

Muscle synergies for the control of single-limb stance with and without visual information in young individuals

*Original*

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1 **TITLE**

2 **Muscle synergies for the control of single-limb stance with and without visual information in**  
3 **young individuals.**

4

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25 **ABSTRACT**

26 **Purpose:** Single-limb stance is a demanding postural task featuring a high number of daily living and  
27 sporting activities. Thus, it is widely used for training and rehabilitation, as well as for balance  
28 assessment. Muscle activations around single joints have been previously described, however, it is  
29 not known which are the muscle synergies used to control posture and how they change between  
30 conditions of normal and lack of visual information.

31 **Methods:** Twenty-two healthy young participants were asked to perform a 30 seconds single-limb  
32 stance task in open-eyes and closed-eyes condition while standing on a force platform with the  
33 dominant limb. Muscle synergies were extracted from the electromyographical recordings of 13  
34 muscles of the lower limb, hip, and back. The optimal number of synergies, together with the average  
35 recruitment level and balance control strategies were analyzed and compared between the open- and  
36 the closed-eyes condition.

37 **Results:** Four major muscle synergies, two ankle-dominant synergies, one knee-dominant synergy,  
38 and one hip/back-dominant synergy were found. No differences between open- and closed-eyes  
39 conditions were found for the recruitment level, except for the hip/back synergy, which significantly  
40 decreased ( $p = 0.02$ ) in the closed-eyes compared to the open-eyes condition. A significant increase  
41 ( $p = 0.03$ ) of the ankle balance strategy was found in the closed-eyes compared to the open-eyes  
42 condition.

43 **Conclusion:** In healthy young individuals, single-limb stance is featured by four major synergies,  
44 both in open- and closed-eyes condition. Future studies should investigate muscle synergies in  
45 participants with other age groups, as well as pathological conditions.

46

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48

49 **Keywords:** balance; postural control; postural adjustments; muscle activations; muscle recruitment;  
50 postural strategies.

51

## 52 INTRODUCTION

53 The ability to maintain single-limb stance is essential during daily living activities and sport practice,  
54 as a single task as well as a component of other more complex tasks. It is a simple but challenging  
55 task for posture control and for this reason it is widely used for training and rehabilitation (Makhlouf  
56 et al. 2018; Youssef et al. 2018). In research and clinical practice, it is widely used as a testing task  
57 as it allows to quantify balance alterations and deficits of the single limb otherwise concealed during  
58 the performance of double limb tasks (Hertel et al. 2002; Riemann et al. 2003; Zumbrunn et al. 2011;  
59 Stensdotter et al. 2015; Scholes et al. 2018; Benedetti et al. 2019).

60 From a physiological point of view, single-limb stance can be considered as a high demanding  
61 postural task for neuromuscular and central nervous systems (CNS) requiring an efficient integration  
62 of somatosensory, visual, and vestibular information with the aim to orchestrate a continuous and  
63 effective motor response to manage a reduced base of support (Ivanenko and Gurfinkel, 2018). The  
64 effectiveness of postural control has been usually expressed by means of mechanical parameters such  
65 as the center of pressure (COP), joints or body segments displacement (Madigan et al. 2006; Doyle  
66 et al. 2007; Caballero et al. 2015). Previous literature has reported the essential role of the ankle for  
67 postural stabilization in particular when tasks show an increase in instability, as in the transition from  
68 double- to single-limb stance (Levin et al. 2012) or from stable to unstable surfaces (Riemann et al.  
69 2003). When the ankle movements are not sufficient to guarantee balance, the involvement of more  
70 proximal joints and body segments has been reported (Riemann et al. 2003; Horak et al. 2006).  
71 Further, an increase in the instability during stance tasks has been also reported in case of a number  
72 of pathological conditions (Smithson et al. 2008; Nilsson et al 2006; Stensdotter et al. 2013) and in  
73 case of abnormal sensitive information (Ageberg et al. 2005; Hazime et al. 2012; Stensdotter et al.  
74 2013). Above all, it has been shown that vision has a key role in posture control and that the lack of  
75 visual feedback or abnormal visual feedback lead to peculiar adaptations in mechanical parameters  
76 featuring postural tasks (Collings et al. 2006; Agostini et al. 2016).

77 Even if mechanical parameters, such as COP or joint displacement, are useful to quantify instability  
78 during postural tasks, they do not give adequate information on motor control. Essential information  
79 for motor control assessment comes from the analysis of muscles activations, which mediates CNS  
80 control and mechanical expression of movement.

81 While a wide number of studies investigated multi-muscles activations during double limb stance, in  
82 the transition from double to single stance or during various stance tasks in response to sudden  
83 perturbations (Robert et al 2008; Torres-Oviedo and Ting 2010; Yang et al. 2015; Yamagata et al.

84 2018; Munoz-Martel et al. 2019), less is known about quiet single-limb stance. Few studies focused  
85 on ankle/foot muscles activation, given their important role as previously described (Kelly et al. 2012;  
86 Kurz et al. 2018).

87 However, the investigation of muscle activations around a single joint is reductive, since it is well  
88 known that CNS organizes motor response to a given task in terms of muscle synergies (Torres-  
89 Oviedo and Ting 2007; Ting and McKay 2007). This means that CNS coordinates the activation of a  
90 set of muscles which are synergistic for a given task, or a number of similar tasks (Torres-Oviedo and  
91 Ting 2010).

92 To the best of the authors' knowledge, it is not known which are muscle synergies used for  
93 maintaining balance condition during single-limb stance and how muscle synergies change in  
94 condition of lack of visual feedback. Since the single-limb stance task is largely used, understanding  
95 which are the muscle synergies adopted by healthy individuals is essential to address future research  
96 as well as training, rehabilitation, and functional assessment, both in healthy and pathological  
97 individuals. Thus, the first aim of this study was to investigate muscle synergies in lower limb and  
98 back muscles during a single-limb stance task without external perturbations in healthy young  
99 individuals. The second aim of this study was to investigate how the lack of visual information affects  
100 steady single-limb stance muscle synergies. Studies on the effects of visual feedback on synergistic  
101 muscle activation during double-limb stance found a change in neural drive to synergistic muscle  
102 groups with the lack of visual information (Danna-Dos-Santos et al. 2015). It is not known how  
103 muscle synergies changes in condition of lack of visual information during single-limb-stance. Since  
104 the ankle is the first joint which acts to maintain postural stability, it is hypothesized that muscles  
105 activations around the ankle joint, and thus ankle-dominant synergies, will be affected by the greater  
106 instability related to the lack of visual information.

107

## 108 MATERIALS AND METHODS

### 109 *Participants*

110 Eleven male participants (age:  $23.9 \pm 2.2$  years; height:  $182 \pm 8.4$  cm; body mass:  $74.5 \pm 10.8$  kg)  
111 and eleven female participants (age:  $24.5 \pm 2.9$  years; height:  $169 \pm 5.8$  cm; body mass:  $57.2 \pm 6.5$   
112 kg) were recruited to participate in the study. Inclusion criteria were: a) age between 20 and 35 years,  
113 b) physical activity level of 2 and 3 according to the Saltin and Grimby scale (Grimby et al. 2015),  
114 thus excluding sedentary individuals and competitive athletes, and c) absence of known neurological  
115 diseases. Exclusion criteria were a) previous injuries or surgery, and b) abnormalities in lower limb  
116 and foot joints.

117 Each participant signed an informed consent before participating in the study. The study was  
118 conducted in accordance with the Declaration of Helsinki and received ethical approval from the  
119 Ethical Committee of the Rizzoli Orthopedic Institute (PG n. 0004167).

120

### 121 *Experimental Protocol and Data Analysis*

122 Participants were asked to stand barefoot on a force platform (Dynamic Walkway P6000, BTS  
123 Bioengineering, Milan, Italy) with the dominant limb and to maintain the contralateral knee joint  
124 flexed at approximately  $90^\circ$ . They were asked to look forward, to maintain upper limbs aligned to the  
125 trunk, and to remain as still as possible for at least 30 seconds (Figure 1). Minimal arms movements  
126 were allowed; however, participants were asked to minimize them as much as possible. They  
127 performed the task in both opens eyes (OE) and closed eyes (CE) conditions. Two trials for each  
128 condition were performed in random order and with two minutes of rest between the trials. Muscle  
129 activations were recorded from 13 muscles of the dominant limb and trunk through electromyography  
130 wireless probes (BTS FreeEMG 1000, BTS Bioengineering, Milan, Italy) fixed on EMG electrodes  
131 (Ag/AgCl) applied over Tibialis Anterior (TA), Peroneus Longus (PL), Peroneus Brevis (PB), Soleus  
132 (SO), Lateral Gastrocnemius (LG), Vastus Medialis (VM), Vastus Lateralis (VL), Rectus Femoris  
133 (RF), Biceps Femoris (BF), Semitendinosus (ST), Gluteus Medius (GM), Longissimus Dorsii  
134 Omolateral to the dominant lower limb (LDO), and Longissimus Dorsii of Contralateral side (LDC)  
135 in accordance with SENIAM recommendations (Hermens et al. 2000). To reduce the skin impedance,  
136 before electrode application, the skin area was shaved and cleaned with ethyl alcohol. A footswitch  
137 (FSW) was placed under the first metatarsal head of the non-dominant foot. Force platform, EMG,

138 and FSW signals were part of the same integrated system and were recorded with a 1000 Hz sampling  
139 rate.



140

141

Figure 1. One of the participants performing the single-limb stance task.

142

### 143 *Segmentation of single-limb stance epochs*

144 The segmentation of the EMG time-instants relative to the beginning and the end of the single-limb  
145 stance task was performed considering the FSW signals. More specifically, the FSW signals were  
146 used to detect the time-instants when the subject moved from the double- to the single-limb stance  
147 (beginning of the task) and vice versa (end of the task).

148 First, the FSW signals were amplitude-normalized to obtain signals that range between 0 and 1, where  
149 0 corresponds to an open FSW (foot not touching the force platform) and 1 corresponds to a closed

150 FSW (foot on the force platform). The beginning of the single-limb stance task was detected in  
151 correspondence of a 1-to-0 transition, while the end was detected in correspondence of a 0-to-1  
152 transition. Moreover, to avoid the segmentation of excessive unipedal perturbations due to double- to  
153 single-limb stance transition (and vice versa), the beginning and the end of the single-limb stance  
154 were set 5 seconds after and before the previously detected time-instants, respectively.

155

### 156 *Muscle synergy extraction and sorting*

157 Muscle synergy extraction and sorting procedures were performed in accordance with our previous  
158 study (Ghislieri et al. 2020). Briefly, the segmented EMG signals corresponding to single-limb stance  
159 tasks were high-pass filtered at a cut-off frequency of 35 Hz through an 8<sup>th</sup> order zero-lag IIR  
160 Butterworth digital filter, full-wave rectified, and low-pass filtered at a cut-off frequency of 12 Hz  
161 through a 5<sup>th</sup> order zero-lag IIR Butterworth digital filter (Torricelli et al. 2016). The EMG amplitude  
162 was normalized to the global maximum activation of each muscle recorded for each trial of each  
163 condition to ensure the equally weighted contribution of all the observed muscles in the muscle  
164 synergy assessment (Torricelli et al. 2016).

165 The original data matrix containing the envelopes of the segmented EMG signals was then factorized  
166 into low-dimensional elements using the Non-negative Matrix Factorization (NMF) algorithm (Lee  
167 et al. 1999; Torres-Oviedo et al. 2007). The NMF models the original data matrix as the linear  
168 combination of two low-dimensional elements (Zelik et al. 2014): the time-independent weight  
169 vectors ( $W$ ) modeling the spatial component of the motor control and the time-dependent activation  
170 coefficients ( $C(t)$ ) modeling the temporal component of the motor control, as detailed in (1):

$$M(t) = \sum_{k=1}^N C(t)_k \cdot W_k + e \quad (1)$$

171 where  $N$  represents the number of muscle synergies needed to accurately assess the motor control and  
172  $e$  is the reconstruction error.

173 The reconstruction accuracy of the original EMG signals for each number of synergies from 1 to 8  
174 was computed through the total Variance Accounted For ( $tVAF$ ), defined as the uncentered Pearson's  
175 correlation coefficient. The  $tVAF$  was used to select the optimal number of muscle synergies ( $N_{opt}$ )  
176 needed to properly reconstruct the original EMG signals and to accurately assess the motor control  
177 strategies. As detailed in our previous work (Ghislieri et al. 2020), the  $N_{opt}$  was selected by  
178 consecutively applying a global criterion on each number of synergies from 1 to 8 (least number of



179 synergies granting a  $tVAF \geq 90\%$ ) and a local criterion on the number of muscle synergies selected  
180 through the global criterion ( $VAF \geq 75\%$  for each of the observed muscles) (Clark et al. 2010; Torres-  
181 Oviedo et al. 2007; Ting et al. 2010).

182 Muscle synergies extracted from each trial of each condition were then sorted through a  $k$ -means  
183 clustering algorithm applied to the weight vectors ( $W$ ) by setting the  $k$  value equals to  $N_{opt}$  (Steele et  
184 al. 2015). Once the weight vectors were sorted, the activation coefficient vectors ( $C(t)$ ) were ordered  
185 consequently.

186

### 187 *Muscle synergy analysis*

188 Muscle synergies extracted from the segmented EMG signals during the two different task conditions  
189 (OE and CE) were quantitatively compared in terms of (i) the optimal number of muscle synergies  
190 ( $N_{opt}$ ), (ii) the average recruitment levels ( $Recr$ ), and (iii) balance control strategies ( $S$ ).

#### 191 *i. Optimal number of muscle synergies ( $N_{opt}$ )*

192 The optimal number of muscle synergies ( $N_{opt}$ ) was selected for each trial of each task  
193 condition by choosing the smallest number of muscle synergies ( $N$ ) which guarantees  
194  $tVAF \geq 90\%$  (global criterion) and  $VAF \geq 75\%$  (local criterion) for each of the acquired  
195 muscle.

#### 196 *ii. Average recruitment levels ( $Recr$ )*

197 Since no typical cyclostationary processes can be assessed during a single-limb stance  
198 task, the activation coefficients ( $C(t)$ ) were compared in terms of average recruitment level  
199 ( $Recr_k$ ), defined as the average (over time) of each activation coefficient vector  $C(t)_k$   
200 (Torres-Oviedo et al. 2007; Chvatal et al. 2013). The recruitment level values range  
201 between 0 (no recruitment) and 1 (maximum recruitment) and quantify how much a  
202 specific muscle synergy is activated in the execution of the task.

#### 203 *iii. Balance control strategies ( $S$ )*

204 Three different balance control strategies were defined considering the acquired muscles:  
205 ankle control, knee control, and hip/trunk control strategy. The ankle control strategy  
206 ( $S_{ankle}$ ) was mainly identified by the activation of 5 leg muscles (PL, PB, LG, TA, and  
207 SO), the knee control strategy ( $S_{knee}$ ) by the activation of 3 shank muscles (VM, VL, and  
208 RF), and the hip/trunk control strategy ( $S_{hip}$ ) by the activation of 5 muscles of the proximal  
209 lower limb and the trunk (BF, ST, GM, LDO, and LDC). The computation of the balance  
210 control strategies is described in detail in our previous study (Ghislieri et al. 2020).

## 211 *Statistical analysis*

212 To assess statistically significant changes in the optimal number of muscle synergies considering the  
213 two different task conditions (OE and CE), the hypothesis of normality of the distribution was first  
214 tested through the Lilliefors test. If the normality hypothesis was rejected, the Wilcoxon signed-rank  
215 test was performed, otherwise, a two-tailed paired Student's *t*-test was performed. Two-way ANOVA  
216 for repeated measures followed by *post-hoc* analysis with Tukey adjustment for multiple comparisons  
217 was performed to evaluate the differences between conditions (OE and CE) and muscle synergies  
218 (factors: condition and synergies), for both the average recruitment levels (*Recr*) and balance control  
219 strategies (*S*). For the weight vectors (*W*), an analogous two-way ANOVA was applied to evaluate  
220 the differences between conditions and muscles. All the levels of significance ( $\alpha$ ) were set equal to  
221 0.05. The statistical analysis was carried out using the Statistical and Machine Learning Toolbox of  
222 MATLAB<sup>®</sup> release R2020b (The MathWorks Inc., Natick, MA, USA).

223

## 224 **RESULTS**

225 As follows, are reported the muscle synergy results computed considering the two different single-  
226 limb stance conditions (OE and CE). An example of the activation coefficients and weight vectors  
227 obtained from one of the participants in the eyes open and eyes closed conditions has been reported  
228 as additional data [see Additional file 1]. More specifically, muscle synergies were compared in terms  
229 of (*i*) the optimal number of muscle synergies, (*ii*) average recruitment levels, and (*iii*) balance control  
230 strategies.

### 231 *i. Optimal number of muscle synergies ( $N_{opt}$ )*

232 The application of the Wilcoxon signed-rank test revealed no statistically significant differences ( $p =$   
233 0.52) in the optimal number of muscle synergies ( $N_{opt}$ ) between the OE and CE conditions. In  
234 particular, 4 muscle synergies were needed to accurately model the motor control strategies during  
235 both the OE and CE conditions.

236 Figure 2 shows the muscle synergies, averaged over the sample population, extracted from the two  
 237 different task conditions: OE represented in blue and CE in red. More specifically, for each muscle  
 238 synergy, the average recruitment levels  $Recr_k$  (on the left) and the weight vectors  $W_k$  (on the right)  
 239 are represented. The asterisk (\*) indicates statistically significant differences between conditions  
 240 (repeated measures two-way ANOVA,  $p < 0.05$ ), both for the average recruitment levels and weight  
 241 vectors.

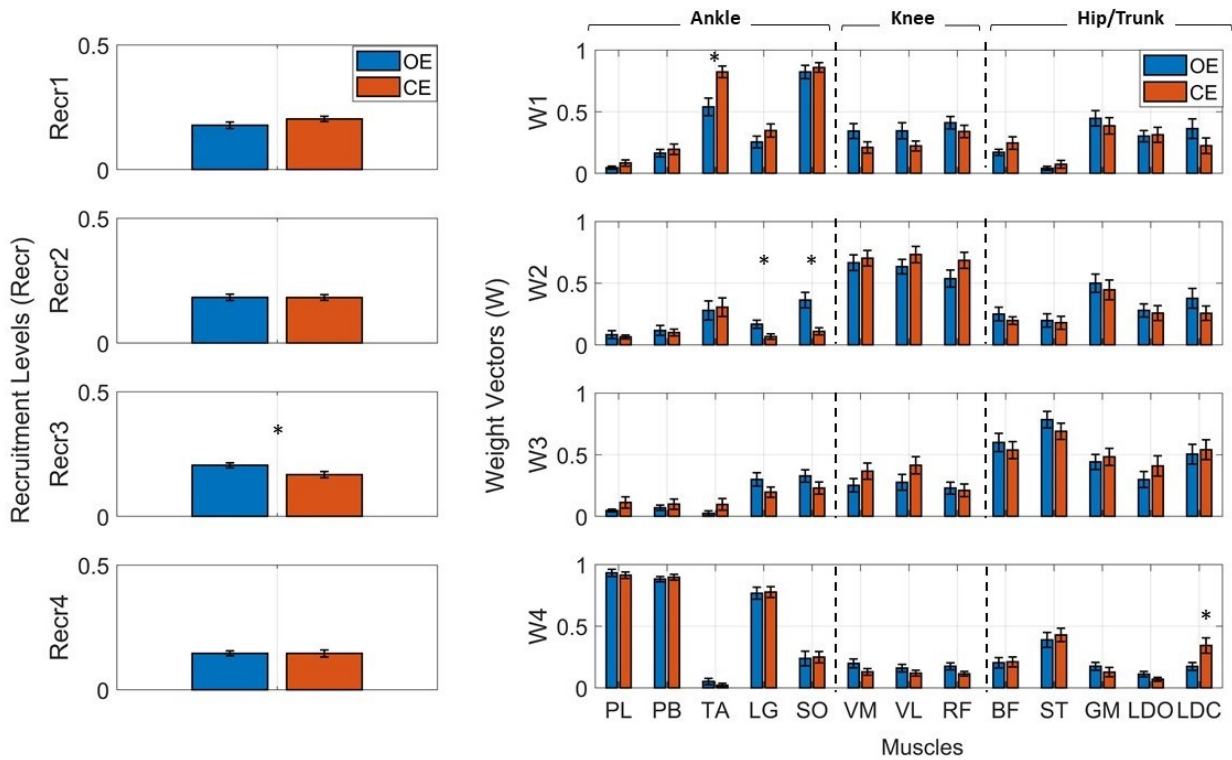


Figure 2. Comparison of muscle synergies extracted during eyes open (OE) and eyes closed (CE) single-limb stance conditions. Color vertical bars represent average recruitment levels  $Recr_k$  (on the left) and weight vectors  $W_k$  (on the right) of the  $k$ -synergy, over the sample population, with the superimposition of the standard errors (black lines). The asterisk (\*) represents a statistically significant differences between conditions, in the weight vectors and average recruitment levels.

242 *ii. Average recruitment levels (Recr)*

243 A statistically significant decrease ( $p = 0.02$ ) was found in the average recruitment level of the third  
 244 muscle synergy  $S_{hip}$  extracted during the CE condition ( $0.17 \pm 0.01$ ) with respect to the OE condition  
 245 ( $0.21 \pm 0.01$ ). No statistically significant differences were detected considering the remaining three  
 246 muscle synergies between OE and CE conditions, suggesting no changes in the recruitment levels of  
 247 those synergies due to the loss of visual feedback.

248 Figure 2 shows the average recruitment levels (on the left), over the sample population, extracted  
 249 during OE and CE single-limb stance conditions.

250 *iii. Balance control strategies (S)*

251 As shown in Figure 2, the first and the fourth muscle synergies can be mainly associated to an ankle  
 252 control strategy, since the muscles mainly enrolled are those belonging to the leg (PL, PB, LGS, TA,  
 253 and SO), the second muscle synergy to a knee control strategy and the third muscle synergy to a  
 254 hip/trunk control strategy. Two-way ANOVA for repeated measures revealed a statistically  
 255 significant increase ( $p = 0.03$ ) of the ankle control strategies ( $S_{ankle}$ ) during the CE condition ( $0.52 \pm$   
 256  $0.06$ ) with respect to the OE condition ( $0.47 \pm 0.06$ ). No additional statistically significant differences  
 257 were detected considering the remaining two balance control strategies ( $S_{knee}$  and  $S_{hip}$ ) between  
 258 conditions.

259 Table 1 represents the values of the balance control strategies, averaged over the sample population,  
 260 with the indication of the statistically significant changes between OE and CE conditions.

261

TABLE I  
 BALANCE CONTROL STRATEGIES (S) AVERAGED  
 ON THE SAMPLE POPULATION

BALANCE CONTROL STRATEGIES	AVERAGE BALANCE CONTROL STRATEGIES (MEAN $\pm$ STANDARD DEVIATION)		
	OE	CE	ANOVA (P-VALUE)
$S_{ankle}$	$0.47 \pm 0.06$	$0.52 \pm 0.06$	<b>0.03</b>
$S_{knee}$	$0.61 \pm 0.24$	$0.71 \pm 0.25$	0.22
$S_{hip}$	$0.53 \pm 0.15$	$0.53 \pm 0.17$	0.89

$S_{ankle}$ : ankle control strategy,  $S_{knee}$ : knee control strategy, and  $S_{hip}$ : hip/trunk control strategy.

262

263

264 **DISCUSSION**

265 The results of this study show that four major muscle synergies are used during single-limb stance,  
266 i.e., two ankle-dominant synergies, one knee-dominant synergy, and one hip/back-dominant synergy,  
267 in an open-eyes as well as in closed-eyes condition. In addition, there is no difference in the  
268 recruitment level between the open-eyes and closed-eyes conditions, except for the hip/back synergy,  
269 which showed a decreased activation in the closed-eyes compared to the open-eyes condition. At the  
270 same time an increase in the ankle balance strategy was found in the closed-eyes compared to the  
271 open-eyes condition, confirming the initial hypothesis of this study.

272 Since the work by Horak and Nashner (1986), it is widely recognized the essential role of the ankle  
273 for the control of upright posture and for the maintenance of posture when balance is challenged by  
274 perturbations of the supporting surface. In these circumstances, muscles around the ankle joint  
275 provide the first activation strategy for balance maintenance (Horak 2006). In our study, no  
276 perturbations were applied to the supporting surface and participants were required to maintain a quiet  
277 stance. The key role of the ankle for the control of posture in quiet stance is confirmed by the  
278 observation of two ankle-dominant synergies adopted by the participants in this study. The first ankle-  
279 dominant synergy (W1) is mainly featured by the tibialis anterior and the soleus muscle activation.  
280 The second ankle dominant synergy (W4) is mainly featured by peroneus longus and brevis muscles  
281 and gastrocnemius lateralis muscle activations. The two synergies may reflect the activations related  
282 to anterior posterior sway and medio-lateral sway, respectively, which may occur during a single-  
283 limb stance task. In particular, the co-activation of antagonist muscles, in this case tibialis anterior  
284 and soleus, might represent a strategy to cope with reduced base of support, with the aim to reduce  
285 movement variability and maintaining stability. Previous studies found an increase in tibialis anterior  
286 and soleus muscles activation in particular in older adults to compensate for reduced vision (Benjuya  
287 et al. 2004) or decreased tendon stiffness (Baudry et al. 2012), and both in children and elderly which  
288 showed a diminished postural steadiness when compared with young adults (Kurz et al. 2017).

289 Literature reports that as difficult the task becomes as higher is the involvement of more proximal  
290 joints for the maintenance of balance, in particular the hip (Horak 2006; Riemann et al. 2003). In  
291 experimental settings, the difficulty of the task is usually increased by increasing the magnitude of a  
292 perturbation, by decreasing the magnitude of the supporting surface or by changing the features of  
293 the supporting surface (Yamagata et al. 2008; Torres-Oviedo and Ting 2010; Yang et al. 2015;  
294 Riemann et al. 2003). For example, it has been reported that by moving from a stable to an unstable  
295 surface, the angular displacement of the ankle was stable across all the testing condition, with the

296 knee and hip displacement arising when the difficulty of the task was higher (Riemann et al. 2003;  
297 Creath et al. 2005).

298 In our study, the difficulty of the task was not modified throughout the experiment and the support  
299 surface was not unstable. However, standing on a single limb might be considered as a *per se* difficult  
300 task because of the reduced base of support in comparison to the common double-limbs stance.  
301 Usually, when the support base is reduced, a precaution strategy consisting in moving forward the  
302 center of mass is adopted to avoid falling backwards. This explains the presence of the hip/back  
303 muscle synergy (mainly featured by hamstrings and back muscles) adopted by the participants of our  
304 study. It should be also mentioned that, in a condition of quiet stance, the co-existence of the hip  
305 strategy with the ankle strategy has been reported (Creath et al. 2005), highlighting that the two  
306 strategies are not different entities, but one predominates depending upon the task and conditions of  
307 the environment (Creath et al. 2005). It is reasonable to think that the participants of the present study  
308 used the hip/back synergy to compensate for ankle dorsiflexion used to move forward the center of  
309 mass to manage the reduced base of support.

310 The essential role of quadriceps muscle for balance control during single-limb stance tasks is  
311 highlighted by the presence of the knee-dominant synergy (W2) used by the participants in this study.  
312 In fact, the knee-dominant synergy was probably used when the ankle synergy was not effective for  
313 the maintenance of balance, but the condition did not require yet the involvement of the hip or the  
314 back synergy. These results highlight the fine coordination between ankle muscles and quadriceps  
315 muscle. It was observed that when the knee-dominant synergy was used, ankle muscles had in general  
316 a low activation. This was especially observed in the closed-eyes condition, when the lack of visual  
317 information led to an increase in the difficulty of the task. In fact, it was observed a significantly  
318 lower activation of the soleus and gastrocnemius muscles when the knee-dominant synergy was used.  
319 This observation arises two possible speculations. The first is that the knee synergy is used when the  
320 ankle synergy is not sufficient for balance control. The second is that knee-synergy may be effective  
321 alone to guarantee stability during single-limb stance in some circumstances. At the same time when  
322 ankle-dominant synergies are used, a low activation of the quadriceps is observed in particular when  
323 the ankle synergy is featured by evertor muscles activation. This could be explained by the fact that  
324 this synergy is mainly used to manage with medio-lateral displacement. This observation is further  
325 confirmed by the higher activation of back muscles of the contra-lateral side for back stabilization in  
326 the mediolateral direction.

327 However, despite some differences in the closed- compared to the open-eyes condition, the number  
328 of synergies used is the same between the conditions, as well as the level of recruitment. This is in

329 accordance with previous literature reporting the stability of muscle synergies adopted between tasks  
330 with the variation of the visual feedback (Peterka 2002; Yang et al. 2015). It has been shown that in  
331 general the lack or the disturbance of vision does not affect synergies because during standing postural  
332 control mostly relies on proprioceptive feedback (Peterka 2002; Yang et al. 2015). In fact, the results  
333 of previous investigations show that proprioceptive disturbance, but not visual disturbances, affected  
334 the regulation of muscle synergies (Yang et al. 2015) and the increase in body sway (Peterka 2002).

335 The reduction in the recruitment level of the hip/back synergy in closed- compared to the open-eyes  
336 condition seems not in accordance with previous literature, reporting a major involvement of  
337 proximal joints as the difficulty of the task increases (Horak and Nashner, 1986; Riemann et al. 2003;  
338 Creath et al. 2005). However, in the present study, an increase in the involvement of the ankle-  
339 dominant synergy for balance control has been observed in the closed-eyes condition. This result  
340 confirms the initial hypothesis of this study on the increase in muscles activations around the ankle  
341 joint. It is likely to think that this modulation aimed at decreasing the degrees of movement to increase  
342 stability, was probably sufficient to maintain balance and the use of muscle synergies involving  
343 proximal joints and segments was not determinant for the outcome of the task.

344 The observation of a change in the modulation of some of the muscle activations in the closed-eyes  
345 condition is in accordance with previous literature. A decrease in synergistic muscle coherence was  
346 observed during double-limb stance in a closed-eyes compared to an open-eyes condition (Danna-  
347 Dos-Santos et al. 2015), thus showing that the lack of visual feedback and the reliance on other  
348 sources of afferent information affects the generation of neural inputs on synergistic muscles.  
349 Regarding the results of the present study, it can be thus speculated that the lack of visual information  
350 affects the modulation of muscle activation, without altering the type and numbers of synergies  
351 adopted. For example, an increase in the ankle balance strategy was found in the closed-eyes  
352 compared to the open-eyes condition. It is plausible to think that the lack of the visual feedback led  
353 to a sensory reweighting for the control of posture, shifting the sensory information arising from  
354 vision with an increased proprioceptive information arising from the ankle joint and ankle movements  
355 (Kabbaligere et al., 2017).

356 Accordingly, the results of this study suggest also that muscle synergies are probably not exclusively  
357 managed throughout a feedforward control, but can be modulated with a feedback control based on  
358 the signals arising from sensory receptors, with the aim to correct movement errors which may occur  
359 in some circumstances. It is likely to think that the maintenance of the single-limb stance in this study  
360 was controlled with pre-programmed muscle synergies. However, the difficulty of the task leading to  
361 continuous losses and recovery of balance probably needs a continuous movement correction based

362 on a feedback control relying on information arising from sensory receptors. Animal studies have  
363 reported organized patterns of muscles activations in response to focal stimulation of the spinal cord  
364 (Tresch et al., 1999; Saltiel et al., 2001; Lemay and Grill, 2004; D'Avella and Bizzi, 2005), thus  
365 suggesting that a feedback control may be launched at spinal level in response to specific sensory  
366 stimuli to modulate the centrally organized synergy recruitment. It is likely to think that similar  
367 patterns may regulate muscle synergies also in humans.

368 Finally, it should be mentioned that in this study biceps femoris and semitendinosus, which are two-  
369 joints muscles, were grouped in the hip/back synergy, and not into the knee synergy. This is related  
370 to the fact that as for the nature of the task, hamstrings muscles were more deputed to the hip extension  
371 than to knee flexion (Bourne et al., 2016; Hegyi et al., 2021). At the same time, quadriceps muscle,  
372 which is also a two-joints muscle, was grouped only in the knee synergy. This is related to the fact  
373 that participants were asked to stand in an upright posture with the hip joint in full extension. In the  
374 latter position, the quadriceps (and in particular the RF) has a reduced activation and thus a lower  
375 involvement in the hip joint control (Ema et al., 2016).

376 To the best of the authors' knowledge, this is the first study investigating muscle synergies deputed  
377 to the maintenance of posture during a single-limb stance task without external perturbations, in an  
378 open-eyes and closed-eyes condition. Due to the large use of this kind of task in clinical practice, both  
379 for rehabilitation and functional assessment, as well as in sport practice for training and testing, the  
380 results of the present study give important information on motor control of this kind of task in healthy  
381 individuals. Future studies should investigate muscle synergies also in other populations to  
382 investigate the effects of orthopedic and neurologic pathologies on muscle synergies, as well as the  
383 effect of rehabilitation and training.

384 The main limitation of this study is that we recruited only healthy young individuals, and thus the  
385 results cannot be generalized to all healthy individuals. Future studies should identify muscle  
386 synergies used for single-limb stance also in other age groups. A second limitation of the study was  
387 that muscle synergies for the transition between double- and single-limb stance (and vice versa) were  
388 not analyzed, thus the results of the present study have to be considered exclusively for steady single-  
389 limb stance tasks.

## 390 **CONCLUSIONS**

391 In conclusion, single-limb stance is featured by four major muscle synergies, two ankle-dominant,  
392 one knee-dominant and one hip/back-dominant. The lack of visual feedback did not affect the number  
393 of synergies used. In general, an increase of activation of the ankle muscles and a decrease in the



394 recruitment of the hip/back synergy was observed in the absence of visual information in comparison  
395 to the normal vision condition. To the best of the authors knowledge, this is the first study providing  
396 information on muscle synergies adopted during single-limb stance which is a task featuring a number  
397 of daily living activities, as well as training and rehabilitation exercises. Future studies should  
398 investigate muscle synergies during single-limb stance also in other age groups, and it seems of high  
399 clinical relevance to investigate synergies on orthopedic and neurologic patients to address clinical  
400 practice and rehabilitation interventions.

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405 **Abbreviations:** BF, biceps femoris; C, activation coefficients; CE, closed eyes; CNS, central nervous  
406 system; COP, center of pressure; EMG, electromyography; FSW, footswitch; GM, gluteus medius;  
407 LDC, longissimus dorsii of contralateral side; LDO, longissimus dorsii omolateral to the dominant  
408 lower limb; LG, lateral gastrocnemius; M, models of the original EMG envelopes; N, number of  
409 synergies; NMF, non-negative matrix factorization; OE, open eyes; PB, peroneus brevis; PL,  
410 peroneus longus; Recr, average recruitment level; RF, rectus femoris; S, balance control strategies;  
411 SO, soleus; ST, semitendinosus; TA, tibialis anterior; VAF, variance accounted for; VL, vastus  
412 lateralis; VM, vastus medialis; W, weight vectors.

413

414

415 **DECLARATIONS**

416

417 **Ethics approval and consent to participate.**

418 Each participant signed an informed consent before participating in the study. The study was  
419 conducted in accordance with the Declaration of Helsinki and received ethical approval from the  
420 Ethical Committee of the Rizzoli Orthopedic Institute (PG n. 0004167).

421

422 **Consent for publication.**

423 A written consent for publication was received from the participants.

424

425 **Competing interests.**

426 The authors declare no competing interests.

427

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430

431 **Authors' contributions.**

432 LL, MGB, MK, VA and MG contributed to the study conception and design. Material preparation,  
433 data collection and analysis were performed by LL, GB, LB, MG and VA. All authors contributed to  
434 the interpretation of the data. LL and MG wrote the first draft of the manuscript, which MGB, MK,  
435 VA, GB and LB critically revised. All authors read and approved the final manuscript.

436

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438 N.A.

439

440 **Data and material availability.**

441 All data and material are available from the corresponding author upon reasonable request.

442

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596



597 **Additional data**

598 File name: Additional file 1.doc

599 Title: Example of muscle synergies in one of the participants.

600 Description of data: Activation coefficients and weight vectors obtained from a representative healthy  
601 subject of the sample population considering two different task conditions: eyes open and eyes closed  
602 conditions.

603