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Functional capacity in adults with cerebral palsy: Lower limb muscle strength matters.

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Running Head: Functional capacity in cerebral palsy.

Title: Functional capacity in adults with cerebral palsy: Lower limb muscle strength matters.

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Study performed at the Centre for Sensorimotor Performance, School of Human Movement and Nutrition Sciences, The University of Queensland, St Lucia, Australia.

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Title: Functional capacity in adults with cerebral palsy: Lower limb muscle strength matters.

Abstract

Objective: To investigate the relationship between lower limb muscle strength, passive muscle properties and functional capacity outcomes in adults with cerebral palsy (CP).

Design: Cross-sectional study.

Setting: Tertiary institution biomechanics laboratory.

Participants: Sample of 33 adults with spastic-type CP with a mean age of 25 (range, 15-51) years; mean ± SD body mass 70.15 ± 21.35 kg; Gross Motor Function Classification System (GMFCS) level I n=20, level II n=13.

Interventions: Not applicable.

Main Outcome Measures: Six-minute walk test (6MWT) distance (m); lateral step-up (LSU) test performance (total repetitions); timed up-stairs (TUS) performance (s); maximum voluntary isometric strength of plantar flexors (PF) and dorsiflexors (DF) (Nm.kg⁻¹); and passive ankle joint and muscle stiffness.
Results: Maximum isometric PF strength independently explained 61% of variance in 6MWT performance; 57% of variance in LSU test performance; and 50% of variance in TUS test performance. GMFCS level was significantly and independently related to all three functional capacity outcomes, and age was retained as a significant independent predictor of LSU, and TUS test performance. Passive medial gastrocnemius muscle fascicle stiffness and ankle joint stiffness were not significantly related to functional capacity measures in any of the multiple regression models.

Conclusions: Low isometric PF strength was the most important independent variable related to distance walked on the 6MWT, fewer repetitions on the LSU test, and slower TUS test performance. These findings suggest lower isometric muscle strength contributes to the decline in functional capacity in adults with CP.

Keywords: Cerebral Palsy, muscle strength, walk test, muscle weakness, aging.

Ambulant young adults with cerebral palsy (CP) experience a decline in physical activity, aerobic capacity and walking ability into adulthood that is accelerated compared to typically developing (TD) adults, with a substantial reduction in mobility occurring before 40 years of age.\textsuperscript{1-5} There is a paucity of knowledge about the individual factors that contribute to the decline in function in adults with CP, however musculoskeletal adaptations such as muscle weakness\textsuperscript{6,7} and stiffness\textsuperscript{1,8} likely interact with the natural effects of ageing in some manner to limit functional capacity. There is a lack of understanding about how musculoskeletal development progresses through adulthood in individuals with CP and whether any decline in physical mobility is related to deterioration of muscle.

Recent evidence suggests that a reduction in muscle strength and alterations in muscle structure, rather than increased spasticity, are associated with impaired gait function and measures of gross mobility,\textsuperscript{1,9-11} with strength accounting for up to 69\% of the variance in gross motor function on the Gross Motor Function Measure (GMFM-66) score.\textsuperscript{12} The moderate relationship (adjusted $R^2 = 21–35\%$) between isometric lower leg strength and mobility capacity in children with CP that is not seen in TD individuals reinforces the importance of muscular strength to function in individuals with CP.\textsuperscript{13} Improvements in ankle muscle strength in young children with CP using resistance exercise programmes has been associated with improvements in the GMFM-66 walking, running, and jumping dimension ($r=0.84$).\textsuperscript{14} Similarly in adults with CP, improvements in ankle dorsiflexor (DF) function and range of movement during gait has been shown following explosive resistance training.\textsuperscript{15} Although the adult CP literature is scarce regarding the relationship between plantar flexor (PF) strength and function, taken together, these findings suggest that muscle strength may play a key role in effective mobility and functional capacity in adults with CP as they age.
The resistance to passive movement in the absence of muscle activation is defined as passive stiffness, which may be due to structural musculoskeletal alterations rather than reflex-mediated mechanisms.\textsuperscript{16-18} Adults with CP have significantly increased passive ankle and PF muscle stiffness compared to TD adults.\textsuperscript{1} Furthermore, this increased passive stiffness of the ankle muscles, along with muscle weakness, has been related to impaired gait function in the adults with CP.\textsuperscript{1} While these biomechanical parameters have been associated at a univariate level to impaired toe-lift, reduced gait-velocity, and active range of movement in adults with CP,\textsuperscript{1} whether they are related to different measures of functional capacity and their relative influence on different functional tasks remains unknown.

A number of functional capacity measures have been used to assess walking ability and performance of gross motor tasks in individuals with CP.\textsuperscript{13,19-21} The six-minute walk test (6MWT) has been used as a measure of walking capacity and endurance in individuals with CP,\textsuperscript{20-22} however, it is difficult to determine the impact of musculoskeletal adaptations on walking capacity assessed using the 6MWT without objectively quantifying strength and muscle properties. Other functional capacity assessments related to strength, coordination, power, and speed of movement are the lateral step-up (LSU) test and timed up-stairs (TUS) test.\textsuperscript{13,23} In children with CP, isometric strength of the leg muscles has explained 28\% and 35\% of the variance in LSU and TUS test performance respectively.\textsuperscript{13} How these various tasks are impacted by a further reduction in muscle strength and increased passive stiffness with ageing in individuals with CP is unknown.

The quantification of the relationships between muscle strength, passive stiffness and functional capacity outcomes using multiple regression analysis will increase our understanding of the muscular factors underpinning the functional decline in adults with CP.
The aim of this study was to investigate the relationships between lower limb muscle strength, passive stiffness, and functional capacity outcomes in adults with CP. It is likely that functional decline in adults with CP is impacted by the interaction of multiple factors, rather than one isolated characteristic (i.e. strength). We hypothesised that lower isometric muscle strength, increased passive muscle and ankle joint stiffness, and older age would be associated with reduced functional capacity outcomes.

**Methods**

Thirty-three community dwelling adults with spastic-type CP (18 M, 15 F; mean age 25 years, range 15-51 years; mean ± SD body mass 70.15 ± 21.35 kg) participated in the study. Participants were recruited across South East Queensland and Sydney, Australia from the Queensland Cerebral Palsy Register; Cerebral Palsy League Queensland; Brisbane Paralympic Football Program; Cerebral Palsy Alliance; and expression of interest advertising. An estimated effect size of 0.4, and an alpha level of 0.05 was used in an a-priori sample size calculation with a power of 0.80, determining that 36 subjects were required to include 4 factors into each model. The Gross Motor Function Classification System (GMFCS) levels of participants were established during the pre-screening process in an interview, with the following classification: Level I, n=18; Level II, n=15. Although developed for children with CP, the GMFCS has been used previously to classify the gross motor ability of adults with CP. Thirteen participants had received lower limb orthopaedic surgeries in the past, with 15 participants also having previously received intramuscular Botulinum toxin-A injections to the lower limbs. This study was provided ethical clearance from the Human Research Ethics
Committees at The University of Queensland (201400066; 2014000229) and The Cerebral Palsy Alliance (2015-09-02). Written and verbal information was provided to all participants prior to the study commencing, and signed written consent (or assent if under 18 years) was obtained. All data were collected between August 2014 and April 2017.

Functional capacity

The 6MWT was used to assess walking capacity and endurance measured as the maximum distance participants could walk during a 6-minute period on a 30m, flat, non-slippery track. The LSU test was used as a composite measure of functional strength, coordination, power, and speed of movement performed on the left and right legs. Participants were required to stand with one leg on a 21cm box placed laterally to them, and the other leg on the ground. One repetition was recorded if the participant raised their standing leg from the start position to within 5 degrees of full extension on the box, and participants were instructed to complete as many repetitions as possible in 30s. The combined total of left and right leg repetitions was used in the analysis. The TUS test was used to assess mobility capacity walking up-stairs as a surrogate measure of a power task that occurs regularly in daily life. Participants were assessed on the time taken to ascend a six-step set of stairs as quickly as possible (without running), using a handrail if required, with the fastest time recorded from three trials used for subsequent analysis. The LSU and TUS tests were recorded on a video camera and analysed offline using freely available PC-based video analysis software.

Functional capacity outcomes were chosen a priori, and statistical regression analyses were only conducted on
these three outcomes. No participants wore ankle-foot orthoses during the functional capacity assessments.

Dynamometry and passive movement assessments

Participants laid prone on an adjustable plinth with their foot secured to a footplate of an isokinetic dynamometer. Detailed information regarding participant setup and instrumentation used in this study have been reported elsewhere. Briefly, passive maximum ankle dorsiflexion range of motion was set in the dynamometer using a manually applied torque threshold greater than 20 Nm. Passive ankle joint torque and angle were measured during three slow passive trials at 10 deg.s⁻¹ while electromyography (EMG) and B-mode ultrasound data of medial gastrocnemius (MG) muscle fascicles were simultaneously collected (Supplementary file 1). In a small subset of participants (n=4) the ankle was rotated in a similar footplate and at a similar speed, however, this was undertaken using manual rotation whilst simultaneously measuring the torque generated using a torque transducer and footplate angle using an accelerometer.

Muscle activity levels were recorded from MG, lateral gastrocnemius (LG), soleus (SOL), and tibialis anterior (TA) using a differential surface EMG system. Ultrasound images of MG muscle fascicles were acquired at 80Hz using a PC-based ultrasound scanner and a linear transducer fixed to the skin using elastic wrap. Changes in muscle fascicle length and pennation angle were measured offline using semi-automated tracking algorithm.

Participants performed two unilateral maximum voluntary isometric contractions (MVIC) of
the ankle PF and DF at five angles corresponding to 5%, 25%, 50%, 75%, and 95% of the
range between maximum PF and maximum DF. The trial that produced maximum torque was
used for subsequent analysis. The passive torque measured at the same joint angle was
subtracted from the maximum torque recorded during each MVIC to calculate the active
torque produced by each participant. All torque measurements were corrected for the torque
generated by gravity acting on both the foot and the footplate, using the geometric fitting
method described in detailed by Barber et al.8

Passive properties

Medial gastrocnemius fascicle slack length was defined as the measured fascicle length (mm)
from the slow passive ankle rotation at an ankle joint PF torque of 1Nm. Fascicle stiffness
was calculated by fitting an exponential function to the mean muscle fascicle versus joint
torque curve for passive ankle rotations. The stiffness value (k) is the coefficient obtained
from the resultant exponential fit. Ankle slack angle (degrees) and ankle joint stiffness was
calculated using the same procedure, fitting an exponential equation to the mean ankle angle
versus joint torque curve (Supplementary file 2).

Statistical analysis
Descriptive statistics are presented as means (SD) for normally distributed continuous variables, median (range) for non-parametric continuous variables, and as frequency (%) for categorical variables. Differences in outcome measures between GMFCS levels were assessed using a Student’s T test, or the Mann-Whitney U test if the variable was non-parametric. The LSU and TUS test data was log transformed prior to regression analyses to account for their skewed distribution. Independent variables included age, sex, GMFCS, PF and DF MVIC, CP subtype (unilateral or bilateral), MG muscle fascicle and ankle joint passive stiffness, MG muscle fascicle slack length and ankle joint slack angle. The three functional capacity outcome measures were used as dependent variables. Multivariate regression of each functional capacity outcome was performed in a two-step process. Firstly, univariate associations between each of the functional capacity measures and independent variables were established. All independent variables with a univariate linear regression significance level of $P \leq 0.20$ were retained. Secondly, variables that were retained from the univariate analyses were entered into a series of multiple linear regression models on a stepwise basis in order of the strength of their association. Cook’s distance value was used to diagnose and remove outliers. All regression models were assessed for multicollinearity, heteroscedasticity and normality of residuals. There were no missing data.

**Results**

The personal demographics and characteristics categorised by GMFCS level are presented in Table 1. Descriptive statistics of the independent and dependent variables categorised by GMFCS level are presented in Table 2. There were significant differences between GMFCS
levels across all three functional capacity measures, with GMFCS level II walking less on the 6MWT, performing less repetitions on the LSU test, and taking longer to ascend six stairs compared to participants who were GMFCS level I. All participants were able to complete the assessment tasks and no participant reported musculoskeletal pain during assessments.

Six-minute walk test

At a univariate level, there were associations between normalised PF and DF MVIC, MG slack length, height, age, limb involvement, and 6MWT distance that were significant at p<0.20 (Table 3). The final multiple linear regression model retained normalised PF MVIC, and GMFCS level (Table 4), \(F(2, 28) = 50.53, p<0.001\), adjusted \(R^2 = 0.77\). No other independent variables were significant (p\(\leq\)0.05) in the multiple linear regression model.

Lateral step-up test

Univariate linear regression revealed that normalised PF and DF MVIC, MG fascicle stiffness, ankle joint slack angle and MG slack length, age, and limb involvement were associated (p<0.2) with LSU performance (log transformed) (Table 3). Normalised PF MVIC, GMFCS level, and age were retained in the final regression model (Table 4), \(F(3, 26) = 29.36, p<0.001\), adjusted \(R^2 = 0.76\). No other independent variables were significant (p\(\leq\)0.05) in the multiple linear regression model.
Timed up-stairs

Univariate analysis showed normalised PF and DF MVIC, ankle joint and MG fascicle stiffness, ankle joint slack angle and MG fascicle slack length, age and limb involvement to be significantly associated ($p \leq 0.2$) with TUS test performance (log transformed) (Table 3). Normalised PF MVIC, GMFCS level, and age were retained in the final model (Table 4), $F(3, 28) = 27.06$, $p < 0.001$, adjusted $R^2 = 0.72$. No other independent variables were significant ($p \leq 0.05$) in the multiple linear regression model.

Discussion

The multiple linear regression models developed in this study provide insight into potential underlying factors associated with walking ability and functional capacity in adults with CP. The main finding of this study was that maximum isometric PF strength explained 50 – 61% of the variance in 6MWT, LSU test and TUS test performance of adults with CP. Maximum isometric PF strength explained more of the variance in walking capacity (6MWT, 61%) and LSU test performance (57%) compared to the TUS test (50%) indicating the relative importance of the calf muscles to walking ability in adults with CP. Maximum isometric PF strength of adults with CP in this study (0.81 ± 0.51 Nm.kg$^{-1}$) was approximately 50% less than what has been found in TD young adults (1.50 ± 0.37 Nm.kg$^{-1}$), and approximately 35%
less than TD adults aged over 70 years (1.19 ± 0.36 Nm.kg\(^{-1}\)).\(^{32}\) The relative reduction in
muscle strength compared to TD adults is consistent with the literature describing muscle
weakness in individuals with CP compared to their TD counterparts.\(^{7,13,27,33}\) Although not
determined in this study, a loss of muscle volume,\(^{34}\) altered muscle quality,\(^{35}\) and changes in
activation properties\(^{36}\) may explain the relative muscle weakness compared to TD
individuals.

The overall mean distance walked for adults with CP in this study on the 6MWT was 465.10
± 155.40 m, which is in agreement with previously reported 6MWT distances in adults with
CP of similar functional classification.\(^{22,23}\) Typically developed older men and women aged
between 55 and 70 years have been shown to walk greater distances on the 6MWT,\(^{37}\) which
may indicate an accelerated decline in walking capacity in the relatively younger adults with
CP in this study (mean age 25 years) compared to older TD adults. Of our independent
variables, maximum isometric PF strength, and GMFCS level were retained in the final
regression model explaining a combined 77% of the variance in 6MWT distance. The
significant relationship between PF strength and 6MWT in this study when controlling for
GMFCS level, supports the evidence indicating the importance of the calf muscles in
generating the power needed for an effective push-off during gait.\(^{38}\) This finding explains
slightly more variance in 6MWT performance than has been shown in children and
adolescents with CP, where ankle PF and hip flexor strength explained approximately 50% of
the variance in 6MWT distance.\(^{11}\)

Our findings contradict a recent study assessing predictors of 6MWT performance in adults
with CP (mean age 39 ± 12 years), where no relationship was found between lower limb
muscle strength and 6MWT performance.\(^{22}\) The robustness of this finding is limited,
however, due to muscle strength being measured using a subjective manual muscle testing method without objectively quantifying joint torque. Our model also incorporated GMFCS level as an independent variable that was significantly associated with 6MWT distance in the final model. This finding may be considered intuitive as adults with CP who have higher gross motor function walk significantly greater distances on the 6MWT. Age has been previously reported as a significant independent predictor of 6MWT distance in TD older adults. Age, however, was not a significant independent predictor of 6MWT in this study. This finding is in agreement with the limited 6MWT literature in adults with CP, where no effect of age was found in a sample with a greater mean age (mean, SD 39 ± 12 years). The exclusion of age from our final 6MWT regression model may have been due to the relatively young to middle-age distribution of the participants, along with the presence of more important predictor variables.

Maximum isometric PF strength explained the most variance in LSU test performance (adjusted $R^2$=57%) and TUS test (adjusted $R^2$=50%). For the LSU and TUS tests, GMFCS and age were also significantly and independently associated with test outcomes. There was, however, less variance explained by PF strength for the TUS regression model than for the 6MWT and LSU test. Explanations for this include the potential use of the handrail for balance ascending stairs, as well as the likelihood for different neuromuscular demands and contribution of PF strength required in the TUS test. There was more variance explained by maximum PF strength in our sample of older adults for the LSU and TUS tests than has been shown in children with CP (28 – 35%). There is potentially greater deterioration in other physiological variables with ageing in individuals with CP, such as reduced rate of force development, reduced muscle activation capacity and increased co-contraction.
culminating in a greater influence of muscle strength on these functional capacity outcomes compared to younger individuals.

Clinically, joint stiffness (contracture) and muscle weakness are considered detrimental to functional capacity in individuals with CP. Even though MG muscle fascicle stiffness was higher in the GMFCS II group compared to GMFCS level I group, there was no significant relationship between passive muscle stiffness and any of the functional capacity outcomes in the multiple regression analyses. While passive stiffness has been shown to be moderately correlated to reduced toe-lift during gait (r=-0.48),\(^1\) compensatory movement strategies (altered kinematics) may be used by the individual to maintain walking and overall functional capacity.

The significant relationship between PF strength and functional capacity outcomes found in this study indicates the potential for interventions targeting muscle weakness that may lead to improved function. Muscle weakness in adults with CP highlighted in this study indicates the need for early intervention to prevent the rapid decline in muscle health (muscle volume, quality, and function) that may occur before 30 years of age in adults with CP. To date, however, there is a lack of evidence linking an increase in lower limb strength following strength training interventions to improved functional capacity in adults with CP.\(^23,40\) The paucity of studies that have implemented robust progressive resistance training programs in individuals with CP, as well as the lack of objective neuromuscular outcome measures following the interventions, makes it difficult to draw conclusions regarding the effectiveness of strength training in this population.\(^41\) Given the high multimorbidity rates in middle-aged adults with CP, even without improvements in functional capacity following muscle strengthening interventions, there may be benefits to overall health wellbeing by maintaining
muscle mass and strength across the lifespan.\textsuperscript{42} The design of robust exercise training interventions in skeletally mature individuals with CP,\textsuperscript{28} with objective neuromuscular and functional outcome measures, show the most potential to further our understanding of the link between neuromuscular adaptation and functional capacity in this population.

### Study Limitations

It is not possible to establish a cause and effect relationship using a regression approach. The generalisability of the findings in this study are limited to the independent variables that were measured and included in the regression analyses in adults with CP classified as GMFCS level I or II. It is plausible that other potential predictors of neuromuscular performance, such as activation capacity, play a role in the diminished functional capacity in adults with CP that were not captured in this study. The LSU and TUS tests also contain elements of strength endurance, balance, coordination and power that were not measured in isolation in this study. Due to the plasticity of the human musculoskeletal system, alternative strategies and mechanisms might contribute to accomplishing functional capacity outcomes, overcoming any detriment in isolated torque generating capacity and increased passive stiffness. Musculoskeletal pain may impact functional capacity outcomes in some individuals with CP, and quantification of pain levels may be considered for inclusion in future studies. Nine participants had previous lower limb muscle-tendon surgery that may have influenced isometric strength and functional capacity outcomes not specifically accounted for in the regression analyses. Due to the time since surgery (5-40 years); age of participants; and
heterogeneity of surgical procedures, it was not possible to determine the impact of the prior surgical interventions on the outcome measures in this study.

Conclusions

The results from this study further our understanding of how the deterioration of muscle strength is independently associated with functional decline in adulthood for people with CP. We therefore suggest that the relationship between muscle deterioration and functional decline with ageing in CP is primarily driven by a loss of muscle strength that can be measured objectively. Appropriately designed interventions to enhance muscle strength, such as progressive resistance training, might slow the decline in functional capacity and improve overall muscle health in individuals with CP as they age.

Acknowledgements

We gratefully acknowledge the support of Professor Robert Ware for statistical advice, and Miss Shari O’Brien and Mr Ben van Dorsselaer for technical assistance during data collection.
Conflict of Interest

The authors declare no conflict of interest, either real or perceived.

Funding sources

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References


Suppliers List

a. Kinovea open source video analysis software (v.0.8.15), France.

b. Biodex Isokinetic Dynamometer System 3. Biodex Medical Systems, Inc. 20 Ramsey Road, Shirley, NY, 11967-4704, USA.

c. Neurolog multichannel surface EMG system. 37 Hydeway, Welwyn Garden City, Hertfordshire, AL7 3BE, UK.

d. Telemed Echo Blaster 64 EXT-1T Ultrasound System and LV7.5/65/64D transducer. Telemed Medical Systems, Dariaus ir Gireno str. 42, Vilnius LT-02189, Lithuania.
Table 1. Participant demographics and characteristics categorised by GMFCS level.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall (n=33)</th>
<th>GMFCS I (n=18)</th>
<th>GMFCS II (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, (range)</td>
<td>25 (15 – 51)</td>
<td>24 (15 – 41)</td>
<td>27 (17 – 51)</td>
</tr>
<tr>
<td>Sex: M/F</td>
<td>18/15</td>
<td>10/8</td>
<td>8/7</td>
</tr>
<tr>
<td>Height, cm</td>
<td>166.67 (12.68)</td>
<td>170.85 (10.00)</td>
<td>164.15 (12.53)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>70.15 (21.35)</td>
<td>72.85 (19.12)</td>
<td>69.41 (26.10)</td>
</tr>
<tr>
<td>Lower limb involvement, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>20 (61)</td>
<td>13 (72)</td>
<td>7 (47)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>13 (39)</td>
<td>5 (27)</td>
<td>8 (53)</td>
</tr>
<tr>
<td>Previous lower limb surgery, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle-tendon lengthening</td>
<td>9 (27)</td>
<td>3 (17)</td>
<td>6 (40)</td>
</tr>
<tr>
<td>Osteotomy</td>
<td>3 (9)</td>
<td>0 (0)</td>
<td>3 (20)</td>
</tr>
<tr>
<td>Previous intramuscular botulinum toxin-A injection, n (%)</td>
<td>15 (45)</td>
<td>8 (44)</td>
<td>7 (47)</td>
</tr>
<tr>
<td>Use of mobility aid during functional capacity assessments, n (%)</td>
<td>5 (15)</td>
<td>0 (0)</td>
<td>5 (33)</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
<td>--------</td>
<td>-------</td>
<td>--------</td>
</tr>
</tbody>
</table>

Data are mean (SD) unless otherwise stated. *Significantly different to GMFCS Level I at p<0.05.
Table 2. Descriptive statistics of outcome measures categorised by GMFCS level.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Overall (n=33)</th>
<th>GMFCS I (n=18)</th>
<th>GMFCS II (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6MWT distance (m)</td>
<td>465.10 (155.40)</td>
<td>564.78 (112.98)</td>
<td>345.48 (107.65)†</td>
</tr>
<tr>
<td>LSU repetitions (n), median (range)</td>
<td>27.0 (4.0 – 74)</td>
<td>51.0 (22.0 – 74.0)</td>
<td>18.0 (4.0 – 38.0)†</td>
</tr>
<tr>
<td>TUS (s), median (range)</td>
<td>3.77 (2.30 – 16.78)</td>
<td>3.20 (2.30 – 4.93)</td>
<td>7.57 (3.0 – 16.78)†</td>
</tr>
<tr>
<td>MVIC (Nm.kg$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF</td>
<td>0.81 (0.51)</td>
<td>1.01 (0.52)</td>
<td>0.57 (0.40)*</td>
</tr>
<tr>
<td>DF</td>
<td>0.15 (0.09)</td>
<td>0.18 (0.08)</td>
<td>0.11 (0.09)*</td>
</tr>
<tr>
<td>Ankle joint stiffness (k)</td>
<td>0.10 (0.03)</td>
<td>0.09 (0.03)</td>
<td>0.11 (0.03)</td>
</tr>
<tr>
<td>MG fascicle stiffness (k)</td>
<td>0.75 (0.88)</td>
<td>0.55 (0.49)</td>
<td>1.00 (1.16)</td>
</tr>
<tr>
<td>Ankle joint slack angle (PF, deg)</td>
<td>17.44 (13.02)</td>
<td>19.94 (12.73)</td>
<td>15.15 (13.42)</td>
</tr>
<tr>
<td>MG fascicle slack length (mm)</td>
<td>44.15 (12.10)</td>
<td>47.28 (10.32)</td>
<td>40.38 (13.34)</td>
</tr>
</tbody>
</table>

Data are mean (SD) unless otherwise stated. *Significantly different to GMFCS Level I at p<0.05; †Significantly different to GMFCS Level I at p<0.001.
Table 3. Univariate analysis of relationships between independent and dependent variables. Significant correlations ($r$) at $p<0.05$ are indicated in bold.

<table>
<thead>
<tr>
<th></th>
<th>Six-minute walk test distance (m)</th>
<th>Lateral step-up test (total repetitions)</th>
<th>Timed up-stairs test (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>-0.41</td>
<td>-0.58</td>
<td>0.56</td>
</tr>
<tr>
<td>Height (m)</td>
<td>0.41</td>
<td>0.27</td>
<td>-0.23</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.01</td>
<td>-0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>PF MVIC (Nm.kg$^{-1}$)</td>
<td>0.65</td>
<td>0.71</td>
<td>-0.73</td>
</tr>
<tr>
<td>DF MVIC (Nm.kg$^{-1}$)</td>
<td>0.39</td>
<td>0.45</td>
<td>-0.53</td>
</tr>
<tr>
<td>Ankle stiffness (k)</td>
<td>-0.20</td>
<td>-0.27</td>
<td>0.37</td>
</tr>
<tr>
<td>MG fascicle stiffness (k)</td>
<td>-0.32</td>
<td>-0.39</td>
<td>0.40</td>
</tr>
<tr>
<td>Ankle slack angle (deg)</td>
<td>-0.30</td>
<td>-0.46</td>
<td>0.46</td>
</tr>
<tr>
<td>MG fascicle slack length (mm)</td>
<td>0.39</td>
<td>0.38</td>
<td>-0.49</td>
</tr>
</tbody>
</table>

For the timed up-stairs test, a negative correlation indicates a faster time.
Table 4. Results of multiple regression models. Adjusted $R^2$ values indicate the cumulative $R^2$ value for the regression model as each independent variable was entered in a stepwise process.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>n</th>
<th>Independent variables entered stepwise into final model</th>
<th>Regression coefficient ± SE</th>
<th>$p$ Value</th>
<th>Adjusted $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Six-minute walk test</td>
<td>32 (1 outlier removed)</td>
<td>PF MVIC (Nm.kg$^{-1}$)</td>
<td>164.34 ± 29.45</td>
<td>&lt;0.001</td>
<td>0.61</td>
</tr>
<tr>
<td>Lateral step-up test (Log transformed)</td>
<td>30 (3 outliers removed)</td>
<td>PF MVIC (Nm.kg$^{-1}$)</td>
<td>0.27 ± 0.08</td>
<td>0.002</td>
<td>0.57</td>
</tr>
<tr>
<td>Timed up-stairs test (Log transformed)</td>
<td>32 (1 outlier removed)</td>
<td>PF MVIC (Nm.kg$^{-1}$)</td>
<td>-0.17 ± 0.05</td>
<td>0.003</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Note: PF MVIC = Peak Force Maximum Isometric Contract; GMFCS level = Gross Motor Function Classification System level; Age is in years.
Supplementary Files: Figure Legends

Supplementary file Figure 1. Representative data of (A) ankle joint angle, (B) ankle joint torque, and (C) normalised EMG during a passive ankle rotation from PF to DF in the dynamometer. Black line, MG EMG; red line, LG EMG; blue line, SOL EMG; grey line, TA EMG.

Supplementary file Figure 2. Representative data of (A) ankle joint torque versus ankle joint angle, and (B) ankle joint torque versus MG muscle fascicle length during a passive ankle rotation from PF to DF in the dynamometer. Red line, trial 1; blue line, trial 2; black dashed line, mean of all trials; grey dashed line, exponential fit of the mean curve.