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Combining muscle morphology and neuromotor symptoms to explain abnormal gait at the ankle joint level in cerebral palsy

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ABSTRACT

Background: Individuals with spastic cerebral palsy (CP) have neuromotor symptoms contributing towards their gait patterns. However, the role of altered muscle morphology alongside these symptoms is yet to be fully investigated.
Research question: To what extent can medial gastrocnemius and tibialis anterior volume and echo-intensity, plantar/dorsiflexion strength and selective motor control, plantarflexion spasticity and passive ankle dorsiflexion explain abnormal ankle gait.
Method: In thirty children and adolescents with spastic CP (8.6 ± 3.4 y/mo) and ten typically developing peers (9.9 ± 2.4 y/mo), normalised muscle volume and echo-intensity were estimated. Both cohorts also underwent three-dimensional gait analysis, whilst for participants with spastic CP, plantar/dorsi-flexion strength and selective motor control, plantarflexion spasticity and maximum ankle dorsiflexion were also measured. The combined contribution of these parameters towards five clinically meaningful features of gait were evaluated, using backwards multiple regression analyses.
Results: With respect to the typically developing cohort, the participants with spastic CP had deficits in normalised medial gastrocnemius and tibialis anterior volume of 40% and 33%, and increased echo-intensity values of 19% and 16%, respectively. The backwards multiple regression analyses revealed that the combination of reduced ankle dorsiflexion, muscle volume, plantarflexion strength and dorsiflexion selective motor control could account for 12–62% of the variance in the chosen features of gait.
Significance: The combination of altered muscle morphology and neuromotor symptoms partly explained abnormal gait at the ankle in children with spastic CP. Both should be considered as important measures for informed treatment decision-making, but further work is required to better unravel the complex pathophysiology.

1. Introduction

Children and adolescents with spastic cerebral palsy (CP) present with various neuromotor symptoms, due to the initial brain lesion and resulting lack of spinal cord development [1]. This ranges from reduced strength and selective motor control, to the presence of hyperactive stretch reflexes (spasticity) and reduced joint range of motion [2]. Due to advances and availability of medical imaging techniques, altered muscle morphology, such as reduced volumes and alterations to the internal muscle properties have been identified [3–7]. It has been suggested that neuromotor symptoms and altered muscle morphology all contribute towards function, such as gait, leading to abnormal joint

Abbreviations: CP, cerebral palsy; 3DfUS, three-dimensional freehand ultrasound; GMFCS, gross motor function classification system; 3DGA, three-dimensional gait analysis; GVS, gait variable score

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loading, possible damage to the muscle tissue, bony deformations and increased energy expenditure [2]. However, as the aforementioned impairments may all be epi-phenomena of the initial brain injury, associations may be found as a consequence of the overall magnitude of functional impairments. This can pose a challenge when attempting to determine the symptoms most clinically relevant for treatment planning. A more robust approach is to use a multiple regression analysis, where the combined contribution of neuromotor symptoms and altered muscle morphology towards abnormal gait can be assessed, taking any collinearity between impairments into consideration [8]. This would negate the issue of associations due to the magnitude of functional impairments, and may reveal which impairments should be targeted for treatment, hopefully leading to more optimised gait.

Several investigations previously explored the association between neuromotor symptoms with abnormal gait, the latter assessed using three-dimensional gait analysis (3DGA). A thorough investigation including various measures of neuromotor symptoms found that strength and selective motor control had significant associations with abnormal gait, but not reduced joint range of motion or spasticity [8]. Other investigations assessed the combined contribution of neuromotor symptoms, where strength was found to account for 69% of the gross motor function variability, and eight percent for spasticity [9]. Selective motor control measured on a more robust scale was also found to account for 60% of the overall kinematic gait deviation measured by the gait profile score [10]. For altered muscle morphology, only linear correlations with abnormal gait in spastic CP have been explored. The volume of the plantarflexor muscles, estimated using magnetic resonance imaging, correlated with plantarflexor work during terminal stance [11], whilst another investigation found tibialis anterior anatomical cross-sectional area, estimated using two-dimensional ultrasound, to correlate with maximum ankle dorsiflexion during the swing phase of gait [12]. However, as magnetic resonance imaging is expensive and imposing for young children, it has reduced clinical feasibility. Two-dimensional ultrasound is a more cost-effective and user-friendly alternative, but has operator errors not seen in magnetic resonance imaging, usually attributed to the location of where the image is taken on the leg [13]. These factors may contribute towards the limited exploration between altered muscle morphology and abnormal gait, despite being a primary impairment in spastic CP [14,15].

An innovative medical imaging technique was recently established to overcome some of the aforementioned limitations of magnetic resonance imaging and two-dimensional ultrasonography, but still feasible for use in the clinical setting. Reconstructions of muscles can be created in minimal time using three-dimensional freehand ultrasonography (3DfUS), a static-condition technique combining B-mode ultrasonography with motion-tracking data [16]. From the reconstructions, muscle volume can be reliably estimated, together with an indirect estimation of alterations to the internal muscle properties, according to the echo-intensity of the ultrasound images [6,7]. When validated with magnetic resonance imaging, a high correlation was found between echo-intensity and intramuscular non-contractile tissue for both medial gastrocnemius and tibialis anterior muscles [17]. In an elderly population, echo-intensity was also shown to correlate with reduced isometric force- and rate of torque-generation [18], two impairments also present in individuals with spastic CP [19,20]. However, the relation between muscle volume and echo-intensity is yet to be thoroughly investigated in children with spastic CP, along with the relation between echo-intensity and abnormal gait.

Therefore, the first aim of this investigation was to compare medial gastrocnemius and tibialis anterior volume and echo-intensity between children with spastic CP, and typically developing peers. Based on previous findings, it was hypothesised that the children with spastic CP have smaller volumes (when normalised to body mass) and higher echo-intensity values. Linear correlations between normalised muscle volume and echo-intensity, in both muscles, was also explored. It was hypothesised that reduced normalised volume associates with increased echo-intensity, for both muscles, but only in the cohort of children with spastic CP. The second aim was to explore the combined contribution of neuromotor symptoms and altered muscle morphology towards abnormal gait at the ankle. To achieve this aim, linear regressions between medial gastrocnemius and tibialis anterior normalised volume, echo-intensity, plantar- and dorsiflexion strength and selective motor control, plantarflexion spasticity and passive ankle dorsiflexion, with four clinically relevant discrete features of gait at the ankle, were explored. The measures that had significant linear regressions were then used as independent variables in backwards multiple regression analyses to assess the combined contribution towards the corresponding gait feature. An additional gait variable that expresses the amount of ankle deviation over an entire gait cycle was also used, and explored by all the outcomes measures that had significant linear regressions. It was hypothesised that a combination of neuromotor symptoms and altered muscle morphology significantly explain the chosen features of abormal gait at the ankle.

2. Method

2.1. Participants

Children and adolescents diagnosed with spastic CP and typically developing peers were recruited through the University Hospital of Pellenberg between January 2015 to August 2017. The initial diagnosis of spastic CP was confirmed by a neuro-paediatrician, based on imaging of the brain and a clinical examination. All participants were aged between 5–17 years. The following inclusion criteria were also applied to the group of children with spastic CP a) GMFCS levels I—II, b) no previous orthopaedic or neurological surgery, and c) no botulinum neurotoxin-A injections six months prior to the measurement. This resulted in a total of 30 participants with spastic CP (mean age 8 years 6 months (3:4); mean body mass 31.9 kg (16.5); 21 males, 9 females), and 10 typically developing participants (mean age 9 years 9 months (2:4); mean body mass 34.1 kg (9.4), 8 males, 2 females). The experimental protocol was approved by the local Ethical-Committee (s56041), and written informed consent was acquired from all parents and guardians, and children over the age of 12.

2.2. Instrumentation

A 3DfUS method was used for this investigation [16]. This consisted of a Telemed-Echoblaster 128 Ext-1Z B-mode ultrasound system with a 5.9 cm 10 MHz linear US transducer (HL9.0/60/128Z) Telemed Medical Systems, Milan, Italy) and an OptiTrack V120:Trio motion-tracking system with three fixed optical cameras (NaturalPoint, Corvallis, OR, USA). The ultrasound acquisition acquisition settings remained unaltered throughout, with a sample rate of 30 Hz. A validated conversion equation was applied to the echo-intensity values [21], making them comparable to an earlier investigation from our research group [22]. The motion-tracking system had a 1.0 mm spatial resolution, and a sample rate of 120 Hz. To synchronize the US and motion-tracking data, a signal trigger was used [16].

The 3DGA technique consists of a 10–15 infrared motion-tracking camera system with a spatial resolution of 0.1 mm and an acquisition rate of 100 Hz (Version 1.7.1 Vicon, Oxford Metrics Group, Oxford, UK), and two AMTI force plates (Advanced Mechanical Technology, Inc., Watertown, Massachusetts) integrated in the walkway.

2.3. Acquisition

Age and body mass were recorded for all participants. The most affected leg for each participant with spastic CP was assessed, whilst a coin-flip determined the measured leg in the typically developing cohort. Each participant with spastic CP also underwent a full clinical examination. Plantar- and dorsiflexion strength was graded using manual muscle testing [23], whilst selective motor control was graded on a five-point scale [2]. The Modified Ashworth and Modified Tardieu Scales were used to grade plantarflexor spasticity [24,25], and maximal ankle dorsiflexion with the knee extended was measured with a goniometer.

The medial gastrocnemius was acquired using 3DfUS in a prone lying position, with a triangular cushion placed under the lower leg to provide approximately 25° of knee flexion. The sagittal plane knee and ankle angles were then measured with a goniometer. The same setup was applied for the tibialis anterior, but in a supine position. Movement of the leg was prevented by a cushioned strap over the thigh. Two physiotherapists experienced with 3DfUS and visual identification of the muscles acquired all the data, using sufficient gel to avoid excess pressure on the leg. Care was taken to hold the ultrasound probe perpendicular to the deep aponeuroses of the muscles, enhancing the visibility of the muscle border and minimising erroneous echoes [26]. In cases of visually detected muscle contraction or movement of the leg, the acquisition was repeated.

Each participant also walked at a self-selected velocity along a 10-meter walkway. Kinematics and kinetics were collected using the VICON software Nexus with the lower limb PlugInGait marker set model (Version 1.7.1 Vicon, Oxford Metrics Group, Oxford, UK). A minimum of three valid barefoot walking trials were collected [11], determined by good marker visibility and entire foot contact on the force plates.

2.4. Processing

The 3DfUS data was reconstructed into a three-dimensional voxel array using a custom-made software package [16], and processed using HOROS (www.horosproject.org). Muscle volume and echo-intensity were estimated by drawing equally spaced transverse plane segmentations along the inside of the muscle border, summing 10% of all the available ultrasound images. An interpolation was applied to create segmentations in the remaining 90% of images [7]. This reduced the time required to extract muscle volume and echo-intensity, whilst also maintaining sufficient reliability (standard error of measurement values for estimating medial gastrocnemius volume and echo-intensity are 2.6 ml and 3.3 values, respectively [7]). Muscle volume was reported in millilitres (ml), and normalised to body mass (ml/kg) to enable interparticipant comparisons. Echo-intensity was reported on an 8-bit greyscale (256 values), where black represented a value of 0 and white represented a value of 255, with in-between values 254 shades of grey. Using ultrasound, non-contractile properties such as tendons and adipose tissue appear lighter (hyper-echoic), whilst fluid and bones appear darker (hypo-echoic).

Gait cycle events of initial contact and toe off were visually determined using the software Nexus 1.7.1. Three-dimensional kinematic data were calculated for the pelvis, hip, knee, ankle and foot. All joint and segment orientation angles and power were decomposed into the sagittal, coronal and transverse planes using the lower limb PlugInGait model. From the continuous waveforms of the sagittal plane kinematics and kinetics, organized in gait cycles, four discrete features based on different aspects of the gait cycle, and one measure representative of the overall ankle deviation during the entire gait cycle, were selected based on clinical relevance [27]. All features were automatically extracted from the kinematic and kinetic continuous waveforms using custom-made MATLAB software (Version 2016a, Mathworks, Natick, MA, USA) and each outcome parameter was calculated as the mean from a minimum of three gait cycles [11]. The gait features included: 1) ankle angle at initial contact; 2) ankle angle at fifty percent of the stance phase (midstance); 3) maximum dorsiflexion angle during swing; 4) peak ankle power generation during terminal stance, and 5) sagittal plane ankle kinematic deviation, according to the gait variable score (GVS) [28]. Joint angles were expressed in degrees and peak ankle power was reported in watts, and normalised to body mass (W/kg) to enable interparticipant comparisons

2.5. Statistical analysis

Data were analysed in SPSS (Version 22, SPSS, Inc., Chicago, Illinois). Normal distribution was assessed by visually inspecting the symmetry of each histogram. Equality of variance was assessed using Levene's test, and treated accordingly. An independent samples student's *t*-test and Cohen's d effect size compared differences between the spastic CP and typically developing cohorts. A Pearson's correlation coefficient was used to explore the strength of the correlation between normalised muscles volumes with echo-intensity. Linear regression analyses were used to explore the strength of the associations between neuromotor symptoms and altered muscle morphology with the chosen features of gait [29]. Normalised tibialis anterior volume and echo-intensity, dorsiflexion manual muscle testing and selective motor control, and maximum dorsiflexion were associated with ankle angle at initial contact and maximum dorsiflexion angle during swing. Normalised medial gastrocnemius volume and echo-intensity, plantarflexion manual muscle testing, selective motor control, modified Ashworth and Tardieu scales, and maximum dorsiflexion were associated with the ankle angle at midstance and peak ankle power generation. Subsequently, backwards multiple regression analyses were performed for the four discrete features of gait, where only the corresponding outcome measures that had significant linear regressions were used as independent variables (parsimonious statistical approach). An additional backwards multiple regression analysis was performed to evaluate the explained variance of the GVS of the ankle. This was based on the included independent variables in the four multiple regression analyses for the four discrete features of gait. For each backwards multiple regression analysis, the statistical significance ($\leq$.100) and adjusted R-squared was reported, along with the included independent variables, their part correlation and collinearity statistics (tolerance and variance inflation factor).

3. Results

An overview of the clinical information related to the participants with spastic CP can be found in Table 1. No significant differences in age (p = .266) or body mass (p = .684) were found between the cohorts, but muscle volumes (absolute and normalised), echo-intensity, and the four discrete features of gait at the ankle were significantly different (Table 2). The participants with spastic CP presented with deficits of normalised medial gastrocnemius and tibialis anterior volume of 40% and 33%, and echo-intensity increases of 19% and 16%, respectively. Normalised muscle volume and echo-intensity were significantly correlated in the participants with spastic CP (medial gastrocnemius: r = -0.64, p ≤ .001; tibialis anterior: r = -.53, p = .003), but not in the typically developing cohort (medial gastrocnemius: r = -.17,

Table 1
Overview of the clinical information related to the spastic CP participants.

Characteristics		Spastic Cerebral Palsy (n = 30)
Topographical Paresis		19 unilateral / 11 bilateral
Gross motor function classification system level		20 I / 10 II
Previous botulinum neurotoxin-A interventions		Yes: 20 / No: 10
Number of botulinum neurotoxin-A interventions		3 (IQR 2-5)
Manual muscle testing	Plantarflexion	4 (IQR 3.6-4)
	Dorsiflexion	3.6 (IQR 3.3-4)
Selective motor control	Plantarflexion	2 (IQR 1.5-2)
	Dorsiflexion	1.5 (IQR 1-1.5)
Spasticity (plantarflexors)	Modified Ashworth scale	2 (IQR 1.5-2)
	Modified Tardieu scale	18 (IQR 14-25)
Maximum dorsiflexion (degrees)		5 (IQR 0-10)

Table 2
Comparison of mean values between cohorts for the chosen outcome parameters.

Outcome Parameters		Typically developing (n = 10)	spastic cerebral palsy (n = 30)	Independent samples t-test (p < .050)	Cohen's d Effect Size
MG	volume (ml)	68.1 (20.6)	41.0 (29.2)	.010	1.072
	volume (ml/kg)	2.0 (0.3)	1.2 (0.4)	≤.001	2.263
	echo-intensity (0-255)	101.9 (7.0)	121.6 (9.3)	≤.001	−2.393
TA	volume (ml)	41.7 (12.8)	25.2 (14.7)	.003	1.197
	volume (ml/kg)	1.2 (0.1)	0.8 (0.1)	≤.001	3.999
	echo-intensity (0-255)	110.5 (5.5)	128.2 (7.8)	≤.001	−2.622
Angle at initial contact (°)		1.6 (2.3)	−5.4 (8.7)	≤.001	1.100
Angle at midstance (°)		11.5 (1.7)	5.3 (10.6)	.004	0.817
Peak ankle power (W)		131.8 (36.7)	72.8 (49.5)	.001	1.354
Peak ankle power (W/kg)		3.9 (0.7)	2.2 (0.6)	≤.001	2.608
Maximum angle in swing (°)		4.7 (2.6)	−2.4 (10.2)	.001	0.954

MG, medial gastrocnemius; TA, tibialis anterior.

p = .639; tibialis anterior: r = -.10, p = .795) (Fig.1).

The linear regression exploration (Table 3) revealed that peak ankle power was significantly associated with normalised medial gastrocnemius volume (r = 0.46), medial gastrocnemius echo-intensity (r = –.42) and manual muscle testing (r = .39). The ankle angle at midstance significantly associated with normalised medial gastrocnemius volume (r = .46), medial gastrocnemius echo-intensity (r = -.59), selective motor control (r = .39) and maximum dorsiflexion (r = .52). The ankle angle at initial contact significantly associated with normalised tibialis anterior volume (r = .70), tibialis anterior echo-intensity (r = -.40), manual muscle testing (r = .42), selective motor control (r = .52) and maximum dorsiflexion (r = .62). Maximum dorsiflexion during swing significantly associated with normalised tibialis anterior volume (r = .68), tibialis anterior echo-intensity (r = -.46), selective motor control (r = .48) and maximum dorsiflexion (r = .59).

All backwards multiple regression analyses (Table 4) were significant, and no evidence of collinearity was identified between the independent variables. The first analysis indicated that 62% (p ≤ .001) of the variance in ankle angle at initial contact could be explained by normalised tibialis anterior volume (part r = .31), selective motor control (part r = .20) and maximum dorsiflexion (part r = .37). The second analysis revealed that 48% (p ≤ .001) of the variance in ankle angle at midstance could be explained by normalised medial gastrocnemius volume (part r = .47) and maximum dorsiflexion ((part r = .61). The third analysis revealed that 12% (p = .046) of the variance in peak ankle power during push-off could be explained by plantarflexion manual muscle testing (part r = .39). The fourth analysis revealed that 53% (p ≤ .001) of the variance in maximum ankle angle during swing could be explained by maximum dorsiflexion (part r = .48) and normalised tibialis anterior volume (part r = .32). The fifth analysis revealed that 36% (p = .002) of the variance in the ankle GVS could be explained by normalised medial gastrocnemius volume (part r = .55) and maximum dorsiflexion (part r = .42).

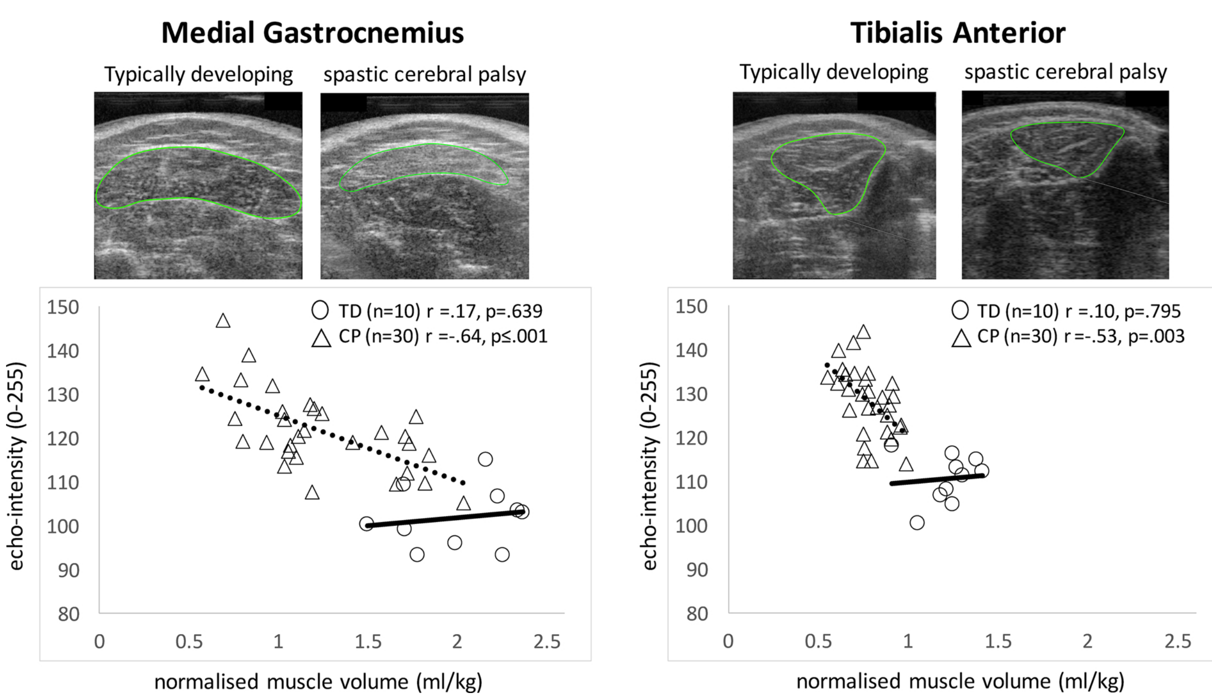

Fig. 1. Upper image: Transverse plane muscle border segmentations at fifty percent of the muscle length in a typically developing and spastic cerebral palsy gross motor function classification system level II child (matched according to body mass). Lower image: Correlations between echo-intensity and normalised muscle volume for the medial gastrocnemius and tibialis anterior. Separate regression lines were added for both spastic cerebral palsy and typically developing cohorts. TD, typical developing; CP, cerebral palsy.

Table 3
Linear regressions between clinical neuro-motor symptoms and lower limb muscle morphology with features of gait at the ankle, in the participants with spastic cerebral palsy (n = 30).

clinical neuro-motor symptoms and lower limb muscle morphology		Peak ankle power		Ankle angle at midstance	
		r	p < .050	r	p < .050
normalised medial gastrocnemius volume		.46	.011	.46	.011
medial gastrocnemius echo-intensity		−.42	.020	−.59	.001
Plantarflexion	manual muscle testing	.39	.046	.34	.080
	selective motor control	.22	.262	.39	.047
	modified Ashworth scale	−.20	.296	−.34	.063
	modified Tardieu scale	.09	.661	.14	.485
Maximum dorsiflexion		.20	.287	.52	.003
clinical neuro-motor symptoms and lower limb muscle morphology		**Ankle angle at initial contact**		**Maximum ankle angle during swing**	
		r	p < .050	r	p < .050
normalised tibialis anterior volume		.70	≤.001	.68	≤.001
tibialis anterior echo-intensity		−.40	.029	−.46	.011
Dorsiflexion	manual muscle testing	.42	.025	.35	.061
	selective motor control	.52	.004	.48	.008
Maximum dorsiflexion		.62	≤.001	.59	.001

4. Discussion

The first aim of this investigation was to compare medial gastrocnemius and tibialis anterior volume and echo-intensity between children and adolescents with spastic CP and typically developing peers. The cohort of children and adolescents with spastic CP had significantly smaller and hyperechoic muscles with respect to the typically developing cohort, confirming the first hypothesis. The alterations to muscle morphology were comparable to previous findings [5,6,30–32], in the sense that their muscles were smaller, with altered internal properties. In the cohort of children and adolescents with spastic CP, significant correlations were also found between normalised volume and echo-intensity, for both muscles, thus confirming the second hypothesis. The underlying mechanism behind these correlations are yet to be fully understood. Muscles of individuals with spastic CP do not grow at the same rate as typically developing muscles [32], possibly due to impaired satellite cell function in adding new sarcomeres [33]. A sequential stress on the muscle belly due to an increase in bone length may alter the passive properties of the muscle, likely the connective tissue [34–37]. This may be true for the medial gastrocnemius muscle, but is unlikely to explain the altered internal properties of the tibialis anterior. Another possible explanation is concerning the neurological aspect of spastic CP, where reduced voluntary activation and regular stimulation of the muscle leads to a lack of myofibrillar growth [15], and a proliferation of adipose tissue due to atrophy [3,4,31]. This would also explain why alterations to both muscle volume and internal properties increase with respect to GMFCS levels [4,5]. Future investigations in younger children may reveal the process that leads to altered internal properties. Yet, in the meantime, early intervention promoting active muscle use is likely to be beneficial to maximise muscle function in individuals with spastic CP.

The second aim of this investigation was to explore the combined contribution of neuromotor symptoms and altered muscle morphology towards abnormal gait at the ankle. The linear regression explorations confirmed that muscle volume and echo-intensity significantly associated with the chosen features of gait. There was a trend for medial gastrocnemius echo-intensity, rather than volume, to have stronger associations with the corresponding features of gait, whilst for the tibialis anterior, volume had stronger associations than echo-intensity. It is not yet clear if this has a clinical relevance with respect to differing muscle function. For the backwards multiple regression analyses, only plantarflexion manual muscle testing could explain 12% of the variance in peak ankle power during push-off. This increased for ankle angle at midstance, where a combination of normalised medial gastrocnemius volume and maximum dorsiflexion could explain 48% of the variance. A smaller muscle volume could be inferred as an inability of the muscle to generate sufficient force for a controlled progression of the tibia over the foot [11,38,39]. This would be in line with earlier findings in children with spastic CP, where eccentric medial gastrocnemius lengthening occurred during midstance [38,39]. In combination with this, forward progression of the tibia over the foot is also dependent on sufficient range of motion, represented here by the maximum dorsiflexion. This was also confirmed by the significant reduction in midstance dorsiflexion angle in the cohort of spastic CP individuals with respect to the typically developing cohort. The backwards multiple regression analysis with ankle angle at initial contact as the dependant variable, revealed that a combination of normalised tibialis anterior volume, dorsiflexion selective motor control and maximum dorsiflexion could explain 62% of the variance. The inclusion of these impairments fit the clinical picture, as sufficient strength, combined with good voluntary muscle activation are required to dorsiflex the ankle. However, both of these impairments can be influenced by insufficient ankle dorsiflexion, which would result in more plantarflexion at initial contact. For the backwards multiple regression analysis of maximum ankle angle during swing, a combination of maximum ankle dorsiflexion and

Table 4
Results of the backwards multiple regression models for each feature of gait at the ankle, in the participants with spastic cerebral palsy (n = 30).

Dependant Variables	Retained Independent Variables	Model summary Adjusted R^2	p value (≤.100)	Part Correlations	Collinearity statistics	
					Tolerance	VIF
Ankle angle at initial contact	Normalised tibialis anterior volume	.62	≤.001	.31	.593	1.687
	Dorsiflexion selective motor control			.20	.716	1.396
	Maximum dorsiflexion with knee extension			.37	.800	1.251
Ankle angle at midstance	Normalised medial gastrocnemius volume	.48	≤.001	.47	.973	1.027
	Maximum dorsiflexion with knee extension			.61	.973	1.027
Peak ankle power during push-off	Plantarflexion manual muscle testing	.12	.046	.39	–	–
Max ankle angle during swing	Maximum dorsiflexion with knee extension	.53	≤.001	.48	.807	1.239
	Normalised tibialis anterior volume			.32	.807	1.239
Ankle gait variable score	Normalised medial gastrocnemius volume	.36	.002	.55	.973	1.027
	Maximum dorsiflexion with knee extension			.42	.973	1.027

VIF, variance inflation factor.

normalised tibialis anterior volume could explain 53% of the variance. Lastly, the GVS at the ankle was included to provide a measure of overall kinematic impairment over the gait cycle. All of the significant independent variables from the linear regression analyses were included in a backwards multiple regression model, with the ankle GVS as the dependant variable. It was found that a combination of normalised medial gastrocnemius volume and maximum ankle dorsiflexion could explain 36% of the variance. The overall findings of the backwards multiple regression analyses suggest that insufficient ankle dorsiflexion, reduced muscle volume, followed by plantarflexion strength and dorsiflexion selective motor control, are the biggest contributors towards pathological gait at the ankle. Therefore, the third hypothesis was accepted. The effects of targeting these impairments are not well known. Hence, future investigations should evaluate to what extent interventions to reduce these impairments lead to a normalisation of gait at the ankle.

4.1. Limitations

The reliability for estimating tibialis anterior volume and echo-intensity using 3DfUS are yet to be reported, but based on the mean difference between the two investigated cohorts, and previous errors in literature for the medial gastrocnemius [7], it is likely that the errors will be smaller. The cohort of children and adolescents with spastic CP were relatively high functioning (GMFCS level I-II) and heterogeneous with respect to topographical impairment and previous botulinum neurotoxin-A interventions, which may be considered a limitation. However, the findings were in line with previous investigations [11,12], increasing the generalisability of the conclusions. Future investigations should also evaluate other muscles and joints, assess neuromotor symptoms with more objective and sensitive measurement tools, and explore a non-parsimonious statistical approach. This may lead to a higher explained variance of the impairments influencing gait in the pathology of spastic CP.

In conclusion, earlier findings regarding altered medial gastrocnemius and tibialis anterior muscle morphology in children and adolescents with spastic cerebral palsy were confirmed. Furthermore, several novel findings were identified. The tibialis anterior muscle in the cohort of children and adolescents with spastic cerebral palsy appeared hyper-echoic with respect to the typically developing cohort, despite not being considered as clinically involved as the medial gastrocnemius. Reduced normalised muscle volume was related to increased echo-intensity in both muscles, but the underlying mechanism that leads to the correlation is yet to be fully understood. With respect to abormal gait at the ankle, insufficient ankle dorsiflexion and reduced muscle volume, followed by plantarflexion strength and dorsiflexion selective motor control, had the highest combined explanation of variance ranging from 12%-62%. However, further investigation is still required to better unravel the underlying mechanism leading to abnormal gait in children and adolescents with spastic cerebral palsy.

Conflict of interest

The funders of this investigation had no involvement in the study design, data collection, data analysis, manuscript preparation or publication designs. The authors have stated that they had no interests that might be perceived as posing a conflict or bias.

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