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Multimodal characterisation of skeletal muscle properties in children with and without cerebral palsy using a non-invasive approach: an observational study protocol

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ABSTRACT

Introduction Children with spastic cerebral palsy (CP) often exhibit significant changes in their skeletal muscles due to altered neural control and atypical mechanical loading. While muscle morphology, in particular muscle size, has been extensively studied, other important properties, such as passive stiffness and muscle composition, remain poorly characterised. Previous studies have typically examined these features in isolation, leaving the interactions among morphology, composition and passive stiffness largely unexplored. This protocol addresses these gaps by providing a comprehensive, multimodal assessment of gastrocnemius muscle properties within a single cohort, which will be systematically compared with control data from typically developing (TD) children.

Methods and analysis This protocol outlines a study to characterise gastrocnemius muscle properties in children with spastic CP and TD peers across different age groups. Muscle size will be measured using three-dimensional freehand ultrasound, composition using quantitative MRI and passive muscle tissue stiffness using shear wave elastography. Additionally, isometric and functional plantar flexion strength will be evaluated through handheld dynamometry and functional strength assessments, respectively. Differences in muscle characteristics between CP and TD children will be assessed, and the relationships among muscle properties will be examined. Exploratory analyses will also evaluate potential associations with isometric and functional plantar flexor strength. This integrated approach will provide a detailed multimodal characterisation of age-related alterations in the gastrocnemius muscle in children with CP.

Trial registration number NCT06991725.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study uniquely examines the interplay between muscle morphology, composition and passive stiffness, addressing an important gap in current literature.
⇒ The inclusion of a typically developing control group enhances interpretability and clinical relevance.
⇒ The combination of quantitative imaging and functional outcomes strengthens the potential to bridge the gap between basic muscle properties and clinical performance.
⇒ The cross-sectional design limits the ability to draw conclusions about causal relationships or longitudinal changes over time.

INTRODUCTION

Skeletal muscle is a highly adaptable tissue, capable of structural and compositional changes in response to altered neural input and mechanical loading. In cerebral palsy (CP), a non-progressive brain lesion occurring during gestation or early childhood,[1] these adaptations are particularly pronounced. CP affects approximately 1.6–3.4 per 1000 newborns and is the most common cause of childhood disability.[2] Spastic CP, the most common subtype,[3] is characterised by impaired corticospinal connectivity, resulting in neuromotor deficits such as spasticity, muscle weakness and reduced selective motor control. These neuromotor symptoms commonly affect the lower limb muscles, with the gastrocnemius being one of the most susceptible,[4 5] leading to functional limitations.[6 7]

A significant portion of the functional problems associated with spastic CP may be attributed to the secondary intrinsic muscular adaptations.[8 9] Muscle size is the most investigated, supported by the emergence of valid and reliable muscle imaging techniques, such as three-dimensional freehand ultrasound (3DfUS).[10 11] Previous studies have identified significant evidence of muscle size deficits in children with CP,[12–15] which can already be present as early as 6 months of age.[16] Muscle size, next to selective motor

control, has been associated with the frequently observed symptom of muscle weakness in children with CP.[6 17–19] However, muscle size accounts for only a small proportion of muscle strength deficits,[19] as it does not directly reflect the amount of contractile tissue. Since muscles also include non-contractile components, such as fat and collagen, muscle composition may play a crucial role in understanding muscle weakness and related functional impairments.[20]

A frequently used method for identifying fat and collagen content within the muscle is muscle biopsies, a technique that is rather invasive and not optimally suited for (young) children. Moreover, the resulting quantification only provides information on a small portion of the muscle, which is characterised by a large heterogeneity in CP. In recent years, advancements in quantitative MRI (qMRI) have created new possibilities for assessing the non-contractile aspects of muscle tissue in a non-invasive way across the entire muscle.[21] Previous studies using the Dixon method, a qMRI technique that can quantify intramuscular fat content, have shown higher levels of fat infiltration in the plantar flexors of individuals with CP compared with typically developing (TD) peers.[22–25] However, only a limited number of studies have been conducted in children, with just two identified to date.[24 25] Of these, only one study examined the regional distribution of intramuscular fat along the length of the muscle.[24] Nonetheless, the small study populations and the wide age ranges, together with the inability to compare Gross Motor Function Classification System (GMFCS) levels, limit interpretation of the potential effects of age and GMFCS level on intramuscular fat infiltration in children with CP. This highlights a clear gap in our understanding of intramuscular fat infiltration in this population.

To complement fat quantification, additional qMRI techniques may provide further insight into muscle tissue composition. For example, T2 mapping has been widely applied to evaluate active muscle damage in various neuromuscular disorders.[26] However, to date, the application of T2 mapping in children with CP has been applied exclusively in the context of follow-up after botulinum toxin treatment,[27] and no studies have yet compared children with CP to TD peers in the absence of therapeutic interventions, leaving its potential to provide complementary information on muscle tissue alterations unknown. Moreover, no studies using qMRI for quantifying collagen tissue have been conducted in the CP population, although previous work has reported strong associations between T1ρ relaxation times and collagen-related measures in the vastus lateralis muscle when comparing the healthy and anterior cruciate ligament (ACL)-deficient limb, with significantly longer T1ρ relaxation times observed in the ACL-deficient limb. These findings suggest that T1ρ may serve as a non-invasive imaging marker of collagen-related extracellular matrix composition in skeletal muscle.[28] To date, only studies using muscle biopsies have reported a 3- to 5-fold higher amount of fibrillar collagens in the semitendinosus muscle of children with CP exhibiting significant muscle contractures, compared with TD peers.[29 30]

Increased amounts of collagen in the extracellular matrix may possibly lead to increased passive muscle stiffness, which is defined as the resistance of a muscle to stretch in the absence of active contraction.[31] Passive muscle stiffness can cause shortened muscle lengths (MLs) and eventually the development of contractures. Recent ultrasound elastography advancements, in particular shear wave elastography (SWE), can directly quantify passive muscle stiffness. Overall, previous literature indicates increased passive muscle stiffness in children with CP, although some inconsistencies remain, and study populations are generally small.[32–35] Moreover, the regional distribution of muscle stiffness along the ML remains insufficiently studied, despite its potential relevance for targeted interventions.

Given the concurrent presence of altered muscle characteristics (ie, reduced muscle size, altered composition and increased stiffness), a comprehensive approach is required to understand their interactions and their relations to muscle function. The overall objective of the current study protocol is to provide an integrated characterisation of muscle stiffness, composition and morphology, complemented by (functional) strength measurements in children with CP, with comparisons to TD children. This multimodal approach may enhance our understanding of muscle adaptation in CP and help to elucidate muscular factors associated with functional impairments.

In the first phase, muscle composition and passive muscle stiffness will be defined in children with CP compared with TD peers, next to the well-known reduced muscle size and muscle weakness, in different age groups and across different GMFCS levels. It is hypothesised that collagen and fat content, as well as passive stiffness, are higher in children with CP, with the highest values expected in the oldest age category and in children with higher GMFCS levels. In a subsequent phase, measurements of muscle size, stiffness and composition will be related to plantar flexion strength and function to investigate their combined associations with muscle performance in CP and TD children.

METHODS AND ANALYSIS

Design and objective

This protocol describes a cross-sectional descriptive research design, consisting of three different imaging techniques and strength assessments to provide a complete picture of the medial gastrocnemius of children with CP. To allow comparison with healthy muscle, age- and sex-matched TD children will serve as the control group.

Study population

Children with CP will be recruited through the Cerebral Palsy Reference Centre of the University Hospitals Leuven

(Belgium). They will be eligible for inclusion if they meet the following criteria: (1) diagnosis of spastic CP, (2) uni- or bilateral involvement, (3) a classification of level I, II or III on the GMFCS scale[36] and (4) aged between 2 and 16 years. The following exclusion will be applied: (1) diagnosis of dyskinesia or ataxia, (2) severe co-morbidities, such as cognitive problems, (3) botulinum toxin treatment in the past 10 months, (4) history of orthopaedic surgery during the past 2 years, (5) previous surgery in the medial gastrocnemius muscle at any timepoint, (6) severe ankle deformities and (7) weight-for-height (WFH) values above or below 2 SD from the typical mean. Age and sex-matched TD children will be recruited through family, students and acquaintances. The following exclusion criteria will apply: (1) (history of) musculoskeletal or neuromotor problems, (2) WFH values above or below 2 SD of the typical mean and (3) sport participation in the same sport more than 5 hours per week. Written informed consent will be obtained from the parents or legal guardians of all participants. In addition, consent will also be obtained from participants aged 12 years and older.

All children will be divided into three age groups: pre-school (2–6 years), grade-school (7–11 years) and teen-aged (12–16 years). These categories will enable assessment of developmental changes and age-specific differences in muscle properties between CP and TD children. For children with CP, each age group will include an equal number of children with GMFCS levels I and II+III. Pubertal status will be taken into account using the Tanner staging scale.[37] For children with CP, the most involved leg will be selected for the assessments, while for TD children a random leg will be chosen.

Study set-up and materials

Anthropometric measurements

Body weight (kilogram) and height (metre) will be measured for each participant to better describe the characteristics of the study population and to allow normalisation of specific muscle parameters that are subject to growth, such as muscle size properties.

Muscle size and architecture

Muscle size will be assessed using a well-described reliable 3DfUS protocol,[10] combining 2D ultrasonography (Telemed SmartUS B-mode ultrasound device, Telemed Ltd, Vilnius, Lithuania) with a synchronised motion-tracking system (Optitrack V120:Trio, NaturalPoint Inc., Corvallis, Oregon, US). To ensure a resting position of the ankle, the participant will lie prone with their lower leg supported on a triangle cushion, allowing the foot to hang freely over the edge without contacting the table (figure 1). Joint position angles of the knee and ankle will be measured using goniometry. A substantial amount of acoustic coupling gel will be applied in combination with the Portico, a custom-designed plastic holder incorporating a gel pad, to minimise muscle deformation during ultrasound assessment.[38] Four markers on the US probe will be tracked as it is swept over the medial gastrocnemius muscle, allowing for the creation of a 3D reconstruction. From the acquired three-dimensional images, muscle morphological variables will be extracted, including muscle volume (MV, millilitre), ML (millimetre), tendon length (TL, millimetre), muscle tendon unit length (MTUL, millimetre) and anatomical cross-sectional area (CSA, square millimetre). MV will be defined as the three-dimensional volume of the medial gastrocnemius muscle belly, which will be calculated using the cubic planimetry method.[39] ML will be calculated as the distance from the most superficial aspect of the medial femoral condyle to the most proximal point of the muscle-tendon junction, and TL as the distance from the most proximal point of the muscle-tendon junction to the most proximal point of the insertion (calcaneus). MTUL will be defined as the distance from the most superficial aspect of the medial femoral condyle to the most proximal point of the insertion of the tendon (calcaneus). Anatomical CSA will be determined as the two-dimensional CSA measured at 50% of muscle belly length, oriented perpendicular to the muscle's longitudinal axis. Data will be collected and processed using STRADWIN software (V.6.0). Echo intensity (EI; grey scale, 0–256) will serve as an indicator of muscle quality and reflect the ratio of contractile to non-contractile tissue within the muscle. Additionally, the medial gastrocnemius muscle architecture will be characterised by fascicle length (FL) and pennation angle (PA), measured in the fascicle plane (ie, with the ultrasound probe aligned parallel to the muscle fascicles). FL will be defined as the distance between the intersection of the fascicle with the superficial aponeurosis and its intersection with the deep aponeurosis, while the PA represents the angle between the fascicle and the deep aponeurosis. Muscle architecture parameters will be obtained using the B-mode setting of the Supersonic Imagine Aixplorer ultrasound system (see below) due to its high B-mode

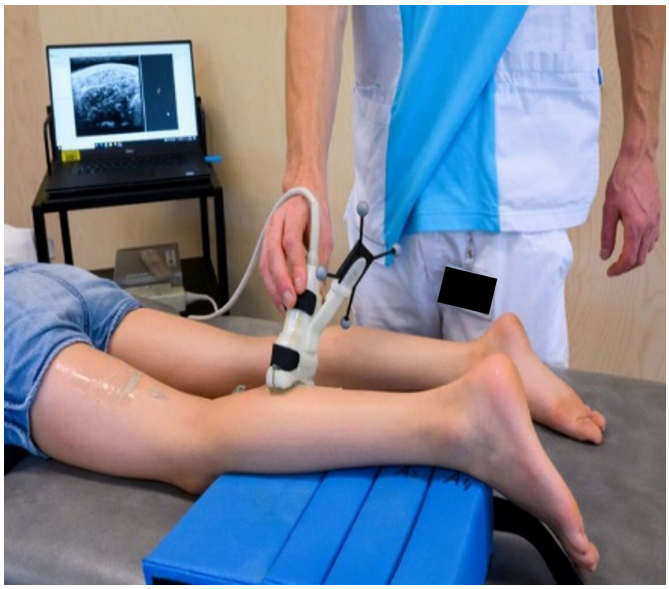


Figure 1 Illustration of 3DfUS measurement set-up. 3DfUS, three-dimensional freehand ultrasound.

resolution. MV, ML, CSA and EI will serve as primary outcomes, while TL, MTUL, FL and PA will serve as secondary outcomes.

Muscle stiffness

Passive muscle stiffness will be assessed using SWE with the Supersonic Imagine Aixplorer ultrasound system (Aix-en-Provence, France). The set-up is similar to that for 3DfUS to ensure muscle rest length. The medial femoral condyle and the muscle-tendon junction will be identified and marked to define two locations for SWE. The muscle mid-belly will be defined as the point located halfway (50%) between these two anatomical landmarks. The distal 75% region of the muscle will be identified by marking the midpoint between the mid-belly and the muscle-tendon junction, corresponding to 75% of the total ML. SWE measurements will be performed at both locations, with the probe aligned parallel to the muscle fascicle orientation. Since the reliability of SWE measurements in children has shown variable results in the literature, a preliminary reliability study will be conducted. Two repeated measurements will be performed within a 2-week interval in a sample of 10 TD children to evaluate reproducibility. Data quality will be assessed with colour-coded maps, and results will be calculated using ELASTOGUI open software (University of Nantes, France) developed for MATLAB (V.R2023b, MathWorks Inc., Natick, MA, US). The primary outcome is shear modulus (kPa) in the mid-belly region, with regional differences between the mid-belly and distal region defined as the secondary outcome. Shear wave speed outcomes (m/s), obtained from the same SWE data, will also be reported as an alternative unit.

Muscle composition

Prior to participation in the MRI scan, parents or legal guardians of the children will complete a standard MRI safety screening questionnaire. MRI scans will only be conducted in children who are able to cooperate and are able to remain still during the procedure. All children will be scanned using a 3.0 Tesla MRI scanner (Achieva dStream, Philips Healthcare, The Netherlands). An MRI protocol including anatomical reference, Dixon, T1ρ mapping and T2 mapping will be applied to investigate muscle composition. Children will be positioned supine, with the foot placed in a custom-made wooden fixture that stabilises the foot in a fixed position during data acquisition and prevents deformation of the calf muscle. Small sandbags will be added to ensure a stable position of the wooden fixture (figure 2). An anterior coil (dStream Torso coil, Philips Healthcare, The Netherlands), supported by an MR-compatible support frame, will be placed over the legs to maximise signal reception and ensure high-quality imaging (figure 2). The MRI protocol takes 15 min, with audio entertainment to improve compliance. All acquisitions are performed in the transversal plane along the medial gastrocnemius ML. T1-weighted images will be obtained for anatomical reference. Intramuscular fat fraction (average fat fraction over the muscle belly length) and fat distribution (proximal, mid-belly, distal) will be determined by the Dixon method, while collagen content will be assessed at the mid-belly region using T1ρ mapping. A composite spin lock preparation pulse is used in the T1ρ mapping to minimise B0 and B1 field inhomogeneity. Finally, a quantitative evaluation of active muscle damage is assessed using T2 mapping. Detailed sequence parameters are listed in table 1. To evaluate the repeatability of these sequences, a reliability study will be conducted in a sample of 10 TD children, with two scans repeated at a maximum interval of 2 weeks. Muscle segmentation will be conducted in 3D Slicer (V.5.6). Custom-made MATLAB scripts will be used for subsequent data analysis. The primary outcomes include intramuscular fat fraction (%) and T2 relaxation time (milliseconds) as well as T1ρ relaxation time (milliseconds) in the mid-belly, while the regional distribution of intramuscular fat fraction (%) will serve as a secondary outcome.

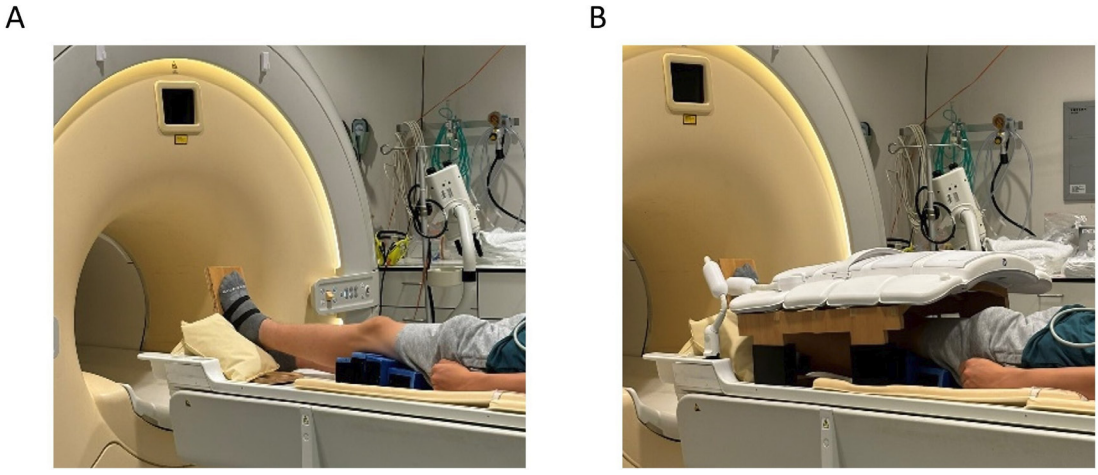


Figure 2 A. Leg placement with foot supported in wooden fixture B. Complete MRI set-up with anterior coil

Table 1 Overview of sequence parameters

	Anatomical reference	Dixon	T1ρ mapping	T2 mapping
Sequence name	T1W_TSE	3D multi-echo GRE	3D GRE	MS multi-echo SE
TR (ms)	601	10	5.3	3000
TE (ms)	20	1.5/2.5/3.5/4.5/5.5/6.5	2.8	13/26/39/52/65/78
FOV (mm^3)	455×301×318	300×200×320	280×142×40	448×224×360
Voxel size (mm^3)	0.8×0.8×6.0	1.3×1.3×4.0	0.55×0.54×10.0	1.6×2.0×10.0
Matrix	568×355	232×154×160	508×263	280×112
Slices	53	160	8	15
Notes		Flip angle 3°	Flip angle 10°, spin lock amplitude 300 Hz, spin lock times 0/5/10/15/20/25/30/40 ms, shot TR 3500 ms	Flip angle 90°/180°

FOV, field of view; GRE, gradient echo; SE, spin echo; TE, echo time; TR, repetition time; TSE, turbo spin echo.

Muscle strength

For assessing isometric strength, participants will be positioned supine with their arms crossed over their chest, their knees in maximal extension within their available range of motion and their feet extending slightly beyond the edge of the examination table. A handheld dynamometer will be placed at the level of the metatarsal heads (figure 3), while the foot is in a neutral ankle position. The distance between the centre of the dynamometer and the lateral malleolus will be measured and defined as the lever arm. Each participant will be instructed to perform three maximal isometric contractions against the handheld dynamometer after which the scores will be averaged. The primary outcomes are maximal plantar flexor torque (Newton x metre) and normalised maximal plantar flexor torque, which will be normalised to the body weight of the child.



Figure 3 Illustration of isometric strength assessment set-up.

Plantar flexion function

Selected activities from reliable functional strength tests (bilateral heel raises (figure 4) and standing long jumps (figure 4))[40] will be used to classify children with CP with mildly or severely altered plantar flexion function, based on histogram analysis (primary outcome). These tests will only be conducted in children who are able to safely complete them. It is anticipated that younger children or those with lower motor function (eg, GMFCS level III) may not be able to perform one or both tests in a standardised manner. In such cases, inability to perform the test will be documented, and these participants will be excluded from analyses related to that specific outcome.

As a muscle endurance test, children will perform as many bilateral heel rises as possible within 30 s, avoiding compensatory movements such as not fully lowering the heels or bending the knees. Each participant will complete three repetitions after which the mean score will be calculated. Next, children will perform a standing long jump test as a measure of lower limb power, jumping from a stationary position and aiming to jump as far forward as possible, with both feet leaving the ground simultaneously and maintaining a stable standing position on landing. Each participant will complete three trials, and the mean value will be calculated. The jump distance (in centimetres) will be normalised to the child's height.

Power analysis

The power analysis was performed to detect a significant interaction effect of group x age using two-way ANOVA (G*Power V.3.1, power=0.80). Effect sizes (ESs) were estimated based on previously reported differences in fat fraction and shear modulus between CP and TD children[24 32–34]. The overall lowest ES (Cohen's d 1.1, for FF) was used and converted to a 0.55 Cohen's f ES. The number of groups was set to nine (3 subject groups x 3 age groups) and the numerator df to four (3 subject groups −1) x (3 age groups −1). The α-level was 0.006 (0.05/8) to correct for multiple testing of seven outcomes (average

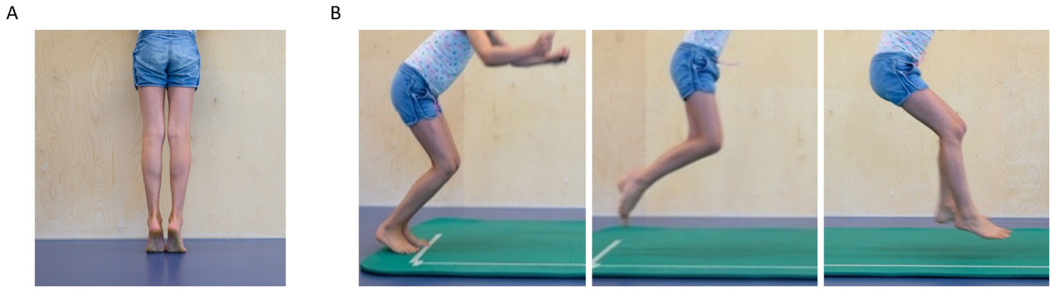


Figure 4 A. Bilateral heel-rises. B. Standing long jumps.

fat fraction, T2 relaxation time, T1ρ relaxation time in the mid-belly, shear modulus in the mid-belly, MV, ML, CSA, EI), and we accounted for a 15% potential loss of data caused by technical problems or non-compliance of participants. Power analyses revealed that a total sample size of 81 children (9 TD, 9 CP-GMFCS I, 9 CP-GMFCS II+III per age group) provides sufficient power to test all hypotheses (figure 5).

Statistical analysis plan

First, descriptive statistics will summarise all outcomes by subject (CP-GMFCS I, CP-GMFCS II+III, TD) and age group (pre-school, grade-school, teen-aged). To define intrinsic muscle composition, size, stiffness and weakness of the medial gastrocnemius in children with CP, a two-way ANOVA will be used to assess the effects of subject and age group. For non-normal distributions or missing data, linear mixed models will be used with subject and age group and their interaction as fixed effects. Additionally, linear mixed models will evaluate the effects of muscle region (proximal, mid-belly, distal), subject group (pooled CP and TD) and age group with random intercept effects for inter-regional dependence. A sub-analysis on grade-school and teen-aged children will examine the impact of sex and pubertal stage on the CP-TD differences as secondary covariates.

To explore associations among muscle size, muscle composition and muscle stiffness, simple linear correlation analyses (Pearson or Spearman, as appropriate) will be conducted. Multivariable linear regression analyses will then be used to examine the joint associations of these muscle parameters with measures of muscle strength and functional performance. For this analysis, the CP subgroups will be pooled and analysed as a single group to ensure that the models have sufficient power to include three to five independent variables per outcome (rule of thumb). The main analyses will be performed on the CP dataset, while a secondary exploratory analysis will be performed on the TD dataset to aid data interpretation. Multicollinearity will be assessed by examining variance inflation factors and pairwise correlations among independent variables, with correlations greater than 0.70–0.80 considered indicative of potential multicollinearity.[41]

PATIENT AND PUBLIC INVOLVEMENT STATEMENT

Patients and the public were not involved in the design or conduct of this study. The results will be disseminated to

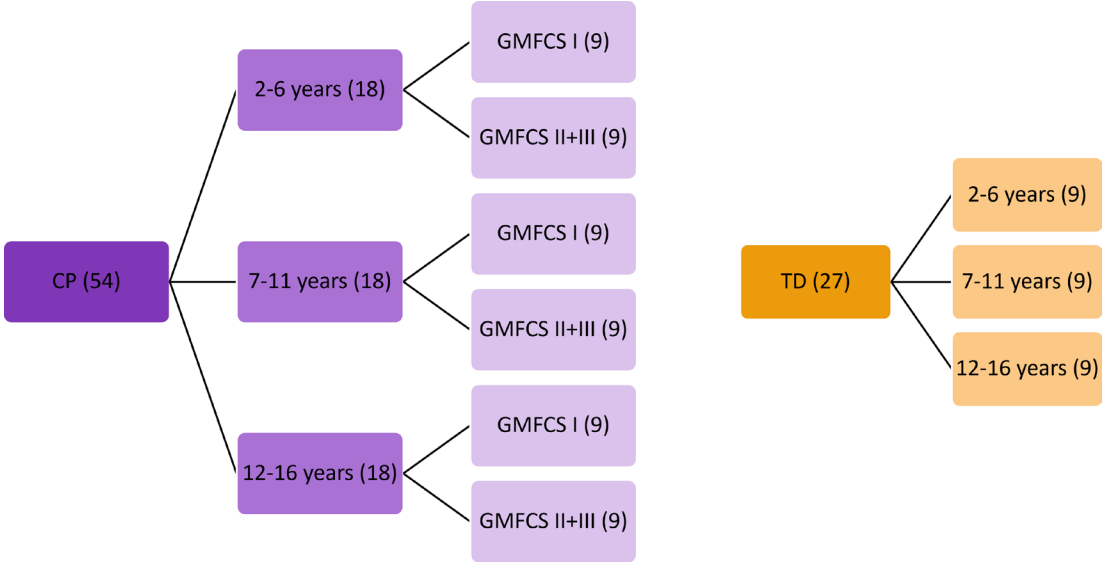


Figure 5 Overview of participant distribution by age and GMFCS level. CP, cerebral palsy; GMFCS, gross motor function classification system; TD, typically developing.

participants in an accessible format through reports and newsletters.

ETHICS AND DISSEMINATION

The study is conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of University Hospitals Leuven / Catholic University Leuven (S62187). During data collection, all participant data will be pseudonymised. The datasets generated from this study will be deposited in the KU Leuven Research Data Repository (RDR), with all participant information anonymised. Study findings will be disseminated to the scientific community and relevant stakeholders via peer-reviewed publications, presentations at national and international conferences, and newsletters.

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Contributors C.L. and K.D. conceived and designed the study protocol. F.C., Z.W., R.P. and T.D. developed the methodology for imaging and data acquisition. C.L. drafted the manuscript. T.D., Z.W., R.P., F.C., A.V.C. and K.D. critically revised the manuscript. F.C., A.V.C. and K.D. supervised the project and acquired funding. K.D. is responsible for the overall content as guarantor. All authors approved the final version of the manuscript. Artificial intelligence tools were used to assist with language editing and improving the clarity of the manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design or conduct of this research but will be involved in its reporting and dissemination.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data sharing not applicable as no datasets generated and/or analysed for this study. No data are available.

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REFERENCES

1 Rosenbaum P, Paneth N, Leviton A. A report: the definition and classification of cerebral palsy April 2006. *Dev Med Child Neurol* 2007;49:8–14.
2 McIntyre S, Goldsmith S, Webb A, *et al*. Global prevalence of cerebral palsy: A systematic analysis. *Dev Med Child Neurol* 2022;64:1494–506.
3 Michael-Asalu A, Taylor G, Campbell H, *et al*. Cerebral Palsy. *Adv Pediatr* 2019;66:189–208.
4 Mathewson MA, Lieber RL. Pathophysiology of muscle contractures in cerebral palsy. *Phys Med Rehabil Clin N Am* 2015;26:57–67.
5 Neptune RR, Kautz SA, Zajac FE. Contributions of the individual ankle plantar flexors to support, forward progression and swing initiation during walking. *J Biomech* 2001;34:1387–98.
6 Eek MN, Beckung E. Walking ability is related to muscle strength in children with cerebral palsy. *Gait Posture* 2008;28:366–71.
7 Schless S-H, Cenni F, Bar-On L, *et al*. Combining muscle morphology and neuromotor symptoms to explain abnormal gait at the ankle joint level in cerebral palsy. *Gait Posture* 2019;68:531–7.
8 Graham HK, Rosenbaum P, Paneth N, *et al*. Cerebral palsy. *Nat Rev Dis Primers* 2016;2:15082.
9 Handsfield GG, Williams S, Khuu S, *et al*. Muscle architecture, growth, and biological Remodelling in cerebral palsy: a narrative review. *BMC Musculoskelet Disord* 2022;23:233.
10 Cenni F, Schless S-H, Bar-On L, *et al*. Reliability of a clinical 3D freehand ultrasound technique: Analyses on healthy and pathological muscles. *Comput Methods Programs Biomed* 2018;156:97–103.
11 Hanssen B, Peeters N, Dewit T, *et al*. Reliability of 3D freehand ultrasound to assess lower limb muscles in children with spastic cerebral palsy and typical development. *J Anat* 2023;242:986–1002.
12 Malaiya R, McNee AE, Fry NR, *et al*. The morphology of the medial gastrocnemius in typically developing children and children with spastic hemiplegic cerebral palsy. *J Electromyogr Kinesiol* 2007;17:657–63.
13 Obst SJ, Boyd R, Read F, *et al*. Quantitative 3-D Ultrasound of the Medial Gastrocnemius Muscle in Children with Unilateral Spastic Cerebral Palsy. *Ultrasound Med Biol* 2017;43:2814–23.
14 Barber LA, Read F, Lovatt Stern J, *et al*. Medial gastrocnemius muscle volume in ambulant children with unilateral and bilateral cerebral palsy aged 2 to 9 years. *Dev Med Child Neurol* 2016;58:1146–52.
15 Schless S-H, Hanssen B, Cenni F, *et al*. Estimating medial gastrocnemius muscle volume in children with spastic cerebral palsy: a cross-sectional investigation. *Dev Med Child Neurol* 2018;60:81–7.
16 De Beukelaer N, Vandekerckhove I, Huyghe E, *et al*. Morphological Medial Gastrocnemius Muscle Growth in Ambulant Children with Spastic Cerebral Palsy: A Prospective Longitudinal Study. *J Clin Med* 2023;12:1564.
17 Goudriaan M, Nieuwenhuys A, Schless S-H, *et al*. A new strength assessment to evaluate the association between muscle weakness and gait pathology in children with cerebral palsy. *PLoS ONE* 2018;13:e0191097.
18 O'Brien SM, Carroll TJ, Barber LA, *et al*. Plantar flexor voluntary activation capacity, strength and function in cerebral palsy. *Eur J Appl Physiol* 2021;121:1733–41.
19 Hanssen B, Peeters N, Vandekerckhove I, *et al*. The Contribution of Decreased Muscle Size to Muscle Weakness in Children With Spastic Cerebral Palsy. *Front Neurol* 2021;12:692582.
20 Howard JJ, Herzog W. Skeletal Muscle in Cerebral Palsy: From Belly to Myofibril. *Front Neurol* 2021;12:620852.
21 Hooijmans MT, Schlaffke L, Bolsterlee B, *et al*. Compositional and Functional MRI of Skeletal Muscle: A Review. Journal of Magnetic Resonance Imaging. 2023.
22 Noble JJ, Charles-Edwards GD, Keevil SF, *et al*. Intramuscular fat in ambulant young adults with bilateral spastic cerebral palsy. *BMC Musculoskelet Disord* 2014;15:236.
23 Svane C, Forman CR, Rasul A, *et al*. Quantitative MRI and Clinical Assessment of Muscle Function in Adults With Cerebral Palsy. *Front Neurol* 2021;12:771375.
24 D'Souza A, Bolsterlee B, Lancaster A, *et al*. Intramuscular fat in children with unilateral cerebral palsy. *Clin Biomech (Bristol)* 2020;80:105183.
25 Akinci D'Antonoli T, Santini F, Deligianni X, *et al*. Combination of Quantitative MRI Fat Fraction and Texture Analysis to Evaluate Spastic Muscles of Children With Cerebral Palsy. *Front Neurol* 2021;12:633808.
26 Yin L, Xie Z-Y, Xu H-Y, *et al*. T2 Mapping and Fat Quantification of Thigh Muscles in Children with Duchenne Muscular Dystrophy. *Curr Med Sci* 2019;39:138–45.
27 Weidensteiner C, Madoerin P, Deligianni X, *et al*. Quantification and Monitoring of the Effect of Botulinum Toxin A on Paretic Calf Muscles of Children With Cerebral Palsy With MRI: A Preliminary Study. *Front Neurol* 2021;12:630435.
28 Noehren B, Hardy PA, Andersen A, *et al*. T1ρ imaging as a non-invasive assessment of collagen remodelling and organization in human skeletal muscle after ligamentous injury. *J Physiol* 2021;599:5229–42.

29 Smith LR, Pichika R, Meza RC, *et al*. Contribution of extracellular matrix components to the stiffness of skeletal muscle contractures in patients with cerebral palsy. *Connect Tissue Res* 2021;62:287–98.
30 Smith LR, Lee KS, Ward SR, *et al*. Hamstring contractures in children with spastic cerebral palsy result from a stiffer extracellular matrix and increased in vivo sarcomere length. *J Physiol* 2011;589:2625–39.
31 Willerslev-Olsen M, Choe Lund M, Lorentzen J, *et al*. Impaired muscle growth precedes development of increased stiffness of the triceps surae musculotendinous unit in children with cerebral palsy. *Dev Med Child Neurol* 2018;60:672–9.
32 Vola EA, Albano M, Di Luise C, *et al*. Use of ultrasound shear wave to measure muscle stiffness in children with cerebral palsy. *J Ultrasound* 2018;21:241–7.
33 Brandenburg JE, Eby SF, Song P, *et al*. Quantifying passive muscle stiffness in children with and without cerebral palsy using ultrasound shear wave elastography. *Dev Med Child Neurol* 2016;58:1288–94.
34 Lallemant-Dudek P, Vergari C, Dubois G, *et al*. Ultrasound shearwave elastography to characterize muscles of healthy and cerebral palsy children. *Sci Rep* 2021;11:3577.
35 Cenni F, Sukanen M, Hernández-Belmonte A, *et al*. The relationship between triceps surae muscle-tendon morphology and shear modulus across passive ankle range of motion in cerebral palsy. *J Biomech* 2025;192:112946.
36 Palisano R, Rosenbaum P, Walter S, *et al*. Development and reliability of a system to classify gross motor function in children with cerebral palsy. *Dev Med Child Neurol* 1997;39:214–23.
37 Tanner J. Growth at Adolescence. *Arch Dis Child* 1956;31:68.
38 Cenni F, Schless S-H, Monari D, *et al*. An innovative solution to reduce muscle deformation during ultrasonography data collection. *J Biomech* 2018;77:194–200.
39 Treece GM, Prager RW, Gee AH, *et al*. Fast surface and volume estimation from non-parallel cross-sections, for freehand three-dimensional ultrasound. *Med Image Anal* 1999;3:141–73.
40 VAN Tittelboom V, Alemdaroğlu-Gürbüz I, Hanssen B, *et al*. Reliability of functional tests of the lower limbs and core stability in children and adolescents with cerebral palsy. *Eur J Phys Rehabil Med* 2021;57:738–46.
41 Slinker BK, Glantz SA. Multiple regression for physiological data analysis: the problem of multicollinearity. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 1985;249:R1–12.