies have assessed  
294 the impact of this approach on biomechanical parameters (Bleyenheuft *et al.*, 2008; Buffenoir *et al.*,

295 2013). The aforementioned studies showed that the passive resistance of the ankle joint was decreased  
296 after a selective block conducted at the superior *soleus* nerve. However, since they used sinusoidal  
297 perturbations applied to the ankle joint at different frequencies, it remains difficult to interpret those  
298 results in the context of classical stretching studies. Future studies, assessing the impact of nerve blocs  
299 of the TS could help to understand the impact of muscle activation ‘at rest’ (such as spastic dystonia) on  
300 the mechanical properties of the TS during stretching.

301 This study was designed to measure the effects of minimal activity on ankle joint and TS muscle  
302 mechanical properties during stretching. As described, a feedback EMG-RMS(TS) was used, based on  
303 an average EMG-RMS for the three heads of the TS, and an amplitude-normalization procedure (i.e.,  
304 maximal EMG-RMS during MVC). This procedure supposes a high sensibility of sEMG to detect very  
305 low levels of muscle activity. Other techniques such as high-density EMG or intramuscular EMG using  
306 fine wires might have been more direct approaches of measurement. We chose our experimental set-up  
307 because it is used in classical stretching studies. The high maximal/baseline ratios, SNR values and  
308 Bland-Altman results make us confident that our procedures were appropriate, in particular for the  
309 lowest conditions (“relaxed” *versus* 1%). Participants reached and maintained the targeted levels during  
310 submaximal conditions (1%, 2% an 5%) using a biofeedback of normalized EMG-RMS(TS) provided  
311 in real time (Figure 1). While the mean values were in accordance with the targets of the feedback, some  
312 inter-individual variability was present (average of  $\pm 0.32\%$  in regard of the targeted level) but, the  
313 ranges of sEMG activity levels reached clearly showed that there was no overlap between conditions.  
314 However, the contributions of GM, GL and SOL to the EMG-RMS(TS) were not constant, in particular  
315 during the 5% condition (Figure 2). It seems that a specific coordination between muscles occurred with  
316 a decrease in the activity of the *gastrocnemii* combined with an increase in the activity of SOL. These  
317 changes in muscle activity for such low levels were not expected to occur. Their mechanical  
318 consequences on joint torque are difficult to evaluate, but did not lead to visible changes in the shear  
319 modulus-angle relationships. However, this point would not alter the main conclusions of the present  
320 paper and the observations for the most active conditions (e.g., 5% of maximal) compared to the  
321 “relaxed” condition. As previously described in the method, the joint axis of rotation was carefully  
322 aligned with the axis of rotation of the ergometer. To our knowledge, the influence of a possible

323 misalignment on the joint torque measured during passive stretching has not be quantified in the  
324 literature. However, considering that the foot was very firmly strapped in the present study, and  
325 movement of the foot are generally lower in passive conditions compared to maximal isometric  
326 contractions, we think that the effect of foot motion was small. It was confirmed in a previous study  
327 (Ates *et al.*, 2018) that found a mean difference in ankle angle of 1.4° (Confidence Interval 95%  
328 1.3°;1.6°) between the ergometer and 3 optoelectronic system during a pilot-testing (n=6, range of -  
329 20°PF to 20°DF). Therefore, this effect was probably small and marginally influenced our results. Due  
330 to potential various joint configuration (e.g., muscle volume, lever arm...) and neuromechanical  
331 properties (eg EMG-force relationships), it is difficult to extrapolate the results of the present study to  
332 other the joints and muscles. Therefore, further studies are needed to examine the effects of muscle  
333 activity during stretching on other joints (e.g., knee torque) with different structure/architecture.

## 334 **CONCLUSION**

335 This study showed that joint stiffness and muscle shear modulus (in particular GM) were higher if  
336 participants activated their TS muscle at 5% of its maximal voluntary , activation (measured by sEMG),  
337 during slow dorsiflexions of the ankle. A lower level of EMG amplitude (2%) had significant influence  
338 on joint torque and shear modulus at the end of the range of dorsiflexion. These results provide new  
339 insights that might be useful for the design of future stretching studies, as 5% has classically been used  
340 as a threshold to describe a “passive state”. A threshold of 1% seems more appropriate for healthy  
341 participants. Further studies are required to set acceptable EMG thresholds for patients.

342

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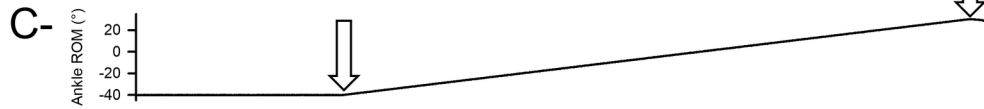
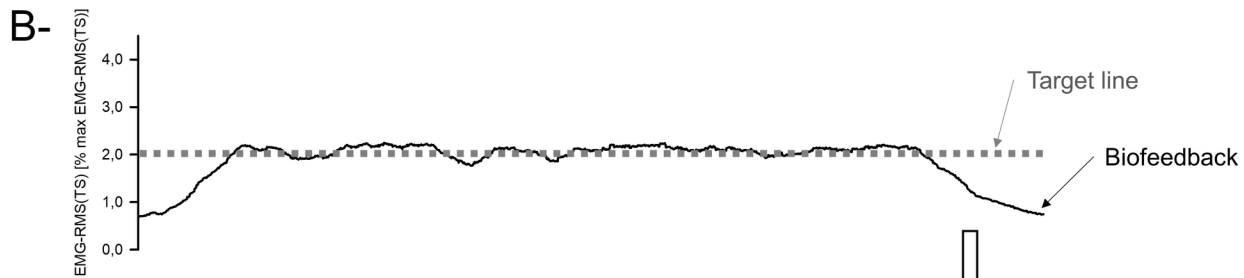
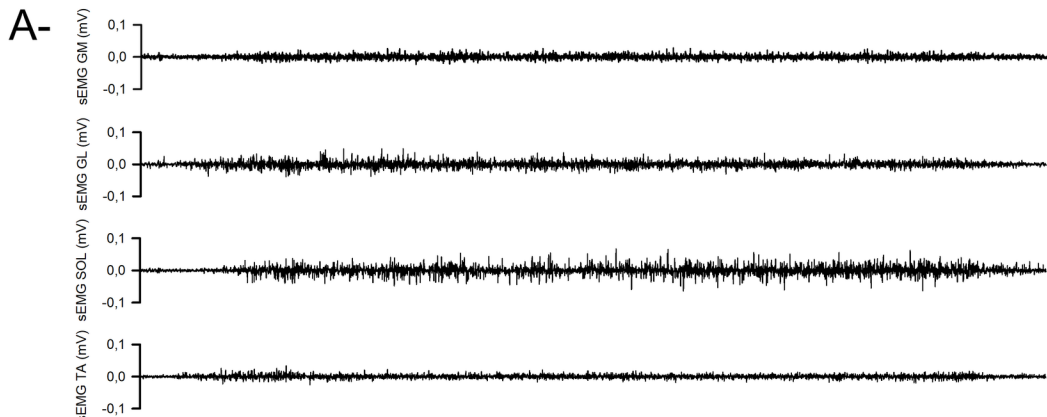
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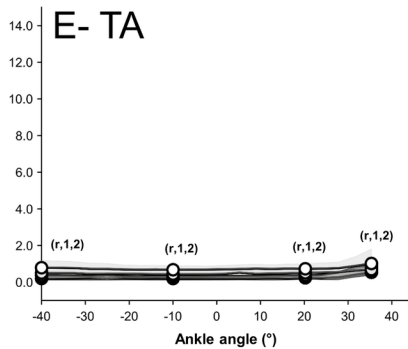
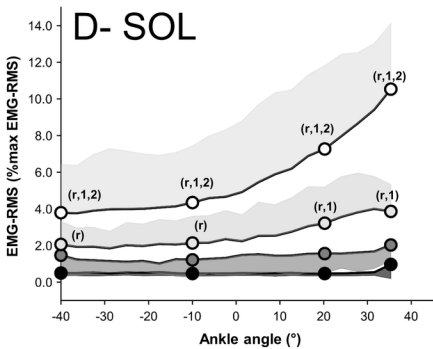
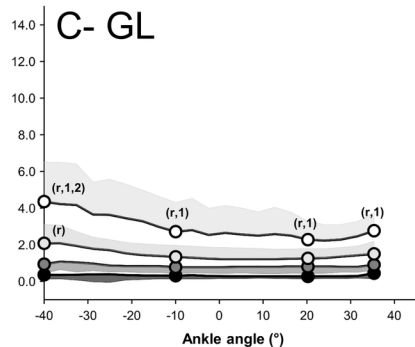
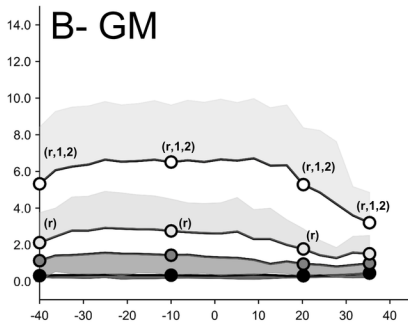
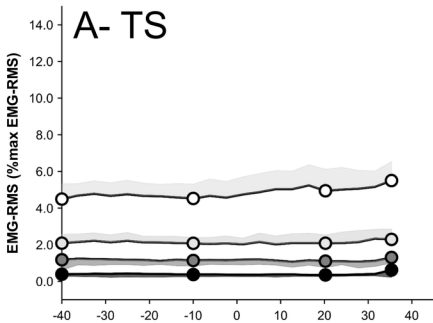
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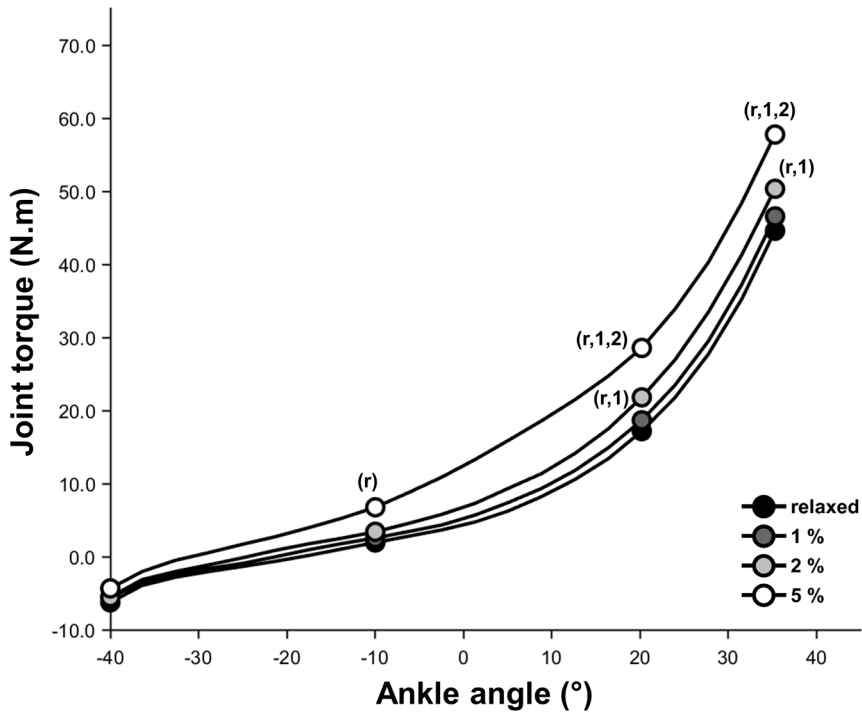
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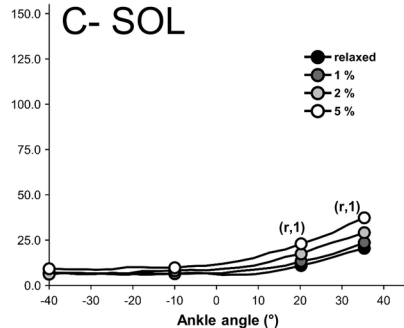
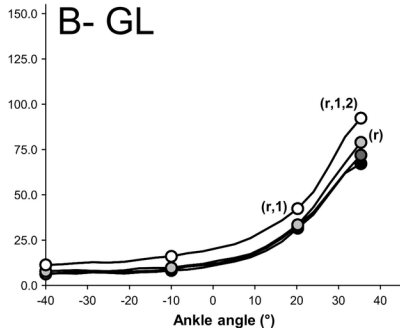
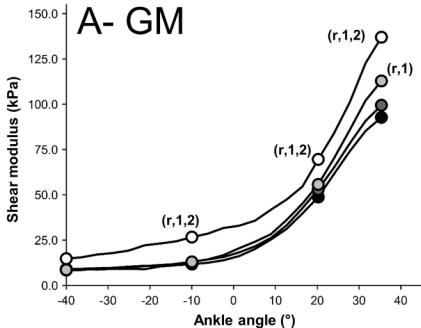
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<b>Muscle</b>	<b>EMG-RMS(baseline)/EMG-RMS(MVC) (%)</b>	<b>Signal to Noise Ratio (dB)</b>
GM	0.35±0.11	37.8±3.1
GL	0.32±0.15	37.6±4.3
SOL	0.44±0.18	33.1±3.7
TA	0.33±0.22	42.7±3.7

**Table 1 – Quality of the myoelectrical activity recorded for each muscle during data collection.**

Data are shown as mean±standard deviation.

Legend: GM: gastrocnemius medialis; GL: gastrocnemius lateralis; SOL: soleus; TA: tibialis anterior; EMG-RMS: root mean square.