








## Association between ankle torque and performance-based tests, self-reported pain, and physical function in patients with knee osteoarthritis

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### ABSTRACT

**Objectives:** This study aimed to investigate the association between ankle torque and performance-based tests, self-reported pain, and physical function in patients with knee osteoarthritis (OA).

**Patients and methods:** The cross-sectional study was conducted with 39 individuals (24 females, 15 males; mean age: 57.3±6.2 years; range, 40 to 65 years) with knee OA between January 2014 and July 2015. Ankle torque was determined using an isokinetic dynamometer. The 40-m fast-paced walk test and a stair climb test were used to assess functional performance. Self-reported pain and physical function were assessed using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). Pearson's correlation coefficients were calculated to test correlations between the dependent variables (40-m fast-paced walk test, stair climb test, WOMAC pain and physical function domains, sex, age, body mass index, and radiologic evidence of OA) and the independent variables (mean plantar flexor torque and dorsiflexor peak torque). A multiple linear regression analysis was applied to quantify the association between the dependent and independent variables.

**Results:** Dorsiflexor and plantar flexor peak torques in the concentric and eccentric modes were negatively correlated with the 40-m fast-paced walk and stair climb tests ( $r=-0.33$  to  $-0.51$ ,  $p\leq 0.05$ ). A negative correlation was found between concentric plantar flexor torque and the WOMAC physical function score ( $r=-0.35$ ,  $p=0.03$ ). No correlation was found between ankle torques and the WOMAC pain score ( $p>0.05$ ). The multiple linear regression analysis showed that the eccentric plantar flexor and dorsiflexor torques were significantly associated with the stair climb test ( $\beta=-0.001$ , 95% confidence interval [CI]:  $-0.001$  to  $0.000$ ,  $p=0.03$ , and  $\beta=-0.002$ , 95% CI:  $-0.004$  to  $0.000$ ,  $p=0.05$ , respectively). No significant associations were found between concentric plantar flexor and dorsiflexor torques and the stair climb test ( $p>0.05$ ). No significant associations were found between the ankle torques and the 40-m fast-paced walk test and WOMAC physical function ( $p>0.05$ ).

**Conclusion:** Ankle torque plays an important role in functional performance. Thus, ankle torque deficit, especially eccentric plantar flexor and dorsiflexor torques, may exert a negative influence on stair climbing performance in patients with knee osteoarthritis.

**Keywords:** Cartilage diseases, correlation of data, knee, muscle strength dynamometer, osteoarthritis, physical functional performance.

The proportion of the population aged 45 years or older with osteoarthritis (OA) diagnosed by a physician is expected to increase from 26.6 to 29.5% by the year 2032.<sup>1</sup> OA is a degenerative chronic disease characterized by

articular cartilage damage. Clinical signs and symptoms include pain, morning stiffness for up to 30 min, swelling, and functional disability. The knee is the weight-bearing joint most frequently affected by OA.<sup>2-4</sup>

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Knee OA is associated with lower muscle capacity. The quadriceps is one of the most compromised muscles in knee OA, which contributes to knee joint instability. Indeed, quadriceps weakness is considered one of the reasons for development and progression of the disease, leading to worse pain symptoms and diminished physical functioning.<sup>5-8</sup> Individuals with knee OA also have hip muscle impairment.<sup>9-11</sup> A systematic review and meta-analysis reported that individuals with symptomatic knee OA have hip strength deficits.<sup>9</sup> Tevald et al.<sup>10</sup> investigated the association between hip muscle strength and functioning in individuals with knee OA and found that isometric hip abductor strength was negatively associated with physical functioning determined on functional tests, such as the get up and go, timed chair-rise, timed stair climb, and descent tests. The results suggest that individuals with knee OA have reduced muscle strength and consequently require a longer time to perform walking, standing, and stair climbing tests. Thus, deficits in hip and knee muscle strength seem to influence the physical functioning of individuals with knee OA.

Ankle muscles play an important role in activities such as walking, jumping/landing tasks, weight-bearing activities, and climbing stairs. Studies have found an association between ankle strength and functional performance in older adults, as lower strength of the plantar flexors and dorsiflexors was related to greater impairment with regard to functional performance.<sup>12,13</sup> Suzuki et al.<sup>14</sup> demonstrated that muscle power generated by the plantar flexors and dorsiflexors is a predictor of functional performance on stair climb and chair rise tests in older women. Although ankle muscle strength seems to be important for physical functioning, few studies have investigated the role of plantar flexors and dorsiflexors in knee OA. Draz and Abdel-Aziem<sup>15</sup> found that individuals with knee OA have lower concentric plantar flexor and dorsiflexor torque peaks compared to healthy individuals, but the authors did not perform an analysis of functional performance in these individuals. Gonçalves et al.<sup>16</sup> also reported lower ankle strength in individuals with knee OA, especially with regards to plantar flexor torque but also failed to investigate how this strength deficit affects functional performance in this population. Vårbakken et al.<sup>17</sup> found that ankle and hip

muscle weakness had a greater impact on the frontal and transverse plane during gait than knee extensor weakness. Therefore, we hypothesized that deficits in plantar flexor and dorsiflexor strength could interfere with functioning in these individuals.

To the best of our knowledge, no previous study has investigated whether plantar flexor and dorsiflexor torques are associated with functioning and symptoms in patients with knee OA. Therefore, the aim of the present study was to determine whether concentric and eccentric plantar flexor and dorsiflexor torques are associated with functional performance (measured using the 40-m fast-paced walk test and stair climb test), self-reported pain, and physical function in patients with knee OA. We hypothesized that the lower ankle torque in these individuals would be associated with impaired functional performance as well as greater self-reported pain and functional disability.

## PATIENTS AND METHODS

This cross-sectional study was conducted at the Physiotherapy Department of the Federal University of São Carlos (UFSCar), Brazil and followed the guidelines of the STROBE statement.<sup>18</sup> The data collection timeframe was from January 2014 to July 2015. Individuals from the general community were recruited through the divulgation of the study on the website of the authors' institution, flyers, as well as local radio, newspapers, and magazines. Individuals with clinical signs of unilateral or bilateral knee OA based on the American College of Rheumatology criteria<sup>19</sup> and radiographic signs Grade 2 or 3 classified according to the criteria proposed by Kellgren and Lawrence<sup>20</sup> were included. The exclusion criteria were those used by Gonçalves et al.:<sup>16</sup> body mass index (BMI) >35 kg/m<sup>2</sup>; having undergone physical therapy for the knee in the previous six months; a history of trauma in the lower limb; a history of ligament and meniscus injuries in the knee; previous knee or hip surgery; a diagnosis of hip OA; systemic arthritic conditions; the use of corticosteroid infiltration in the knees in the previous six months; pain predominantly in another region of the body; any cardiovascular, respiratory, neurological, or musculoskeletal

condition that impeded participation in the proposed evaluations. Eighty-seven individuals were assessed for eligibility. Forty-eight were excluded based on the eligibility criteria or declined to participate. Thus, 39 individuals (24 females, 15 males; mean age:  $57.3 \pm 6.2$  years; range, 40 to 65 years) with knee OA participated in this study (Figure 1).

The participants were instructed not to perform any physical activity beyond habitual activity in the 48 h prior to the tests. The affected limb was selected to perform the strength test. In cases of bilateral knee OA, the more symptomatic limb was evaluated according to the level of pain verified by the numeric pain rating scale.<sup>21</sup>

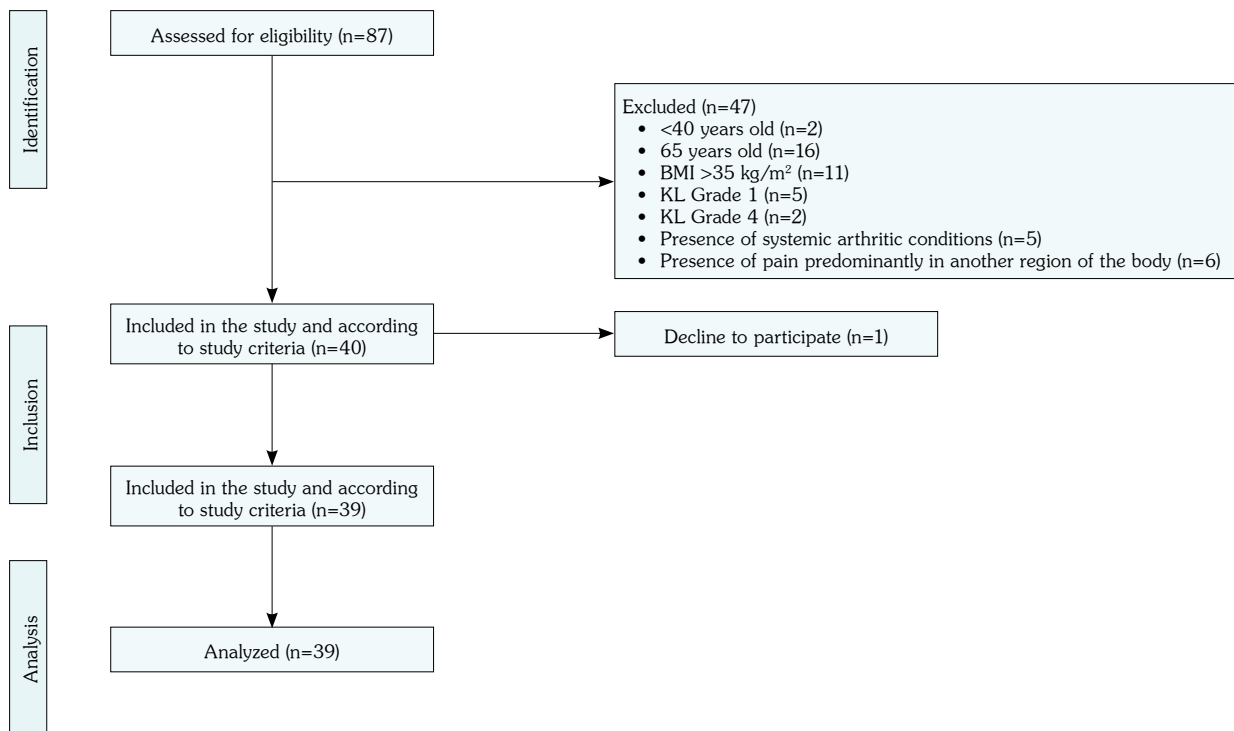
Participants who were included in the study did not receive treatment. Guidance was only given after the end of the study regarding the self-management of their health condition.

### Independent variable

Concentric and eccentric torques during plantar flexion and dorsiflexion were determined using the Biodex Multi-Joint System 3 isokinetic

dynamometer (Biodex Medical Systems 3 Pro, Shirley, NY, USA) and recorded at a sampling frequency of 100 Hz. The isokinetic dynamometer is considered the gold standard for the measurement of muscle strength.<sup>22</sup> Prior to each isokinetic assessment, the dynamometer was calibrated according to the manual provided by the manufacturer.<sup>23</sup> All procedures, including correction for the effect of gravity on the torque measurements, were conducted in accordance with the instruction manual of the equipment.

For the determination of plantar flexor and dorsiflexor torque, the participants were positioned according to the manufacturer's instructions: semi-reclined with the hip flexed at  $70^\circ$  and with the knee maintained at  $30^\circ$  flexion.<sup>23</sup> A pad was placed under the thigh for stabilization. The participants were stabilized with a strap around the hips, two shoulder straps that crossed the chest, a strap across the thigh of the tested leg, and straps across the top of the forefoot and midfoot.<sup>23</sup> The foot was positioned in a bracket such that the axis of rotation of the ankle was aligned with the axis of the lever arm. The range of motion was determined individually in an



**Figure 1.** STROBE flow diagram for patient enrolment into the study. BMI: Body mass index; KL: Kellgren and Lawrence criteria.

attempt to reach a maximum of 10° dorsiflexion and 35° plantar flexion.<sup>16</sup>

Prior to the test, participants performed three submaximal and two maximal contractions to become familiarized with the movements and equipment. The participants were instructed to perform maximum effort on each test. Data collection began after a 3-min rest and consisted of five repetitions of maximum contraction at an angular velocity of 60°/sec.<sup>16</sup>

The concentric and eccentric assessments were initiated in the dorsiflexion position of the ankle. The range of motion for the evaluations was determined for each individual in an attempt to reach a maximum of 10° dorsiflexion and 30° plantar flexion.<sup>24,25</sup> The participants received verbal encouragement during all the trials, but no visual feedback was given during the assessments.<sup>26</sup>

The isokinetic peak torque was normalized by individual body mass (peak torque/body mass [kg]×100). For statistical analysis, we used the mean peak torque of the five trials.

### Outcome measures

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was used to evaluate self-reported pain and physical function.<sup>27</sup> The items are scored using a Likert scale (none=0, mild=1, moderate=2, severe=3, and extreme=4). The pain domain consists of five items (score ranges from 0 to 20 points) and the physical function domain comprises 17 items (score ranges from 0 to 68). Higher scores indicate worse symptoms.<sup>27</sup> A version translated and validated for the Brazilian Portuguese language was used.<sup>28</sup>

Two performance-based tests were conducted: a stair climb test and a 40-m fast-paced walk test. For the stair climb test, the participants were instructed to go up and down a flight of stairs with 11 steps (step rise of 17 cm, step width of 202 cm, and step tread of 31 cm) as quickly and safely as possible.<sup>29</sup> For the 40-m fast-paced walk test, the participants were instructed to walk a distance of 40 m as quickly and safely as possible but without running.<sup>29</sup> For this test, the participants walked along a 10-m track, turned around a cone, and returned to the starting position, repeating the task a second time to complete the distance of

40 m. Prior to the start of the tests, a practice trial was performed to verify comprehension and safety.<sup>29</sup> The performance time was recorded on each test.

### Statistical analysis

The sample size calculation was calculated using the G\*Power software version 3.1.9.2 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) for correlation data. Considering a significance level of  $\alpha=0.05$  and  $\beta=0.2$  as parameters for the correlation analyses and estimated  $r$  of 0.6 for a moderate correlation,<sup>30</sup> a sample size of 37 participants was determined.

The data were analyzed with the aid of IBM SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics (mean, standard deviation, median, maximum, and minimum) were performed for all data and the normality of the data was checked using the Shapiro-Wilk test. Nonnormal data (stair climb test) was transformed by a log transformation. Pearson's correlation coefficient was calculated to analyze correlations between the dependent variables (WOMAC pain and physical function scores, 40-m fast-paced walk and stair climb tests, age, BMI, sex, and radiologic evidence of OA) and independent variables (normalized mean peak ankle torques). Correlation values ( $r$ ) were interpreted as follows: 0.00 to 0.19, none/mild; 0.20 to 0.39, low; 0.40 to 0.69, moderate; 0.70 to 0.89, strong; 0.9 to 1.00, very strong.<sup>30</sup> An alpha level of 0.05 was set for all statistical tests.

A multiple linear regression analysis was applied to quantify the association between the dependent variables (WOMAC physical function score, 40-m fast-paced walk and stair climb tests) and the independent variables (the normalized mean peak ankle torque). Participant characteristics (age, BMI, sex, and radiological evidence of OA) that were related to the performance-based tests and WOMAC domains were included in the multiple linear regression as covariates. The multicollinearity was tested using the variance inflation factor ( $VIF<1.6$ ).

## RESULTS

The demographic data of the sample, WOMAC pain and physical function scores, functional

**Table 1.** Anthropometric variables and characteristics of the participants with knee osteoarthritis, descriptive data of WOMAC domains, performance-based tests, and ankle torque (n=39)

Variables	n	%	Mean±SD
Anthropometric variables and characteristics			
Age (year)			57.3±6.2
Height (m)			1.7±0.1
Mass (kg)			76.7±11.7
BMI (kg/m <sup>2</sup> )			28.1±3.7
Female	24	63.2	
Radiologic evidence of OA			
KL Grade 2	26	66.7	
KL Grade 3	13	33.3	
WOMAC score			
Pain			7.8±4.0
Physical function			25.5±13.8
Performance-based tests			
40m fast-paced walk (sec)			24.8±4.3
Stair climb (sec)			12.3±3.1
Peak torque (Nm/kg×100)			
Plantar flexion			
Concentric			29.0±13.4
Eccentric			53.4±36.0
Dorsiflexion			
Concentric			24.5±7.6
Eccentric			23.2±11.2

SD: Standard deviation; BMI: Body mass index; OA: Osteoarthritis; KL: Kellgren and Lawrence criteria; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

**Table 2.** Correlation (r) and p values between ankle torque and anthropometric variables and characteristics with performance-based tests and WOMAC domains in patients with knee osteoarthritis

	Performance-based tests				WOMAC scores			
	40 m fast-paced walk (sec)		Stair climb (sec)		Pain		Physical function	
	r	p	r	p	r	p	r	p
Ankle torque								
Concentric plantar flexor (Nm/kg×100)	-0.36	0.03*	-0.39	0.02*	-0.15	0.4	-0.35	0.03*
Eccentric plantar flexor (Nm/kg×100)	-0.46	0.003*	-0.47	0.003*	-0.18	0.28	-0.27	0.09
Concentric dorsiflexor (Nm/kg×100)	-0.33	0.04*	-0.38	0.02*	-0.19	0.26	-0.28	0.09
Eccentric dorsiflexor (Nm/kg×100)	-0.49	0.002*	-0.51	0.001*	-0.07	0.66	-0.10	0.56
Age	0.34	0.03*	0.39	0.01*	0.24	0.15	0.12	0.46
Body mass index	0.33	0.04*	0.30	0.07	0.19	0.26	0.32	0.05*
Sex**	0.50	0.001*	0.47	0.003*	0.31	0.05*	0.35	0.03*
Radiologic evidence of OA**	0.15	0.35	0.21	0.20	0.19	0.25	0.12	0.46

WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index; OA: Osteoarthritis; \* p<0.05: Significant relationship; \*\* Point biserial correlation.

**Table 3.** Multiple linear regression analysis results for the association between ankle torque with performance-based tests and WOMAC physical function in patients with knee osteoarthritis

Ankle torque	Performance-based tests						WOMAC score		
	40m fast-paced walk (sec)*			Stair climb (sec)**			Physical function***		
	$\beta$	95% CI	p	$\beta$	95% CI	p	$\beta$	95% CI	p
Concentric plantar flexor (Nm/kg $\times$ 100)	-0.05	-0.15 to 0.05	0.36	-0.001	-0.003 to 0.001	0.26	-0.26	-0.60 to -0.08	0.13
Eccentric plantar flexor (Nm/kg $\times$ 100)	-0.03	-0.07 to 0.003	0.07	-0.001	-0.001 to 0.0001	0.03	-0.04	-0.17 to 0.09	0.51
Concentric dorsiflexor (Nm/kg $\times$ 100)	-0.06	-0.24 to 0.11	0.46	-0.002	-0.005 to 0.001	0.21	-0.26	-0.87 to 0.34	0.38
Eccentric dorsiflexor (Nm/kg $\times$ 100)	-0.10	-0.22 to 0.02	0.10	-0.002	-0.004 to 0.0001	0.05	0.15	-0.28 to 0.58	0.49

WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index; \* Age, sex, and BMI were included as covariates; \*\* Age and sex were included as covariates; \*\*\* Sex and BMI were included as covariates.

performance on the 40-m fast-paced walk and stair climb tests and ankle torques are presented in Table 1.

Significant correlations were found between the dorsiflexor and plantar flexor torques and the performance-based tests. Low negative correlations were found between concentric plantar flexor torque and the 40-m fast-paced walk test ( $r=-0.36$ ,  $p=0.03$ ) and the stair climb test ( $r=-0.39$ ,  $p=0.02$ , Table 2). Moderate negative correlations were found between eccentric plantar flexor and the 40-m fast-paced walk test ( $r=-0.46$ ,  $p=0.003$ ) and the stair climb test ( $r=-0.47$ ,  $p=0.003$ ). Low negative correlations were found between concentric dorsiflexor torque and the 40-m fast-paced walk test ( $r=-0.33$ ,  $p=0.04$ ) and the stair climb test ( $r=-0.38$ ,  $p=0.02$ , Table 2). Moderate negative correlations were found between eccentric dorsiflexor torque and the 40-m fast-paced walk test ( $r=-0.49$ ,  $p=0.002$ ) and the stair climb test ( $r=-0.51$ ,  $p=0.001$ ). These negative correlations indicate that lower muscle strength is related to impaired functional performance on walking and stair climbing tasks.

No correlation was found between the ankle torques and WOMAC pain score ( $p>0.05$ ). A significant correlation was found between concentric plantar flexor torque and the WOMAC physical function score ( $r=-0.35$ ,  $p=0.03$ ). No correlation was found between other ankle torques and the WOMAC physical function score (Table 2).

Results from the multiple linear regression analysis showed that the eccentric plantar flexor and dorsiflexor torques were significantly associated with the stair climb test ( $\beta=-0.001$ , 95% confidence interval [CI]: -0.001 to 0.0001,  $p=0.03$ , and  $\beta=-0.002$ , 95% CI: -0.004 to 0.000,  $p=0.05$ , respectively). No significant associations were found between concentric plantar flexor and dorsiflexor torques and the stair climb test ( $p>0.05$ ). In addition, no significant associations were found between the ankle torques and the 40-m fast-paced walk test and WOMAC physical function (Table 3).

## DISCUSSION

The present investigation is a pioneering study on the association between ankle torque and

performance-based tests, self-reported pain, and self-reported physical functioning in patients with knee OA. Our hypothesis was partially supported, as ankle torque was significantly associated with performance on the 40-m fast-paced walk and stair climb tests in this population but was not associated with self-reported pain and physical functioning, except for the correlation between concentric plantar flexor torque and the WOMAC physical function score. In addition, the multiple linear regression analysis showed that eccentric plantar flexor and dorsiflexor torques were significantly associated with the stair climb test, although the other variables were also correlated.

Studies have demonstrated an association between physical functioning and both knee extensor strength and hip abductor strength.<sup>7,10,31</sup> To the best of our knowledge, however, no study has investigated the relationship between ankle torque and physical functioning.

The plantar flexor and dorsiflexor muscles have an essential role in the gait cycle.<sup>32</sup> Previous studies found a decreased ankle dorsiflexion moment, decreased stride length during gait, and decreased walking speed in individuals with knee OA.<sup>33-38</sup> Moreover, such individuals have lower ankle torque compared to healthy individuals.<sup>15,16</sup> According to our findings, the strength of these muscles seems to influence the performance of this activity of daily living in individuals with knee OA.

We found an association between eccentric plantar flexor and dorsiflexor torques and the stair climbing task, showing that plantar flexor and dorsiflexor torque may influence the performance of this task. Indeed, the literature reports altered ankle kinematics during the task of climbing and descending stairs.<sup>39-41</sup> Hicks-Little et al.<sup>39</sup> found reductions in step and stride in individuals with knee OA when climbing and descending stairs. Igawa and Katsuhira<sup>40</sup> found that individuals with knee OA had less ankle muscle power compared to healthy individuals when descending steps. Gonçalves et al.<sup>41</sup> found an altered plantar flexion angle during stair climbing in individuals with knee OA. Plantar flexor strength is vital in the propulsion phase of climbing stairs and during gait.<sup>32</sup> As climbing and descending stairs is a common complaint among individuals with knee OA,<sup>40</sup> this difficulty could be related to ankle muscle weakness.

Hicks-Little et al.<sup>42</sup> found that individuals with knee OA took a long time to reach the peak of the dorsiflexion angle during the support phase of the gait cycle when descending stairs compared to a healthy group. Therefore, dorsiflexors participate in the swing phase during stair climbing stairs, performing ankle dorsiflexion, and moving the foot away from the step.<sup>32</sup> A meta-analysis showed that patients with knee OA exhibited decreased ankle dorsiflexion during the stair task, took a long time to perform an 11-step stair climb test, and had a longer/slower stair ascending and descending time/velocity.<sup>43</sup> Our results showed that the eccentric dorsiflexor torque was significantly associated with the stair climb test. Thus, dorsiflexor strength seems to affect the ability to ascend and descend stairs in patients with knee OA, impacting functional performance since these individuals take a long time to perform this task. In addition, Suzuki et al.<sup>14</sup> showed that dorsiflexor muscle strength was an independent predictor of stair climbing performance in older women, which is in agreement with our findings.

No correlations were found between ankle torques and pain. Self-reported pain using the WOMAC questionnaire is restricted to the previous 72 h, which may have influenced the noncorrelation with strength, as pain was not evaluated during strength assessment. Previous studies also found no association between quadriceps muscle strength and pain in individuals with knee OA.<sup>44,45</sup> In contrast, Muraki et al.<sup>6</sup> conducted a large population-based cohort study and found that the quadriceps muscle strength was associated with knee pain. The strength of ankle muscles is probably not influenced by pain. However, further studies are needed to investigate this association. Regarding the correlation between the WOMAC physical function score and ankle torques, only a low negative correlation was found with peak concentric plantar flexor torque. These results indicate that lower muscle strength increases the likelihood of self-reported functional deficit.

Our results suggest that ankle torque, particularly the eccentric plantar flexor and dorsiflexor torques, may influence the performance of locomotion tasks in patients with knee OA, as lower ankle torque was associated with a poorer performance on stair

climb test (i.e., a longer time required to perform this task). As individuals with knee OA have impaired ankle torque<sup>15,16</sup> and this reduction in strength can negatively impact locomotion tasks, it would be significant to include ankle muscle exercises in rehabilitation programs for this population. Such exercises could improve muscle strength and, consequently, physical functioning in individuals with knee OA.

The strengths of our study were the use of gold standard instruments for the assessment of muscle strength and functional performance<sup>22,29</sup> and the use of a version of the WOMAC questionnaire translated and validated for Brazilian Portuguese<sup>28</sup> that is specific to individuals with knee or hip OA.<sup>27</sup>

There are some limitations to this study. One limitation is the small sample size since we included many variables in the multiple regression model. Therefore, the results of our study should be interpreted with caution. Our study does not allow us to determine cause-and-effect relationships between plantar flexor/dorsiflexor torques and functional performance on the 40-m fast-paced walk and stair climb tests, as well as self-reported pain and physical functioning. Thus, longitudinal studies are needed to confirm our findings. Second, the pain was not measured during or after the ankle torque assessments and performance-based tests, which may have influenced our results. Future studies should measure pain during performance-based tests and use it as a covariate to gain a better understanding of its effect on performance-based physical functioning.

In conclusion, ankle torque plays a critical role in the functional performance of patients with knee OA. The present data suggest that the deficient strength of these muscles, specifically eccentric plantar flexors and dorsiflexors, may negatively influence stair climbing performance.

**Ethics Committee Approval:** Ethical approval was obtained from institutional review board of the Federal University of São Carlos (date: 03.11.2014, no 573.999, certificate number: 27317214.0.0000.5504). The study was conducted in accordance with the principles of the Declaration of Helsinki.

**Patient Consent for Publication:** All participants provided written informed consent prior to participation.

**Data Sharing Statement:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Author Contributions:** Contributing to the conception and design, collecting data, analyzing and interpreting data, drafting the article and revising it critically for important intellectual content, and approving the final version to be published: C.C.; Contributing to the conception and design, collecting data, drafting the article and revising it critically for important intellectual content, and approving the final version to be published: G.G.H.; Contributing to the collecting data, analyzing and interpreting data, drafting the article and revising it critically for important intellectual content, and approving the final version to be published: S.L.F.A., P.M.; Contributing to the analyzing and interpreting data, drafting the article or revising it critically for important intellectual content, and approving the final version to be published: S.T.O.; Contributing to the drafting the article or revising it critically for important intellectual content, and approving the final version to be published: S.P.R.M.; Contributing to the conception and design, drafting the article or revising it critically for important intellectual content, and approving the final version to be published: M.S.M.

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