DOI: 10.1111/dmcn.15763

#### ORIGINAL ARTICLE

## Longitudinal trajectory of medial gastrocnemius muscle growth in the first years of life

Nathalie De Beukelaer<sup>1,2</sup> | Ines Vandekerckhove<sup>1</sup> | Geert Molenberghs<sup>3,4</sup> | Gunnar Naulaers<sup>5,6</sup> | Liesbeth Thewissen<sup>5,6</sup> | Domiziana Costamagna<sup>1,7</sup> | Anja Van Campenhout<sup>5,8,9</sup> | Kaat Desloovere<sup>1,9</sup> | Els Ortibus<sup>5</sup>

<sup>1</sup>Department of Rehabilitation Sciences, KU Leuven, Leuven, Belgium

<sup>2</sup>Kinesiology Laboratory, Geneva University Hospitals and Geneva University, Geneva, Switzerland

<sup>3</sup>Interuniversity Institute for Biostatistics and Statistical Bioinformatics (I-BIOSTAT), KU Leuven, Leuven, Belgium

<sup>4</sup>Interuniversity Institute for Biostatistics and Statistical Bioinformatics (I-BIOSTAT), Data Science Institute, Hasselt University, Hasselt, Belgium

<sup>5</sup>Department of Development and Regeneration, KU Leuven, Leuven, Belgium

<sup>6</sup>Neonatal Intensive Care Unit, University Hospitals Leuven, Leuven, Belgium

<sup>7</sup>Department of Movement Sciences, KU Leuven, Leuven, Belgium

<sup>8</sup>Department of Orthopedics, University Hospitals Leuven, Leuven, Belgium

<sup>9</sup>Clinical Motion Analysis Laboratory, University Hospitals Leuven, Leuven, Belgium

#### Correspondence

Nathalie De Beukelaer, Weligerveld 1, 3212 Pellenberg, Belgium. Email: nathalie.debeukelaer@kuleuven.be

#### **Funding information**

Fonds Wetenschappelijk Onderzoek, Grant/ Award Number: 1188921N and G0B4619N; KU Leuven, Grant/Award Number: C24/18/103; KU Leuven Biomedical Sciences Group

#### Abstract

**Aim:** To define the longitudinal trajectory of gastrocnemius muscle growth in 6- to 36-month-old children with and without spastic cerebral palsy (SCP) and to compare trajectories by levels of gross motor function (Gross Motor Function Classification System, GMFCS) and presumed brain-lesion timing.

**Method:** Twenty typically developing children and 24 children with SCP (GMFCS levels I–II/III–IV = 15/9), were included (28/16 females/males; mean age at first scan 15.4 months [standard deviation 4.93, range 6.24–23.8]). Three-dimensional free-hand ultrasound was used to repeatedly assess muscle volume, length, and cross-sectional area (CSA), resulting in 138 assessments (mean interval 7.9 months). Brain lesion timing was evaluated with magnetic resonance imaging classification. Linear mixed-effects models defined growth rates, adjusted for GMFCS levels and pre-sumed brain-lesion timing.

**Results:** At age 12 months, children with SCP showed smaller morphological muscle size than typically developing children (5.8 mL vs 9.8 mL, p < 0.001), while subsequently no differences in muscle growth were found between children with and without SCP (muscle volume: 0.65 mL/month vs 0.74 mL/month). However, muscle volume and CSA growth rates were lower in children classified in GMFCS levels III and IV than typically developing children and those classified in GMFCS levels I and II, with differences ranging from -56% to -70% (p < 0.001).

**Interpretation:** Muscle growth is already hampered during infancy in SCP. Muscle size growth further reduces with decreasing functional levels, independently from the brain lesion. Early monitoring of muscle growth combined with early intervention is needed.

Spastic cerebral palsy (SCP) is mostly caused by a nonprogressive lesion in the developing brain, for which predominant pathogenic patterns, i.e. white and/or grey matter involvement, are described.<sup>1,2</sup> Consequently, neuromotor impairments (e.g. spasticity and weakness) arise, combined with gait pathology and hampered morphological muscle growth.<sup>1,3</sup> For a long time, altered muscle morphology was categorized as a secondary phenomenon. However, already around 12 to 15 months, reduced medial gastrocnemius muscle volume was found in children with SCP compared with typically developing infants.<sup>4,5</sup> At these early ages, children without independent walking skills and classified in higher

Abbreviations: CSA, cross-sectional area; SCP, spastic cerebral palsy.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

levels on the Gross Motor Function Classification System (GMFCS) presented larger muscle volume deficits.<sup>4,6</sup> Together, it is suggested that primary (e.g. altered neural input) and secondary (e.g. altered muscle use) factors contribute to hampered muscle growth but the underlying mechanisms are not yet well-known.<sup>3</sup>

Reduced corticospinal input to the spinal cord networks, emerging between 17 and 29 weeks after conception in typical development, results in impeded muscle innervation and consequently altered muscle activation. For example, impaired corticospinal integrity is already reflected in the first months of life of children with SCP by the presence of atypical postural patterns and aberrant movements, which is generally associated with higher GMFCS levels.<sup>7,8</sup> Furthermore, impeded muscle innervation is expected to be interrelated with a disturbed myogenesis.<sup>9</sup> Myogenesis accounts for muscle formation, i.e. increasing fibre number and size during the perinatal period, followed by a marked fibre size increase postnatally.<sup>10</sup> Consequently, the timing of the brain lesion might interfere with the developmental milestones in the normal processes of muscle innervation and maturation, observed with reduced muscle growth from early ages in children with SCP. Therefore, diminished neural input is suggested to be a primary mechanism of impaired morphological muscle growth in children with SCP.<sup>3</sup>

Up to now, most of the studies reporting that young children with SCP have early altered muscle morphology compared with age-matched typically developing children used a cross-sectional study design with heterogenous cohorts.<sup>11</sup> Furthermore, limited evidence showed muscle alterations (e.g. smaller muscle volume) no earlier than the age of 12 to 15 months and should therefore be further confirmed with longitudinal studies.<sup>4,5</sup> Additionally, these morphological muscle alterations were not normalized to account for overall anthropometric growth. Longitudinal studies in very young children with and without SCP are needed to establish the abnormal and normal muscle growth trajectories respectively. Hereto, repeated measurements per individual are vital to improve knowledge on (a)typical macroscopic morphological muscle growth in the early years of life, as they take the variation of muscle development into account.12

The purpose of this study was to define the trajectory of morphological muscle growth in children with and without SCP, aged between 6 and 36 months, by performing repeated three-dimensional freehand ultrasound assessments of the medial gastrocnemius muscle with a minimum interval of 6 months. The specific aims were to (1) investigate the onset of the altered growth trajectories between those with SCP and typically developing children; (2) specify the muscle growth rates from a longitudinal perspective; (3) compare the growth trajectories between typically developing and (3A) GMFCS subgroups and (3B) subgroups for the timing of presumed brain lesion. It was hypothesized that, in children with SCP, the muscle growth trajectories have (1) an onset of deficits before the age of 12 months with (2) lower

#### What this paper adds

- Muscle size is lower in children with spastic cerebral palsy at 12 months.
- Muscle growth rates are slower in children in higher Gross Motor Function Classification System levels.

growth rates (i.e. muscle changes with increasing age) compared with typically developing children. Children classified in (3A) higher GMFCS levels compared with lower GMFCS levels and with typically developing children, and (3B) a presumed early compared with later brain lesion timing and with typically developing children, were expected to show more deviant muscle growth trajectories.

#### **METHOD**

#### **Participants**

Children with (a high-risk for) SCP, aged between 6 months and 2 years, were recruited through the Neonatal Intensive Care Unit, Center for Developmental Disabilities and Cerebral Palsy Reference Center from the University Hospitals Leuven. A paediatric neurologist diagnosed the children with SCP, on the basis of their medical history, abnormal neuroimaging, and neurological signs during clinical examination.<sup>13</sup> Using the GMFCS Expanded & Revised edition, children classified in levels I to IV were eligible for inclusion.<sup>14</sup> To increase homogeneity, we merged (1) children classified in GMFCS level I or II and (2) children in level III, or evolution of level IV to III (further details in Appendix S1). Children who did not have a confirmed diagnosis nor were classified in GMFCS levels I to III at the end of the follow-up period were excluded from data analysis. Other exclusion criteria and the flow-chart are presented in Appendix S2. Children with typical development, i.e. born at term and with no medical history of neurological, orthopaedic, or muscular problems, and aged 6 months to 2 years, were recruited.

The study was conducted between March 2019 and March 2022. The study protocol was approved by the Ethical Committee KU Leuven/UZ Leuven and registered (NCT05197764). All parents provided written informed consent.

#### Muscle assessment

Imaging of the medial gastrocnemius muscle was performed with three-dimensional freehand ultrasound.<sup>15</sup> A Telemed-Echoblaster B-mode ultrasound device (Telemed Ltd, Vilnius, Lithuania) with linear probe, with an attached set of reflective markers, was used. The probe position and orientation were tracked with an OptiTrack V10:Trio motion tracking system (NaturalPoint Inc., Corvallis, Oregon, USA). The Portico, a concave gel pad within a custom-shaped plastic mount, was attached to the probe to minimize muscle deformations.<sup>16</sup> Fixed instrumentation settings as described by Schless et al. were used.<sup>17</sup> The medial gastrocnemius data acquisition and processing were performed using Stradwin software (version 6.0; Department of Engineering, Cambridge University, Cambridge, UK).

All participants were prospectively assessed with threedimensional freehand ultrasound over a 2-year period, ending either at the age of 36 months or by the end of the study period (31st March 2022). The interval between the repeated assessments was minimally 6 months, resulting in a minimum of two to a maximum of five observations per child (Figure 1). The most affected leg in the participants with SCP and a random leg for the typically developing infants were measured. Additional specifications and standardization of the data acquisition are described in Appendix S3.

A single experienced researcher (NDB) performed all muscle acquisitions and processing, after extensive training and being blinded during processing for the patient's characteristics (e.g. GMFCS level). Manual segmentations by drawing along the inside muscle borders were performed on a minimum 5% to 10% of the individual transversal ultrasound images of one muscle belly sweep.<sup>15</sup> After applying a cubic planimetry technique, a three-dimensional image of the muscle belly was produced, resulting in the muscle volume (in millilitres).<sup>18</sup> Muscle belly length (in millimetres) was calculated from the medial femoral condyle to the muscle-tendon junction. A single manual segmented anatomical cross-sectional area (CSA, in square millimetres) at 50% of the muscle mid-belly was also extracted.

To account for anthropometric growth, ratio-scaling was performed in line with previously applied techniques in children from 3 years of age.<sup>11,19</sup> Muscle volume was normalized to the product of body mass and length (in millilitres per kilogram metre). Because the ratio-scaling has not yet been used in children younger than 36 months, this normalization technique was firstly validated in the typically developing group. The ratio-scaling was considered valid when the normalized muscle volume of the typically developing children showed a horizontal slope (regression coefficient=0) in relation to age, indicating that variation in muscle growth due to anthropometric growth was removed. The analyses for other muscle outcomes and subgroup analysis were only further explored when the ratio-scaling was found to be valid.

## Assessment of brain-lesion timing in SCP

Anatomical magnetic resonance imaging (MRI) of the brain was collected during the routine clinical follow-up and classified following the predominant pathogenic patterns<sup>20</sup> (Appendix S4). The predominant white and grey matter injuries were further used to indicate a presumed early (i.e. 20th– 30th week after conception) and late (i.e. 30th–40th week after conception) brain-lesion timing respectively.

#### Statistical analysis

To investigate trajectories by observed muscle outcome (i.e. muscle volume, CSA, and muscle belly lengths), linear mixed-effects models were selected, after exploratory analysis for the linearity, and fitted (Appendix S5).<sup>12</sup> To provide an adequate fitting of the models, the mean structure (i.e. fixed effects) and a covariance structure (i.e. random effects)



**FIGURE 1** Representation of the number of the performed longitudinal assessments at the different ages for the group of (a) typically developing (TD) children (n=20) and (b) children with spastic cerebral palsy (SCP) (n=24). The *x*-axis represents the ages at the time of the repeated assessments. On the *y*-axis, a number coding per row was used for each child and ordered from the youngest to the oldest. Each child is further indicated with a specific colour and symbol. (a) The typically developing group included 69 assessments, with a median (range) of three (two to five) assessments per child and a median (range) time of 6.3 months (4.5–18.3) between the assessments. (b) The SCP group included 69 assessments with a median (range) of three (two to four) assessments per child and a median (range) time of 6.1 months (4.0–12.2) between the assessments. This overview also visualizes the limited number of observations before the age of 12 months, especially in the SCP cohort.

were specified.<sup>12,21</sup> The mean structure consisted of a time effect (increasing age over time), a covariate (1) pathology—typically developing versus SCP, (2) levels of motor functioning—typically developing, GMFCS levels I and II versus GMFCS levels III and IV, or (3) typically developing, presumed lesion timing—early versus late, and an interaction effect between the time effect and covariate. Random effects were used, modelling the variability in starting point and the trajectory between the children, i.e. random intercept and random slope respectively. The workflow to build the linear mixed-effects models as described by Vandekerckhove et al. was applied.<sup>22</sup> With the *j*th observation in child *i*, for covariate *c*, the response for the different muscle outcomes was modelled as follows:

$$\text{Response}_{ii} = (\alpha_1 c + a_{1i}c) + (\beta_1 c + b_{1i}c) \times \text{age}_{ii}c + \varepsilon_{(1)ii}c$$

where  $\alpha_1$  is the intercept,  $a_{1i}$  the random intercept,  $\beta_1$  the regression coefficient,  $b_{1i}$  the random slope, and  $\varepsilon_{(1)ij}$  the measurement error.

An *F*-test assessed the differences of the estimated intercepts (i.e. onset of deficits in muscle morphology) and regression coefficients (i.e. muscle growth rates) between (subgroups for) SCP and typically developing. Critical *p*values were set at less than 0.006 after Bonferroni correction for multiple testing (i.e.  $\alpha$  of 0.05 divided by 9: three main outcomes in three between-group comparisons). The intercept was defined at the age of 12 months by comparing the onset with previous evidence and accounting for sufficient numbers of assessments before and around this cut-off. Percentage difference scores of the fixed effects were calculated to define the deficits of the SCP (sub)groups compared with the typically developing group: ([fixed effect<sub>TD</sub>-fixed effect<sub>TD</sub>)×100. The observed individual profiles and the random-effect and residual covariance structures are added as Appendices S6 to S9. Only chronological ages were used, with explorations for corrected age for preterm birth added as Appendix S10. All analyses and visualizations were performed using SAS version 9.4 (Statistical Analysis Software, SAS Institute Inc., Cary, NC, USA).

### RESULTS

A total of 24 children with SCP and 20 typically developing children were included (Table 1 and Table 2). Sixteen participants with SCP were born preterm ranging from 24 to 35 weeks, including three pairs of twins. All participants with SCP received regular physiotherapy and orthoses if clinically indicated (Appendix S11).

Both for the typically developing children and for those with SCP, the modelled muscle growth trajectories for muscle volume, normalized muscle volume, CSA, and muscle belly lengths are summarized in Figure 2 and Table 3.

# Comparison of onset for deficits in muscle morphology

At the age of 12 months and compared with typically developing children, a significant lower muscle volume, normalized muscle volume, and CSA of 41%, 18%, and 34% respectively

| Characteristics   |               | SCP ( <i>n</i> =24) | Typically developing (n=20) |  |  |
|---|---------------|---------------------|-----------------------------|--|--|
| Sex   | Males         | 15 (63%)            | 13 (65%)                    |  |  |
|   | Females       | 9 (27%)             | 7 (35%)                     |  |  |
| Gestational age   | Weeks         | 30 (26–38)          | 41 (39–41)                  |  |  |
|   | Preterm birth | 16 (67%)            | 0                           |  |  |
| GMFCS level   | I–II          | 15 (63%)            |                             |  |  |
|   | III-IV        | 9 (37%)             |                             |  |  |
| Topography  | Unilateral    | 14 (58%)            |                             |  |  |
|   | Bilateral     | 10 (42%)            |                             |  |  |
| Physiotherapy   |               |                     |                             |  |  |
| Duration (minutes)/sessions per week with range for session |               | 30/2 (0.5-4)        |                             |  |  |
| Gestational age at MRI scan, weeks                          |               | 41 (38–72)          |                             |  |  |
| MRICS   | B (B1/B2/B3)  | 17 (71%) (2/6/5)    |                             |  |  |
|   | C (C1/C2/C3)  | 6 (25%) (0/0/6)     |                             |  |  |
|   | D             | 1 (4%)              |                             |  |  |
|   |               |                     |                             |  |  |

**TABLE 1** General characteristics and baseline data of the included infants.

The frequency of participants per characteristic is presented as n (%). Descriptive statistics are indicated with median (interquartile range) or otherwise specified. During the routine clinical follow-up, a structural magnetic resonance image was obtained either around term age (n = 14, range 36–42 weeks gestational age) or during infancy (n = 10, range 52–124 weeks gestational age) for all participants with SCP. MRICS categories: B (B1, periventricular leukomalacia; B2, sequelae of intraventricular haemorrhage or periventricular haemorrhagic infarct; B3, combination of PVL and IVH sequelae); C (C1, basal ganglia/thalamus; C2, cortico-subcortical lesions only; C3, arterial infarctions [middle cerebral artery/other]); D, miscellaneous.

Abbreviations: GMFCS, Gross Motor Function Classification System; MRICS, Magnetic Resonance Imaging Classification System; SCP, spastic cerebral palsy.

| TABLE 2         | General characteristics of the included infants with SCP per GMFCS subgroup (levels I-II vs III-IV, total 24 children) and subgroups for |
|-----------------|--|
| brain lesion ti | iming (early vs late, total 23 children).  |

|              |            | SCP GMFCS level I–II | SCP GMFCS level III-IV | SCP early | SCP late |
|--------------|------------|----------------------|------------------------|-----------|----------|
| Total sample |            | 15                   | 9                      | 17        | 6        |
| Sex          | Males      | 11 (63%)             | 4 (44%)                | 11 (65%)  | 3 (50%)  |
|              | Females    | 4 (27%)              | 5 (56%)                | 6 (35%)   | 3 (50%)  |
| GMFCS level  | I–II       |                      |                        | 11 (65%)  | 4 (67%)  |
|              | III-IV     |                      |                        | 6 (35%)   | 2 (33%)  |
| Topography   | Unilateral | 11 (63%)             | 3 (33%)                | 8 (47%)   | 5 (83%)  |
|              | Bilateral  | 4 (27%)              | 6 (66%)                | 9 (53%)   | 1 (17%)  |
| MRICS        | В          | 11 (63%)             | 6 (67%)                |           |          |
|              | С          | 4 (27%)              | 2 (22%)                |           |          |
|              | D          | 0 (%)                | 1 (11%)                |           |          |

The frequency of participants per characteristic is presented as *n* (%). MRICS categories: B (B1; PVL; B2, sequelae of IVH or periventricular haemorrhagic infarct; B3, combination of PVL and IVH sequelae); C (C1, basal ganglia/thalamus; C2, cortico-subcortical lesions only; C3, arterial infarctions [middle cerebral artery/other]); D, miscellaneous.

Abbreviations: GMFCS, Gross Motor Function Classification System; IVH, intraventricular haemorrhage; MRICS, Magnetic Resonance Imaging Classification System; PVL, periventricular leukomalacia; SCP, spastic cerebral palsy.



**FIGURE 2** Predicted average trajectory (solid lines) for muscle volume, cross-sectional area, and muscle length for (a) typically developing (TD) children (green) and children with spastic cerebral palsy (SCP) (red), (b) typically developing children and those in Gross Motor Function Classification System (GMFCS) levels I–II (blue) and III–IV (pink), and (c) typically developing children and those with early (orange) and late (grey) brain lesion. The predicted individual trajectories (dashed lines) and observed individual outcomes (dots) are shown.

were presented for the total SCP group (p < 0.001). Subgroup analysis demonstrated lower muscle volume for both subgroups classified in GMFCS levels I and II (6.22 mL) and GMFCS levels III and IV (5.09 mL), and for children with a presumed early lesion (5.96 mL) (p < 0.001), compared with typically developing children (9.81 mL). Additionally, CSA

TABLE 3 Intercepts (at 12 months) and regression coefficients (with 95% CI) for the trajectory in muscle morphology.

|                                    |                                       |  |  |                                | Age 12 months                                    |  | Growth rates   |                            |               |
|------------------------------------|---------------------------------------|--|--|--------------------------------|--|--|--|----------------------------|---------------|
| Outcome                            |                                       | Covariate  | Covariate  |                                | $\alpha_1$                                       |  | $\beta_1$  |                            | 95% CI        |
| Muscle volume                      | (ml)                                  | Typically  | developing   |                                | 9.81   |  | 0.74*  |                            | 0.63-0.84     |
|                                    |                                       | SCP  | SCP  |                                | 5.78   |  | 0.65*  |                            | 0.49-0.81     |
|                                    |                                       | Typically  | Typically developing vs SCP                                |                                | $\Delta = -41\%, p < 0.001$                      | $\Delta = -129$                                      |  | %, <i>p</i> = 0.37         |               |
| Normalized muscle volume (ml/kg•m) |                                       | Typically  | developing   |                                | 1.29   |  | 0.032*   |                            | 0.026-0.038   |
|                                    |                                       | SCP  |  |                                | 1.05   |  | 0.031*   |                            | 0.021-0.041   |
|                                    |                                       | Typically developing vs SCP                                |  | $\Delta = -18\%$ , $p = 0.004$ | $\Delta = -18\%, p = 0.004 \qquad \Delta = -2\%$ |  | 6, <i>p</i> =0.93  |                            |               |
| CSA (mm <sup>2</sup> )             |                                       | Typically  | developing   |                                | 215.6  |  | 7.72*  |                            | 6.67-8.78     |
|                                    |                                       |  | SCP  |                                | 142.2  | 8.64*  |  |                            | 6.61–10.7     |
|                                    |                                       | Typically  | Typically developing vs SCP                                |                                | $\Delta = -34\%, p < 0.001$                      |  | $\Delta = +12\%, p = 0.42$   |                            | 2             |
| Muscle belly let                   | ngth (mm)                             | Typically  | developing   |                                | 85.9   |  | 1.64*  |                            | 1.36-1.91     |
|                                    |                                       | СР   |  |                                | 78.9   |  | 1.24*  |                            | 0.95-1.52     |
|                                    |                                       | Typically  | developing vs  | SCP                            | $\Delta = -8\%, p = 0.02$                        | $\Delta = -2$  |  | .4%, <i>p</i> =0.05        |               |
| Outcome                            | Covariate                             | $\alpha_1$   | $\beta_1$  | CI                             | Covariate  | $\alpha_1$   |  | $\beta_1$                  | 95% CI        |
| Muscle volume<br>(ml)              | Typically developing                  | 9.81   | 0.74*  | 0.63-0.84                      | Typically developing                             | 9.81   |  | 0.74*                      | 0.63-0.84     |
|                                    | SCP I–II                              | 6.22   | 0.83*  | 0.71-0.95                      | SCP early  | 5.96   |  | 0.64*                      | 0.45 - 0.84   |
|                                    | SCP III–IV                            | 5.09   | 0.29*  | 0.18-0.40                      | SCP late   | 5.84   |  | 0.70*                      | 0.49-0.90     |
|                                    | Typically developing vs<br>SCP I–II   | $\Delta = -37\%$<br><i>p</i> < 0.001                       | $\begin{array}{l} \Delta = +12\% \\ p = 0.24 \end{array}$  |                                | Typically developing vs<br>SCP early             | $\begin{array}{l} \Delta = - \\ p < 0. \end{array}$  | $\begin{array}{c} -40\% \\ 0.001 \end{array} \qquad \begin{array}{c} \Delta = -1 \\ p = 0.4 \end{array}$     |                            | 2%<br>I       |
|                                    | Typically developing vs<br>SCP III–IV | $\begin{array}{c} \Delta = -48\% \\ p < 0.001 \end{array}$ | $\Delta = -60\%$<br>$p < 0.001$                            |                                | Typically developing vs<br>SCP late              | $\Delta = -p = 0.$                                   | $\Delta = -69$<br>$0.0139$ $\Delta = -69$<br>p = 0.72  |                            | %<br>2        |
|                                    | SCP I–II vs III–IV                    | $\Delta = -22\%$<br>p = 0.13                               | $\begin{array}{l} \Delta = -65\% \\ p < 0.001 \end{array}$ |                                | SCP early vs late                                | $\Delta = -2$ $p = 0.$                               | 2%<br>94   | $\Delta = +89$ $p = 0.71$  | <b>%</b><br>L |
| CSA (mm <sup>2</sup> )             | Typically developing                  | 216.7  | 7.52*  | 6.42-8.61                      | Typically developing                             | 215.8  | 215.8  |                            | 6.63-8.78     |
|                                    | SCP I–II                              | 145.8  | 10.9*  | 9.32-12.5                      | SCP early  | 141.6  | 1  | 9.14*                      | 6.55-11.7     |
|                                    | SCP III–IV                            | 136.4  | 3.30*  | 2.34-4.37                      | SCP late   | 151.3  | 51.3 7   |                            | 4.95-9.98     |
|                                    | Typically developing vs<br>SCP I–II   | $\Delta = -33\%$<br>$p < 0.001$                            | $\begin{array}{l} \Delta = +45\% \\ p < 0.001 \end{array}$ |                                | Typically developing vs<br>SCP early             | $\begin{array}{l} \Delta = -2 \\ p < 0. \end{array}$ | $\begin{array}{c} -34\% \\ 0.001 \end{array} \qquad \begin{array}{c} \Delta = 18\% \\ p = 0.3\% \end{array}$ |                            | 6<br>)97      |
|                                    | Typically developing vs<br>SCP III–IV | $\begin{array}{l} \Delta = -37\% \\ p < 0.001 \end{array}$ | $\begin{array}{l} \Delta = -56\% \\ p < 0.001 \end{array}$ |                                | Typically developing vs<br>SCP late              | $\Delta = -2$ $p = 0.$                               | 30%<br>007   | $\Delta = -3\%$ $p = 0.86$ | %<br>5        |
|                                    | SCP I–II vs III–IV                    | $\Delta = -7\%$<br>p = 0.59                                | $\begin{array}{l} \Delta = -70\% \\ p < 0.001 \end{array}$ |                                | SCP early vs late                                | $\Delta = + p = 0.$                                  | 6%<br>68   | $\Delta = -18$ $p = 0.36$  | 5%<br>5       |
| Muscle belly                       | Typically developing                  | 85.9   | 1.64*  | 1.37-1.91                      | Typically developing                             | 86.0   |  | 1.62*                      | 1.32-1.93     |
| length<br>(mm)                     | SCP I–II                              | 85.8   | 1.09*  | 0.71-1.47                      | SCP early  | 78.9   |  | 1.13*                      | 0.81-1.45     |
| ()                                 | SCP III–IV                            | 69.2   | 1.34*  | 0.88-1.79                      | SCP late   | 80.3   |  | 1.59*                      | 1.12-2.06     |
|                                    | Typically developing vs<br>SCP I–II   | $\begin{array}{l} \Delta = 0\% \\ p = 0.97 \end{array}$    | $\Delta = -34\%$<br>p = 0.02                               |                                | Typically developing vs<br>SCP early             | $\Delta = -p = 0.$                                   | 8%<br>03   | $\Delta = -30$ $p = 0.03$  | )%<br>3       |
|                                    | Typically developing vs<br>SCP III–IV | $\begin{array}{l} \Delta = -19\% \\ p < 0.001 \end{array}$ | $\begin{array}{l} \Delta = -18\%\\ p = 0.26 \end{array}$   |                                | Typically developing vs<br>SCP late              | $\Delta = -2$ $p = 0.$                               | 7%<br>20   | $\Delta = -2\%$ $p = 0.91$ | 6             |
|                                    | SCP I–II vs III–IV                    | $\Delta = -24\%$<br>p < 0.001                              | $\Delta = +23\%$<br>p=0.40                                 |                                | SCP early vs late                                | $\Delta = +2$ $p = 0.2$                              | 2%<br>77   | $\Delta = +41$ $p = 0.11$  | 1%            |

Estimates of fixed effects in the linear mixed model are presented in each group as well as for the comparison between typically developing children and those with SCP (subgroups). The significance of regression coefficients ( $\beta_1$ ) is indicated with an asterisk, and significant differences ( $\Delta$ ) in intercepts ( $\alpha_1$ ) and regression coefficients ( $\beta_1$ ) between the groups with field colours: green, typically developing vs total SCP group; light yellow, typically developing vs GMFCS levels I–II or early lesion; dark yellow, typically developing vs GMFCS levels III–IV or late lesion; pink, between GMFCS or brain lesion groups (p < 0.01). Typically developing, n = 20 with 69 observations; SCP total, n = 24 with 69 observations; SCP GMFCS levels I–II, n = 15 with 44 observations; SCP GMFCS levels III–IV, n = 9 with 25 observations; SCP early lesion, n = 17 with 50 observations; SCP late lesion, n = 5 with 17 observations.

Abbreviations: CI, confidence interval; CSA, cross-sectional area; GMFCS, Gross Motor Function Classification System; SCP, spastic cerebral palsy.

values were lower for the group of children classified in GMFCS levels I and II (145.8mm<sup>2</sup>, p < 0.001), and in GMFCS levels III and IV (136.4mm<sup>2</sup>), than typically developing

children (216.7mm<sup>2</sup>) and for the subgroup with a presumed early (141.6mm<sup>2</sup>) brain-lesion timing compared with the typically developing group (215.8mm<sup>2</sup>, p<0.001). Lastly, at

the age of 12 months, significantly lower muscle belly lengths were found for those classified in GMFCS levels III and IV (69.2 mm) compared with typically developing children (85.9 mm) (p < 0.001) and with those classified in GMFCS levels I and II (85.8 mm) (p < 0.001).

#### Comparison of muscle growth rates

Although the longitudinal growth rates of the total SCP group did not differ from the data for the typically developing group, those classified in GMFCS levels III and IV showed significantly lower muscle volume and CSA growth rates than typically developing children (-60% and -56% respectively, p < 0.001) and those in GMFCS levels I and II (-65% and -70% respectively, p < 0.001). The CSA growth rate for those classified in GMFCS levels I and II was significantly higher than for typically developing children (+45%, p < 0.001). Compared with the typically developing group, the normalized muscle volume muscle growth rate did not differ in children with SCP.

#### DISCUSSION

This longitudinal study showed linear medial gastrocnemius morphological muscle growth in 6- to 36-month-old children with and without SCP. At 12 months of age, children with SCP presented significantly lower muscle volume, normalized muscle volume, and CSA than typically developing peers, confirming an onset of deficits in muscle morphology during infancy. More muscle deficits for children classified in GMFCS levels III and IV and presumed early brain-lesion timing were found at this infancy onset. Using repeated assessments over a 2-year follow-up, the muscle volume and CSA growth rates were similar between the total SCP and typically developing groups and between subgroups for brain-lesion timing. However, the GMFCS level III and IV subgroup showed lower muscle volume and CSA rates than both the typically developing group and the subgroup of those classified in GMFCS levels I and II.

The onset of altered muscle growth was investigated with the intercept estimated at 12 months of age, confirming an onset of deficits in muscle morphology no later than this age. However, we obtained only a few assessments before the age of 12 months, and, for now, we were unable to confidently set the onset at an earlier age and test whether the muscle deficits occur before 12 months of age. Compared with those typically developing, lower muscle volume and CSA at the age of 12 months were found for both GMFCS subgroups, with larger deficits for higher GMFCS levels (hypothesis 3A). Children with a presumed early brain-lesion timing showed lower muscle sizes (muscle volume and CSA) compared with 12-month-old typically developing infants, partly confirming hypothesis 3B. Interestingly, the current muscle volume deficits of 3.6 up to 4.7 mL at the age of 12 months are remarkably larger than the minimal detectable change of 0.5 mL.<sup>23</sup>

The observed similar growth rates for medial gastrocnemius muscle in children with and without SCP did not confirm our second hypothesis. The previously reported muscle volume growth rates, ranging from 0.28 to 0.32 mL/month, were lower than the current SCP muscle growth rates.<sup>4,5</sup> The discrepancies might be attributed to different methodological approaches. Both previous studies were cross-sectional, while using longitudinal data sets takes the variability of (a) typical muscle growth trajectories into account, i.e. modelling random intercept and slope.<sup>12</sup> Next, older children (i.e. age 69 months) and children with a history of botulinum neurotoxin A treatment were included in previous studies. Notably, the initiation of botulinum neurotoxin A is already acknowledged to hamper growth of the injected muscle in children around 2 to 5 years of age.<sup>24</sup> The current findings suggest a linear increase of muscle size with increasing age for infants and toddlers. However, additional longitudinal assessments of the youngest and oldest ages are required to confirm the linearity of the trajectory in longitudinal muscle growth and to establish growth charts.

Remarkably, those classified in GMFCS levels I and II, compared with typically developing children, demonstrated lower muscle volume and CSA but similar muscle belly lengths at the age of 12 months. After this age of infancy, the muscle belly length growth rate was lower whereas CSA growth rate was higher than in typically developing children. These findings indicate an early progression of reduced muscle lengths for children in lower GMFCS levels. Moreover, they highlight muscle volume growth may be primarily attributed to CSA growth, which probably originates from an increase in the CSA of parallel organized muscle fibres. Interestingly, the subgroup in GMFCS levels I and II included more children with unilateral involvement (Appendix S12). Those children might have experienced a similar dosage of mechanical loading than typically developing children, explaining their similar muscle size growth rates.<sup>25</sup> However, children with unilateral SCP more often present with reduced range of motion in the ankle joint than children with bilateral involvement, which might be related to the reduced muscle belly length growth observed in the GMFCS levels I and II subgroup.<sup>26</sup> In combination with potential increased levels of spasticity up to the age 5 years, a close follow-up of these muscle lengths as well as treatment approaches aiming to preserve the muscle length (e.g. use of ankle-foot orthoses) is advised, especially for those in GMFCS levels I and II.<sup>27,28</sup>

Children classified in GMFCS levels III and IV demonstrated lower muscle volume and CSA growth rates than typically developing children and those in GMFCS levels I and II. The complete muscle trajectory (i.e. onset and growth rates) indicates that children classified in GMFCS levels I and II have an aberrant start in muscle morphology but with subsequent similar muscle growth rates as typically developing children, while children classified in GMFCS levels III and IV continue with reduced muscle growth after an aberrant start. The observed differences between GMFCSlevel subgroups highlight the potential contribution of functional limitations in the muscle growth trajectories. These differences might further result in altered mechanical loading (e.g. fewer weight-bearing activities) and reduced levels of muscle activation, influencing the process of protein synthesis and thus muscle growth.<sup>3</sup>

Lower muscle volume and CSA at the age of 12 months was found only for children with a presumed early lesion compared with the typically developing group. However, the muscle alterations at 12 months and the subsequent growth trajectories did not differ between children with presumed early and late brain lesions, for which hypothesis 3B was not confirmed. Consequently, the specific timing of an interruption in the neuro- and myogenesis process did not clearly induce a different muscle growth trajectory. In addition to the original occurrence of the brain lesion, the lesion extent and location might be important in the presence of the muscle pathology. Further, perinatal and postnatal brain plasticity might emerge for which the postnatal structural or functional changes are influenced by activitybased experiences (e.g. crawling, walking). Therefore, the postnatal refinement of the corticospinal terminations, which are responsible for muscle activation, might induce muscle growth and highlights an emerging feedback loop.<sup>9</sup> These neural adaptations might thus compensate or partly rescue the disturbances in myogenesis as consequence of the initial brain lesion.

However, in the current study, only broad categories based on the predominant pathogenic patterns were included to provide homogenous categorization despite the differences between the ages at the time of the brain MRI. Nevertheless, MRI classification limited the representation of continuous brain changes to only a binary distribution, i.e. presumed early and late occurrence of the brain lesion. Additionally, an unequal distribution for the presumed brain lesion timing was presented, with a limited number of children (6 out of 23) with a presumed late timing. The current results should be interpreted with caution due to the lack of sufficient power. Future research should include larger sample sizes and assess the brain lesion location and extent, and motor cortex-corticospinal tract connections, to fine tune the contribution of the neural input to altered muscle growth.<sup>29</sup> Nevertheless, early motor interventions are urgently needed to target and stimulate muscle growth.<sup>30</sup> The potential impact of different biomechanical loading might be roughly estimated through GMFCS levels. However, measures of gross motor capacity, actual mobility performance, hyperresistance, and muscle weakness should be incorporated, to comprehensively delineate the contribution of biomechanical factors to muscle profiles in SCP. Previous studies indicated that SCP muscle seems to fail in growing at the same rate as skeletal growth.<sup>4</sup> The current modelled trajectories in muscle growth were explored for ratio-scaling of muscle volume to body dimensions and suggested inappropriate exclusion of anthropometric growth in the typically developing children. Further validation of normalization techniques

for muscle sizes and lengths in infancy studies is needed.<sup>11</sup> Furthermore, nutrition, growth factors, and the metabolic capacity have been suggested as important contributors to muscle growth, and should also be investigated.<sup>3</sup> In sum, the origin of altered muscle growth should be considered as multifactorial. Notably, the current study did not consider the factor of preterm birth, for which a corrected age can be applied. The effect of gestation on muscle size was only provisionally described in Appendix S10.

The current study was conducted during the COVID-19 pandemic. This limited the access to paediatric departments, challenging the recruitment of high-risk infants. Future studies should start measuring muscle growth in the first months of life to investigate the direct, initial impact of the neural alterations and the effect of preterm birth by taking corrected age into account. Lastly, threedimensional freehand ultrasound assessments in infants were feasible but movements during data acquisition and changing ankle positions between follow-up assessments might have influenced measurements of muscle lengths. However, reliability data for muscle belly lengths in infants are lacking.

### **CONCLUSION**

This study described the longitudinal trajectory of early medial gastrocnemius morphological muscle growth in children with and without SCP. An early onset of lower muscle size is confirmed and a role for altered neural input as well as levels of motor functioning contributing to altered muscle growth in children with SCP is indicated. We demonstrated that muscle deficits were more pronounced in children with more functional limitations (i.e. in children classified with higher GMFCS levels) and were suggested after a presumed early brain-lesion timing. Hence, muscle growth should be closely monitored from early ages and targeted during early intervention.

#### ACKNOWLEDGEMENTS

We thank the Day Care Centers, the Cerebral Palsy Reference Center, the Neonatology Unit, and the Center for Developmental Disabilities of the University Hospitals Leuven for their support in recruiting the participants. Open access funding provided by Universite de Geneve.

#### FUNDING INFORMATION

Internal KU Leuven grant, Belgium, 3D-MMAP-C24/18/103 for NDB. Internal funding of KU Leuven Biomedical Sciences Group: Fund for Translational Biomedical Research, Belgium, 2019 and the Research Foundation Flanders (FWO), Belgium, G0B4619N. IV was funded through a research fellowship from FWO (1188921 N).

#### CONFLICT OF INTEREST STATEMENT

The authors have stated that they had no interests that might be perceived as posing a conflict or bias.

## DATA AVAILABILITY STATEMENT

Data are available from the corresponding author upon reasonable request.

#### ORCID

Nathalie De Beukelaer <sup>®</sup> https://orcid. org/0000-0002-7800-3354 Ines Vandekerckhove <sup>®</sup> https://orcid. org/0000-0002-6023-3004 Anja Van Campenhout <sup>®</sup> https://orcid. org/0000-0002-8158-5535

#### REFERENCES

- Graham HK, Rosenbaum P, Paneth N, Dan B, Lin J-P, Damiano DL, et al. Cerebral palsy. Nat Rev Dis Prim [Internet]. 2016 Dec 22;2(1):15082. Available from: http://www.nature.com/articles/nrdp2 01582
- 2. Krägeloh-Mann I, Horber V. The role of magnetic resonance imaging in elucidating the pathogenesis of cerebral palsy: A systematic review. Dev Med Child Neurol. 2007;49(2):144–51.
- 3. Gough M, Shortland AP. Early muscle development in children with cerebral palsy. the consequences for further muscle growth, muscle function, and long-term mobility [Internet]. Cerebral Palsy in Infancy: Targeted Activity to Optimize Early Growth and Development. Roberta Shepherd; 2013. 157–73 p. Available from: https://doi.org/10.1016/B978-0-7020-5099-2.00007-8
- Herskind A, Ritterband-Rosenbaum A, Willerslev-Olsen M, Lorentzen J, Hanson L, Lichtwark G, et al. Muscle growth is reduced in 15-month-old children with cerebral palsy. Dev Med Child Neurol [Internet]. 2016 May [cited 2018 Jun 13];58(5):485–91. Available from: http://doi.wiley.com/10.1111/dmcn.12950.
- Willerslev-Olsen M, Choe Lund M, Lorentzen J, Barber L, Kofoed-Hansen M, Nielsen JB. 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(7):672–9.
- Palisano R, Rosenbaum P, Walter S, Russell D, Wood E, Galuppi B. Development and reliability of a system to classify gross motor function in children with cerebral palsy. Dev Med Child Neurol. 1997;39(4):214-23.
- Hadders-Algra M. Neural substrate and clinical significance of general movements: an update. Dev Med Child Neurol. 2018;60(1):39–46.
- Einspieler C, Bos AF, Krieber-Tomantschger M, Alvarado E, Barbosa VM, Bertoncelli N, et al. Cerebral Palsy: Early Markers of Clinical Phenotype and Functional Outcome. J Clin Med [Internet]. 2019 Oct 1 [cited 2022 Jun 30];8(10). Available from: https://pubmed.ncbi.nlm. nih.gov/31590221/
- 9. Clowry GJ. The dependence of spinal cord development on corticospinal input and its significance in understanding and treating spastic cerebral palsy. Vol. 31, Neuroscience and Biobehavioral Reviews. Pergamon; 2007. p. 1114–24.
- Lieber RL, Roberts TJ, Blemker SS, Lee SSM, Herzog W. Skeletal muscle mechanics, energetics and plasticity. J Neuroeng Rehabil [Internet]. 2017 Dec 23 [cited 2018 Jun 13];14(1):108. Available from: http://jneuroengrehab.biomedcentral.com/articles/10.1186/s1298 4-017-0318-y.
- Williams AS, Stott NS, Valentine J, Elliott C, Reid SL. Measuring skeletal muscle morphology and architecture with imaging modalities in children with cerebral palsy: a scoping review. Dev Med Child Neurol. 2020;63(3):263–73.
- 12. Verbeke G, Molenberghs G. Linear Mixed Models for Longitudinal Data. Springer S. New York: Springer-Verlag; 2000.
- Novak I., et al. Early, Accurate Diagnosis and Early Intervention in Cerebral Palsy Advances in Diagnosis and Treatment. 2017;171(9): 897–907.

- Palisano RJ, Rosenbaum P, Bartlett D, Livingston MH. Content validity of the expanded and revised Gross Motor Function Classification System. Dev Med Child Neurol. 2008;50(10):744–50.
- Cenni F, Schless SH, Bar-On L, Aertbeliën E, Bruyninckx H, Hanssen B, et al. Reliability of a clinical 3D freehand ultrasound technique: Analyses on healthy and pathological muscles. Comput Methods Programs Biomed [Internet]. 2018;156:97–103. Available from: https://doi.org/10.1016/j.cmpb.2017.12.023
- Cenni F, Schless SH, Monari D, Bar-On L, Aertbeliën E, Bruyninckx H, et al. An innovative solution to reduce muscle deformation during ultrasonography data collection. J Biomech [Internet]. 2018;77:194–200. Available from: https://doi.org/10.1016/j.jbiom ech.2018.06.002
- Schless SH, Cenni F, Bar-On L, Hanssen B, Kalkman B, O'brien T, et al. Medial gastrocnemius volume and echo-intensity after botulinum neurotoxin A interventions in children with spastic cerebral palsy. Dev Med Child Neurol. 2018;61(7):783–90.
- Treece G, Prager R, Gee A, Berman L. Fast surface and volume estimation from non-parallel cross-sections, for freehand threedimensional ultrasound. Med Image Anal. 1999;3(2):141–73.
- Handsfield GG, Meyer CH, Hart JM, Abel MF, Blemker SS. Relationships of 35 lower limb muscles to height and body mass quantified using MRI. J Biomech. 2014;47(3):631–8.
- Himmelmann K, Horber V, De La Cruz J, Horridge K, Mejaski-Bosnjak V, Hollody K, et al. MRI classification system (MRICS) for children with cerebral palsy: development, reliability, and recommendations. Dev Med Child Neurol [Internet]. 2017 Jan 1 [cited 2018 Aug 20];59(1):57–64. Available from: http://doi.wiley.com/10.1111/ dmcn.13166.
- 21. Verbeke G, Molenberghs G. Linear Mixed Models in Practice: A SAS-Oriented Approach. New York: Springer; 1997.
- 22. Vandekerckhove I, Van den Hauwe M, De Beukelaer N, Stoop E, Goudriaan M, Delporte M, et al. Longitudinal Alterations in Gait Features in Growing Children With Duchenne Muscular Dystrophy. Front Hum Neurosci [Internet]. 2022 Jun 2;16. Available from: https://www.frontiersin.org/articles/10.3389/fnhum.2022.861136/ full.
- 23. Williams SA, Bell M, Kim HK, Salim Al Masruri G, Stott NS, Fernandez J, et al. The reliability and validity of triceps surae muscle volume assessment using freehand three-dimensional ultrasound in typically developing infants. J Anat. 2021;(July):1–12.
- 24. De Beukelaer N, Weide G, Huyghe E, Vandekerckhove I, Hanssen B, Peeters N, et al. Reduced Cross-Sectional Muscle Growth Six Months after Botulinum Toxin Type-A Injection in Children with Spastic Cerebral Palsy. Toxins (Basel). 2022;14(139).
- Theis N. Lower limb muscle growth in unilateral and bilateral cerebral palsy. Dev Med Child Neurol. 2016;58(11):1102–3.
- Nordmark E, Hägglund G, Lauge-Pedersen H, Wagner P, Westbom L. Development of lower limb range of motion from early childhood to adolescence in cerebral palsy: a population-based study. BMC Med [Internet]. 2009 Oct 28 [cited 2022 Jul 11];7. Available from: https:// pubmed.ncbi.nlm.nih.gov/19863779/
- Lindén O, Hägglund G, Rodby-Bousquet E, Wagner P. The development of spasticity with age in 4,162 children with cerebral palsy: a register-based prospective cohort study. Acta Orthop [Internet]. 2019 May 4;90(3):286–91. Available from: https://actaorthop.org/actao/article/view/519
- Morris C, Bowers R, Ross K, Stevens P, Phillips D. Orthotic management of cerebral palsy: recommendations from a consensus conference. NeuroRehabilitation [Internet]. 2011 [cited 2022 Aug 1];28(1):37–46. Available from: https://pubmed.ncbi.nlm.nih.gov/21335676/
- Martin JH. The corticospinal system: From development to motor control. Neuroscientist. 2005;11(2):161–73.
- Morgan C, Fetters L, Adde L, Badawi N, Bancale A, Boyd RN, et al. Early Intervention for Children Aged 0 to 2 Years with or at High Risk of Cerebral Palsy: International Clinical Practice Guideline Based on Systematic Reviews. JAMA Pediatr. 2021;175(8):846–58.

#### SUPPORTING INFORMATION

The following additional material may be found online: Appendix S1: Description of stability and evolutions in GMFCS levels.

**Appendix S2:** Detailed list of exclusion criteria and reasons for drop-out for infants with spastic cerebral palsy.

Appendix S3: Additional specifications and standardization of the data acquisition.

Appendix S4: MRI collection and classification.

Appendix S5: Advantages and specifications of linear mixed models.

Appendix S6: Observed individual profiles for muscle volume, cross-sectional area, and muscle length in typically developing children and children with spastic cerebral palsy. Appendix S7: Observed individual profiles for normalized muscle volume and predicted average trajectory in typically developing children and children with spastic cerebral palsy.

Appendix S8: Observed individual profiles for muscle volume, cross-sectional area, and muscle length in typically developing children and children with spastic cerebral palsy. Appendix S9: Estimates of the random effects variances and residual variances in the linear mixed models.

Appendix S10: Muscle growth trajectory with corrected ages for preterm birth.

Appendix S11: Standard clinical care of all participants with spastic cerebral palsy.

Appendix S12: Observed individual profiles for crosssectional area and muscle length in typically developing children and children with spastic cerebral palsy.

How to cite this article: De Beukelaer N, Vandekerckhove I, Molenberghs G, Naulaers G, Thewissen L, Costamagna D, et al. Longitudinal trajectory of medial gastrocnemius muscle growth in the first years of life. Dev Med Child Neurol. 2024;66:531-540. https://doi.org/10.1111/dmcn.15763

TRANSLATING 2024

78<sup>TH</sup> ANNUAL MEETING

QUEBEC CITY, CANADA | OCTOBER 23-26

ACPDN

# Visit www.aacpdm.org/events/2024 for more information!

