

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

47

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 ± 2.2 years; height: 182 ± 8.4 cm; body mass: 74.5 ± 10.8 kg)
111 and eleven female participants (age: 24.5 ± 2.9 years; height: 169 ± 5.8 cm; body mass: 57.2 ± 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° . 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 8th 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 5th 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 (α) were set equal to
221 0.05. The statistical analysis was carried out using the Statistical and Machine Learning Toolbox of
222 MATLAB[®] 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.

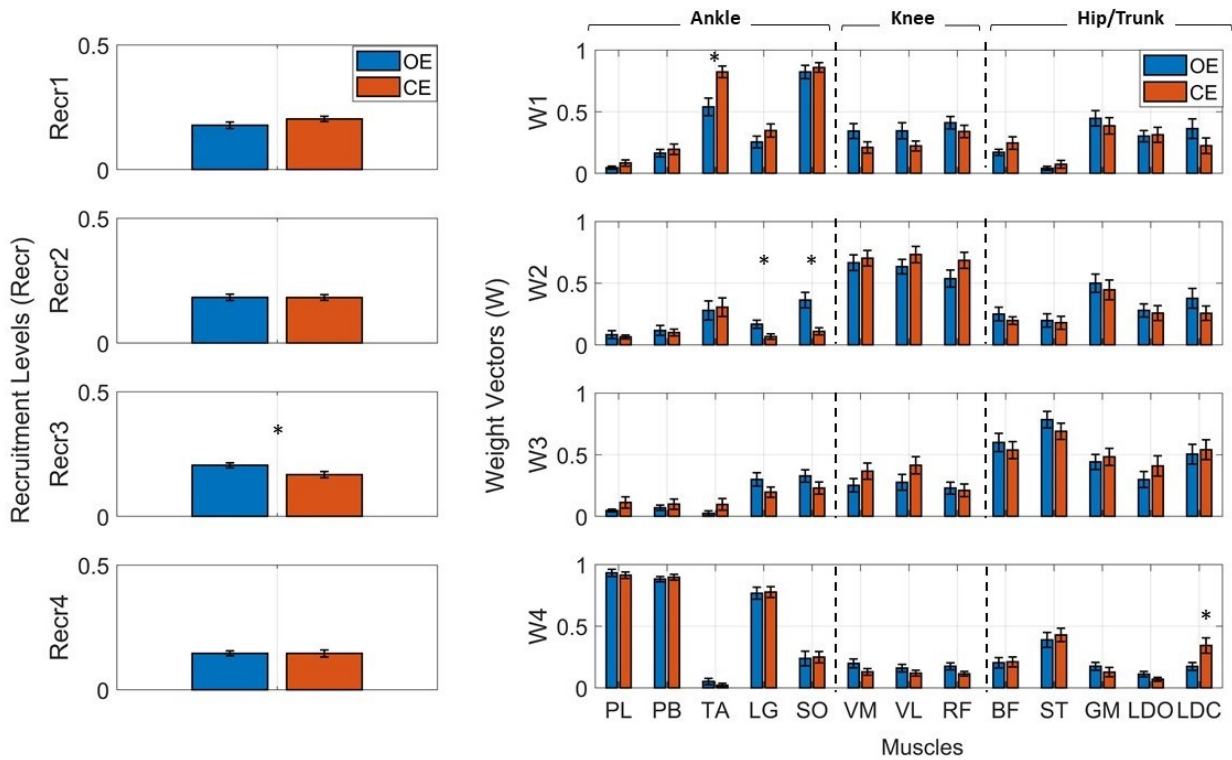


Figure 2. Comparison of muscle synergies extracted during eyes open (OE) and eyes closed (CE) single-limb stance conditions. Color vertical bars represent average recruitment levels $Recr_k$ (on the left) and weight vectors W_k (on the right) of the k -synergy, over the sample population, with the superimposition 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 ± 0.01) with respect to the OE condition
 245 (0.21 ± 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 ± 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 ± 0.06 | 0.52 ± 0.06 | 0.03 |
| S_{knee} | 0.61 ± 0.24 | 0.71 ± 0.25 | 0.22 |
| S_{hip} | 0.53 ± 0.15 | 0.53 ± 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.

401

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403

404

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