Heterogeneity of muscle sizes in the lower limbs of children with cerebral palsy

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ABSTRACT

Introduction: Cerebral palsy (CP) is associated with reduced muscle volumes, but previous studies have reported deficits in only a small number of muscles. The extent of volume deficits across lower limb muscles is not known. This study presents an imaging-based assessment of muscle volume and length deficits in 35 lower limb muscles.

Methods: We imaged and segmented 35 muscles in 10 subjects with CP and 8 typically developing (TD) controls using MRI. Muscle volumes were normalized, and Z-scores were computed using TD data. Volume Z-scores and percent deficits in volume, length, and cross-sectional area are reported.

Results: Muscle volumes are 20% lower on average for subjects with CP. Volume deficits differ significantly between muscles (12%-43%) and display significant heterogeneity across subjects. Distal muscles, especially the soleus, are commonly and severely small.

Discussion: Heterogeneity across muscles and across subjects reinforces the subject-specificity of CP and the need for individualized treatment planning.

Keywords: MRI, cerebral palsy, muscle, heterogeneity, lower limb

INTRODUCTION

Cerebral palsy (CP) is a neuromusculoskeletal disorder caused by a developmental brain injury occurring *in utero* or around birth[1–4]. Among the pathologies associated with CP are impaired gait[5,6], muscle spasticity[7–9], and skeletal malformation[2,10]. In addition, decreased muscle size has been nearly unanimously identified in CP by different research groups using MRI[11–16] and ultrasound[17–21] in various lower limb muscles. The functional consequences of decreased muscle size are diminished capacity to generate muscle force, diminished range of motion, and/or diminished maximum velocity of muscle contraction[22]. Functional deficits will manifest for muscles that are accordingly decreased in size, but all muscles may or may not be similarly reduced in size for CP subjects.

In addition to variability in muscle size within a CP subject, there may be variability in muscle size across subjects. CP is generally regarded as manifesting heterogeneously across subjects[2,10,23], because it can involve different pathologies stemming from the primary neural lesion[4]. In light of the general heterogeneity of CP, it may be that muscle size profiles are also heterogeneous and differ across subjects. The possibility of inter-subject heterogeneity is interesting and may emphasize the importance of subject-specific muscle assessments in treatment planning. The extent of inter-subject variability of muscle sizes is unknown, since previous assessments of CP muscle size have reported only a small number of muscles and have not focused on variance of muscle profiles across subjects. An assessment of the heterogeneity of muscle size profiles in CP is needed to fully appreciate whether heterogeneity within and across subjects' muscles should be a consideration in diagnosing and treating CP.

Previous research has attempted to better understand the role of strength limitations in CP gait pathologies via strength training interventions and computational approaches. Using both

general and targeted strength training interventions, previous studies have reported strength gains in subjects with CP[24–28], but it is unclear whether and how strength gains translate to functional improvements[27,29–32]. Historically, identification of weak muscles for targeted strengthening has relied on qualitative assessments by physiotherapists[33] or analysis of motion capture data[27], which are subjective and do not provide muscle-specific information. Musculoskeletal models may be used clinically in order to estimate muscle-tendon lengths and velocities, which provide insight into the need for tendon lengthening procedures[34–37]. These modeling approaches have also been applied to understanding the role of specific muscles in gait pathologies[31,38,39] but have been limited by an unavailability of CP-specific muscle size data. Non-invasive imaging approaches offer more objective measures of individual muscles. Ultrasound imaging, for instance, has been used clinically as a guide for botulinum toxin injections[40,41]. A comprehensive imaging assessment of CP lower limb muscles may provide more objective muscle size data that can be used to understand CP muscle strength and can be incorporated into musculoskeletal models to empower more CP-specific muscle force simulations.

In this study, we used MRI to assess muscle volumes and lengths in 35 lower limb muscles in subjects with CP. We report volume and length deficits in CP muscles compared to typically developing (TD) controls, and we compute deficits in cross-sectional areas (CSA). To assess individual muscle size deficits in individual subjects, we compute and use Z-scores of muscle volumes normalized to body size. We use these methods to non-invasively identify abnormally small muscles and identify heterogeneity in muscle profiles across CP subjects.

MATERIALS AND METHODS

Subject Characteristics

Ten children with CP with the following characteristics [mean ± SD (range)]: age: 13.9 ± 1.9 (11-17) years, height: 159.1 ± 12.6 (134.5-175.3) cm, body mass: 59.4 ± 16.7 (36.4-96.1) kg, body mass index: 23.2 ± 4.7 (17.6-31.5) kg/m^2 were recruited from University of Virginia Clinics (see Tables 1a and 1b for individual subject characteristics). The CP population was heterogeneous and included hemiplegic and diplegic subjects ranging from levels I to III on the Gross Motor Function Classification System (GMFCS). Also, many subjects previously had surgical interventions including hamstrings release, Achilles lengthening, hamstrings lengthening, and dorsal rhizotomy. No subject had undergone surgery within 12 months prior to scanning. Subject inclusion criteria included: age between 11 and 17 years, the ability to ambulate, the ability to safely undergo MRI, and the ability to remain motionless in the MRI scanner for the duration of the imaging time. Our study was approved by the University of Virginia Institutional Review Board; informed consent was obtained from all subjects' legal guardians and all subjects provided assent. Eight typically developing adolescent controls from a similar age range were recruited and imaged using the same methods as the subjects with CP. The control subject parameters were: age: 14.0 ± 1.5 (12-17), height: 165.7 ± 10.1 (145.4-178.4) cm, body mass: 64.9 ± 12.1 (47.5-83.5) kg, body mass index: 23.5 ± 3.0 (20.2-29.7) kg/m^2.

Imaging and Segmentation

Subjects were scanned on a 3T Siemens (Munich, Germany) Trio MRI Scanner using a 2D multi-slice gradient-echo pulse sequence with an interleaved spiral k-space trajectory[42]. The scanning parameters used were: TE/TR/α: 3.8 ms/ 800 ms/ 90°; FOV: 400 mm × 400 mm; slice

thickness: 5 mm; in-plane spatial resolution: 1.1 mm × 1.1 mm*;* body receiver coil*;* and 4 signal averages. Spectral-spatial excitation pulses were used for fat suppression[43]. Additionally, a Chebyshev approximation was applied for semi-automatic off-resonance correction to compensate for spatial variations of the magnetic field[44]. Contiguous axial images were obtained from the iliac crest to the ankle joint. Scan time was approximately 20 minutes per subject.

Each of 35 muscles in the paretic limb for hemiplegic subjects and the most affected limbs for diplegic subjects were segmented using in-house segmentation and image processing software written in Matlab (The Mathworks Inc., Natick, MA, USA). For diplegic subjects, the most affected limb was identified by clinicians as the limb that experienced shorter duration of single-limb stance and reduced excursions over the gait cycle. Segmentations were performed by a team of 4 trained individuals, each provided with a detailed slice-by-slice segmentation atlas created from 1 of our data sets. Observers identified boundaries of individual muscles in axial slices. A single highly trained observer evaluated and refined all segmentations before further analysis to ensure consistency across users. The volume of each muscle was calculated by summing all of the slice-wise voxel volumes for each muscle; no corrections were made for fatty infiltration or connective tissue inside CP muscle volumes. Total lower limb muscle volume was computed as the sum of all 35 muscles segmented for each subject. Muscle belly lengths were defined as the linear distance from the most superior to the most inferior extent of the muscle, similar to previously published definitions[45,46]. Four muscles in this study displayed *in vivo* curvature such that their linear lengths were not consistent with line-of-action muscle lengths measured in previous anatomical studies[46]. For these muscles (rectus femoris, sartorius, psoas, and semimembranosus) muscle belly lengths were defined as the distance along the muscle's centroid path[47,48].

To examine consistency of segmentation, we assessed inter- and intra-observer variability by assigning the same image data to 2 different observers for segmentation. One observer was later assigned the same image data again. All 3 segmentations were vetted by the highly-trained observer, each on different days. Inter- and intra-observer variability was assessed by computing the percent error in volumes and lengths between the 2 observers and between the 2 trials of the same observer, respectively.

Normalization and Analysis of Muscle Volumes

Subjects with CP are often smaller in body mass and height than their typically developing counterparts[49–63]. It was previously shown that lower limb muscle volumes of healthy subjects scale with the product of height and mass[48]. To reduce the effects of body size on muscle size differences when comparing typically developing and CP muscle volumes, we normalized muscle volumes by the product of height and mass according to equation 1:

$$volume_{norm} = \frac{muscle\ volume}{height \cdot mass} \quad (1)$$

where $volume_{norm}$ denotes the normalized muscle volume of a given muscle and a given subject (i.e. we calculated 35 $volume_{norm}$ for each subject), *muscle volume* is the volume of each muscle obtained from image post-processing in units of cm^3, *height* is subject height in *m*, and *mass* is body mass in *kg*.

To assess how typical or atypical an individual muscle is in subjects with CP, we used Z-scores. A Z-score is the number of standard deviations an individual measurement is from the population mean of that measurement. For normally distributed data, the 95% confidence interval of a measurement resides between Z = -1.96 and Z = +1.96. In this way, Z-scores can be

used to compare individual measurements against confidence intervals of a reference population. For subjects with CP, we computed Z-scores to compare the volume of each muscle against the volumes observed in our typically developing control subjects. Volume Z-scores were computed according to Equation 2:

$$Z_{volume} = \frac{volume_{norm}{}^{CP} - \mu(volume_{norm}{}^{TD})}{\sigma(volume_{norm}{}^{TD})} \quad (2)$$

where Z_{volume} is the Z-score of a normalized muscle volume (i.e. we computed 35 Z_{volume}s for each subject), $volume_{norm}{}^{CP}$ is the normalized muscle volume for a CP subject's muscle (see Equation 1), $\mu(volume_{norm}{}^{TD})$is the population mean of normalized muscle volume in the typically developing control group, and $\sigma(volume_{norm}{}^{TD})$ is the standard deviation of normalized muscle volume in the typically developing control group.

In this case, a volume Z-score is a measure of how many TD standard deviations (σ) a CP muscle is away from the TD mean. We consider Z-scores greater than 2 or less than -2 to be significantly atypical. Ranking of the control subject data revealed that these limits correspond to the 99% confidence interval of the typically developing muscle volume Z-scores. We used Z-scores to determine muscles that were *commonly* significantly small and *severely* significantly small within this population. *Commonly* small muscles were defined as muscles for which 80% or more of subjects presented volume Z-scores that were less than -2. *Severely* small muscles were defined as muscles with a population average volume Z-score that was less than -2.

Normalization and Analysis of Lengths and Cross-Sectional Areas

Geometrically, observed differences in muscle volume may be due to differences in length or in cross-sectional area (CSA); therefore we also examined differences in these 2

parameters. To reduce the effects of body size on muscle length comparisons, we normalized muscle belly lengths by limb length:

$$length_{norm} = \frac{muscle\ length}{limb\ length} \quad (3)$$

where $length_{norm}$ denotes the normalized muscle belly length of a given muscle, *muscle length* is the muscle belly length acquired from images, and *limb length* is the sum of the linear lengths of the femur and tibia.

Muscle cross-sectional areas were computed by dividing muscle volume by muscle length:

$$CSA = \frac{muscle\ volume}{muscle\ length} \quad (4)$$

where *CSA* is the average area of the muscle perpendicular to the direction of the muscle's length. To reduce the effects of body size on muscle CSA, this parameter was normalized by body mass:

$$CSA_{norm} = \frac{CSA}{mass} \quad (5)$$

Statistical Methods

Correlations between total muscle volume and the height-mass product were compared between CP and TD groups using ANCOVA. Values of Z-scores were used to indicate significant differences for individual muscles in individual subjects. Z-scores outside of ±2 were considered to be significantly atypical (see Normalization and Analysis of Muscle Volumes). Group-wise comparisons of normalized muscle sizes—$volume_{norm}$, $length_{norm}$, and CSA_{norm}—warranted a non-parametric test and we used the Wilcoxon rank-sum test. We used Levene's

166 Test to compare variances of measurements between the CP and TD populations and between
167 muscles within the CP population. An alpha value of 0.05 was used for statistical tests and
168 analysis was conducted in Matlab R2013b.
169

RESULTS

Segmentation Variability

Across all muscles in the lower limb, the mean inter-observer variability was 4.7% for volume and 3.2% for length, and the mean intra-observer variability was 4.4% for volume and 1.8% for length. Eight muscles displayed volume variability greater than 10% for either inter- or intra-observer variability: the vastus intermedius (10.5%), flexor digitorum longus (12.1%), piriformis (14.3%), gluteus minimus (19.2%), gemelli (22.2%), pectineus (22.5%), quadratus femorus (22.7%), and obturator internus (26.9%). Three muscles displayed length variability greater than 10%: the gracilis (10.6%), obturator externus (13.7%), and piriformis (38.6%). Variability was higher in small muscles, in muscles with ill-defined or hard to identify boundaries (e.g. vastus intermedius), and in muscles with a medial-lateral orientation. Inter-observer variability was higher than intra-observer variability for all measurements except for vastus intermedius volume and gracilis length.

Total Muscle Volume Deficits in CP

The total muscle volume per height-mass was significantly reduced for CP subjects compared to their TD counterparts. For both TD and CP subjects, lower limb muscle volume scaled well with the product of height and mass, but the slope of this curve was significantly reduced for subjects with CP compared to TD adolescents ($P<0.0001$) (Fig. 1A). There was not a significant difference in the slope of this curve between TD adolescents and the TD adults from Handsfield et al.[48] ($P=0.72$). Normalized muscle volume for CP subjects was 46.2 ± 6.9 $cm^3/kg\text{-}m$ (mean ± SD) compared to 58.5 ± 4.5 $cm^3/kg\text{-}m$ for TD adolescents (Fig. 1B). The difference in variance of normalized muscle volume was not significantly different between CP and TD populations

193 (P=0.11). Although we lacked the statistical power to rigorously compare muscle volume per
194 height mass across GMFCS levels, there was an observable stratification of GMFCS level with
195 normalized muscle volume. Subjects with GMFCS level of I were within the 95% confidence
196 interval for normalized muscle volume of typically developing subjects. All other subjects fell
197 outside of this interval. The 1 subject with a GMFCS level of III had the lowest normalized
198 muscle volume of the CP population (Fig. 1B).
199
200 Individual Muscle Volume Deficits in CP
201 Volume Z-scores of CP muscles revealed significant volume deficits in lower limb muscles. Of
202 the 350 muscles assessed in CP subjects, 117 muscles (33%) were outside of the 2σ confidence
203 interval, and 78 (22%) were more than 2.5σ smaller than the TD mean. Twenty-three muscles in
204 the CP cohort (6.5%) were more than 4σ smaller than the TD mean, indicating a severe degree of
205 muscle volume impairment. By definition of volume Z-score, only 0.5% of the TD volume Z-
206 scores would be expected to be less than -2. In fact, 1 muscle out of 280 (0.4%) TD muscles was
207 below this threshold. Five muscles were found to be *commonly* small in the CP population:
208 soleus and tibialis anterior were significantly small in 9 subjects, while medial gastrocnemius,
209 digital extensors (EDL & EHL), and semimembranosus were each significantly small in 8
210 subjects (Fig. 2B). Eight muscles were *severely* significantly small: soleus (average Z = -4.0),
211 digital extensors (-3.4), medial gastrocnemius (-3.0), tibialis anterior (-2.9), semimembranosus (-
212 2.6), rectus femoris (-2.1), vastus medialis (-2.1), and semitendinosus (-2.1) (Table 2).
213
214 Heterogeneity of Muscle Deficits in CP

Visualization of Z-scores color mapped onto subject-specific lower limb reconstructions (Fig. 3) illustrates the heterogeneity of muscle volume deficits. Heterogeneity of muscles was found to be statistically significant ($P<0.002$) as determined by unequal variance of Z-scores across muscles within the CP population. The most heterogeneous muscles in this study were the gluteus minimus, quadratus femoris, iliacus, semitendinosus, and vastus medialis (Table 2). Subjects who had undergone semitendinosus lengthening or release had significantly smaller normalized semitendinosus volumes than those who had undergone no semitendinosus surgery (0.3 vs. 2.2 cm^3/kg-m, $P<0.05$). Subjects who had undergone Achilles lengthening had significantly smaller normalized soleus volumes (2.0 vs. 2.6 cm^3/kg-m, $P<0.01$) than those who had not undergone the surgery. There were no significant differences in normalized volumes of either gastrocnemius muscle between Achilles surgery groups ($P>0.5$).

Group-wise Comparison of Muscle Volumes

Comparison of normalized muscle volumes across functional muscle groups reveals volume deficits among the CP group. The hip abductors and external rotators were the only 2 functional muscle groups out of 9 that were not small in the CP group compared to the TD group (Fig. 4). The muscle groups with the largest differences were the dorsiflexors (37%), plantarflexors (34%), and hamstrings (29%). Muscles with the largest percent differences were the tibialis anterior (43%), medial gastrocnemius (42%), soleus (39%), lateral gastrocnemius (36%), semitendinosus (35%), and semimembranosus (31%). The ratios of muscle volume for agonist-antagonist pairs for the CP group were similar to the TD sample except for the abductor-adductor ratio, which was markedly larger for the CP group.

238 CSA and Length Deficits in CP

239 Muscles displaying volume deficits present deficits in both CSA and length (Table 3).
240 Statistically significant volume deficits were more often accompanied by significant deficits in
241 CSA than by significant deficits in length (Table 3). Significant CSA deficits were larger on
242 average than significant length deficits. Six muscles displayed significant deficits in volume,
243 CSA, and length. They were the medial gastrocnemius, soleus, lateral gastrocnemius,
244 semitendinosus, digital extensors, and fibularis muscles. Only 1 muscle, the gracilis, displayed
245 significant length deficits that were also greater than CSA deficits.

246

DISCUSSION

The results of the present study show that muscles in subjects with CP are small overall, the magnitude of muscle volume deficits varies across individuals and across muscles, and small muscle volumes are most commonly associated with small cross-sectional areas (CSAs) but in some cases are due to short muscle belly lengths. These results highlight the array of muscle size deficits in the lower limb and indicate a general heterogeneity of muscle deficits across and within CP. Heterogeneity notwithstanding, the most commonly and severely affected muscles among subjects with CP were the soleus, digital extensors, gastrocnemius, tibialis anterior, and semimembranosus—all plantarflexors, dorsiflexors, and one hamstrings muscle.

Inter- and intra-observer analysis of segmentation revealed an average volume discrepancy of approximately 5% and an average length discrepancy of around 3%, which is similar to other published variability assessments[47,82]. High volume variability was associated with muscles that have ill-defined or hard to identify boundaries. Small muscles are also more sensitive to errors since we had a fixed spatial resolution. Muscles with a medial-lateral orientation were difficult to segment since we conducted axial imaging. Variability in length resulted from variations in identifying the most proximal and distal slices of a muscle. With the exception of vastus intermedius volume and gracilis length, inter-observer variability was higher than intra-observer variability, indicating that systematically defining muscle boundaries, origins, and insertions across researchers may improve the precision of segmentation.

Several previous studies have reported muscle volumes in individuals with CP[11,14,15] for several muscle groups and muscles. Our study builds upon this work by reporting volume, length, and CSA deficits in 35 lower limb muscles, including 25 muscles for which volumes in CP were previously unreported. The findings from previous MRI-based measurements of muscle

volumes in CP demonstrated diminished muscle volumes overall. Our results are consistent with these studies, and our group means are especially consistent with those of Noble et al.[15] who had a larger population and studied 9 muscles. Nevertheless, previous studies are not all consistent with regards to which muscles are significantly small. For example, while Oberhofer et al.[14] found substantially diminished volumes in the hamstrings and vasti but not in the plantarflexors, Noble et al.[15] found substantially diminished volumes in the hamstrings and plantarflexors but not in the vasti. We found diminished volumes in the hamstrings, vasti, and plantarflexors. Because sample sizes were relatively small and varied in these studies, disparate results are not entirely surprising. Differences between studies may be related to heterogeneity of muscle volume deficits in CP subjects. The lack of significant soleus volume deficits reported by Oberhofer et al.[14], for example, is interesting considering the magnitude and frequency of soleus deficits we observed. This could be explained by the fact that Oberhofer et al. found a relatively high variance in normalized soleus volume among CP subjects[14]. It may be that heterogeneity within their population influenced the lack of significance in soleus deficit.

In this study, we accounted for the disparate body sizes across subjects by normalizing muscle volumes by the product of height and mass, which is somewhat distinct from previous studies. We previously showed very good scaling of TD muscle volumes with the height-mass product[48]. Previous research into body size scaling of humans has suggested that a parameter of both stature (i.e. height) and size (i.e. mass) are needed to normalize muscle volume, since humans vary widely in size and shape[64,65]. This may be especially true of subjects with CP who are known to be shorter and smaller than their TD counterparts[49–63]. In our study, the height-mass product was a good predictor of muscle volumes in subjects with CP, but the slope of the CP curve was significantly lower than the slope of the TD curve. This indicates reduced muscle

volume for a given body stature and size in CP subjects. To indicate whether normalization technique could contribute to differences between studies, we re-examined muscle volume deficits when volumes were normalized by body mass. Normalization by mass increased the percent deficits of CP muscle volumes for all muscles in the lower limb by an average of 3.5% (Table 4). This difference is small and indicates that normalization by mass results in larger deficits between CP and TD groups. Since muscle volume is shown to scale with height-mass, and CP subjects are shorter than their TD counterparts[56–63], we argue that height-mass may be a more sensitive and appropriate metric for normalizing muscle volumes. Additionally, the use of height-mass to normalize muscle volume is dimensionally consistent with normalizing muscle length by height or limb length and normalizing muscle cross-sectional area by mass, which we have done in this study.

Muscle volume deficits may be due either to CSA or muscle belly length. We found that CSA deficits were larger and more common than length deficits. While muscle shortness was less common in our data, certain subjects displayed substantial deficits in length for muscles such as the gracilis and semitendinosus. This fact should reinforce the inter-subject variability of CP and the need to approach muscle deficiencies with a subject-by-subject perspective. Functionally, physiological cross-sectional area (PCSA) and muscle fiber length are more meaningful parameters than CSA and muscle belly length, as they relate to muscle force capacity[22,66] and range of motion[22], respectively. Holzbaur et al.[47] previously multiplied CSAs by mean values of muscle-length-to-optimal-fiber-length ratios (L_m/L_f^0) from the literature to estimate PCSAs. Similar computation of PCSA for CP subjects would have been questionable, since we did not measure optimal fiber lengths and thus could not account for CP-specific L_m/L_f^0. To our knowledge, there are no reports of CP-specific L_m/L_f^0 for most muscles, although

316 it is known that pennation angles and sarcomere operating ranges may differ from TD
317 subjects[19,67–70]. Future *in vivo* determination of L_m/L_f^0 in CP subjects may shed light on this issue
318 and may be enabled with recent techniques for imaging fibers and sarcomeres *in vivo*[71–74].

319 We found significant heterogeneity in individual normalized muscle volumes among our
320 10 subjects. Our population was heterogeneous in GMFCS level, surgical history, and
321 manifestation of CP, but we did not recruit the full range of possible manifestations of CP. Our
322 inclusion criteria excluded subjects with GMFCS levels of IV or V, since the imaging portion of
323 the study required subjects who could remain motionless for 20 minutes in the scanner.
324 Considering the broad range of severities and manifestations of CP, we believe that muscle size
325 heterogeneity is present in the general CP community. Conversely, it may be that subject
326 populations with CP presentations that are more homogeneous than our population may have less
327 inter-subject heterogeneity of muscle volumes than we found. This would be an interesting
328 finding and would suggest that certain clinical diagnoses are associated with specific muscle
329 volume profiles.

330 We found a statistically significant reduction in normalized muscle volume in subjects
331 who had undergone muscle/tendon surgeries compared to those who had not. While this is an
332 interesting result, we cannot conclude that such surgeries contribute to muscle volume deficits,
333 since this was not a longitudinal study. It may simply be that the subjects with the smallest
334 muscles were candidates for surgery on those muscle-tendon units. Future longitudinal studies of
335 muscle volumes before and after surgery will better indicate how surgery affects muscle
336 volumes.

337 There are several limitations to this study that should be considered. First, we did not
338 distinguish intramuscular fatty infiltration[75] or fibrotic tissue[9] from healthy muscle in the

computation of muscle volume from images. Thus, the reported CP volume measurements likely include non-contractile tissue and over-predict the true volume of functional muscle tissue. To precisely determine the volume of functional contractile muscle tissue, future studies would need to subtract image voxels that correspond to fatty or fibrotic intramuscular tissue[76–78]. Our study population was overwhelmingly male. While there may be gender differences in normalized muscle volumes, our previous studies demonstrated similarity of muscle volume per height-mass in TD males and females[48]. Because MRI is not capable of detecting fiber type distribution within muscle tissue, we were unable to link volume deficits with atrophy of specific fiber types in CP. Previous studies have reported variability in type I fiber distribution in CP[79–81]. It is unclear to what extent this variability is subject- and muscle-specific and how fiber-type deficits relate to bulk muscle volume deficits. Future work linking fiber-type distribution and whole muscle volume deficiencies would be an interesting complement to this study. Lastly, segmentation of muscles from MRI remains a subjective process as it relies on manual user identification of muscle boundaries. Our variability assessment revealed reasonably low variability in volume and length. However, small muscles and muscles with difficult to define origins and insertions were the most sensitive to volume and length discrepancies.

Further technical developments of this *in vivo* muscle analysis may promote increased accessibility for researchers and clinicians. Currently, the measurements presented here rely on a custom rapid MRI sequence[48] that is not widely available on all MRI scanners. This sequence required no accessory coils to be placed on subjects but did require subjects to remain motionless for approximately 20 minutes in the scanner. Application of these methods to more severely affected subjects with CP may require use of accessory coils to reduce scan time or may not be possible for subjects who have difficulty remaining motionless for several minutes. Manual

362 segmentation of muscles is time-intensive and not conducive for the clinical setting. The
363 translation of our MRI sequence to built-in scanner sequences and the development of semi- or
364 fully-automated muscle segmentation routines[83,84] will promote more widespread availability of
365 the techniques presented here. Additionally, we did not link muscle size deficits to common
366 clinical measurements of strength, range of motion, or spasticity. It may be possible to assess
367 heterogeneity of muscle distribution with accurate and systematic use of clinical assessments.
368 Future research may suggest how to use currently available clinical assessments or develop new
369 clinical assessments to understand heterogeneity on a patient-specific basis.

370 Small muscles in CP subjects may be the result of a combination of many factors, such as
371 denervation, spasticity, disuse atrophy, or altered gait mechanics that change the muscle forces
372 needed to produce movement. While the precise causes of muscle volume deficits are not
373 entirely clear, this study showed that muscle volume deficits are heterogeneous in lower limb
374 muscles in adolescents with CP. The effects of muscle volume deficits are reduced strength
375 capacity and/or reduced muscle operating range, among other consequences. In this study,
376 muscle deficits were common and pronounced in the distal hamstrings, plantarflexors, and
377 dorsiflexors, but especially in the soleus. These results implicate reduced plantarflexor power
378 during mid-stance of gait and reduced dorsiflexor power for foot contact and swing as potential
379 common gait pathologies in CP. Gait disorders are mechanically complex, however, and linking
380 of muscle volume deficits with gait pathology is an area that requires further study. Prior
381 research into improving CP gait with strength training has produced mixed results[24,27,29,31].
382 Specifically, some studies have questioned whether increases in muscle strength will translate to
383 improved movement[27,29]. However, in light of the well-established relationship between muscle
384 size and strength capacity, we suggest that Shortland's contention[85] that preservation or increases

in muscle size may prevent degenerative mobility that occurs with growth and aging in CP, reduce energy costs, or delay the onset of fatigue. CP muscle physiology is complex, however, and further study is needed to understand optimal strategies for building or preserving CP muscle size and function.

LIST OF ABBREVIATIONS

CP – Cerebral Palsy

CSA – Cross Sectional Area

EDL – Extensor Digitorum Longus

EHL – Extensor Hallucis Longus

FOV – Field Of View

GMFCS – Gross Motor Function Classification System

L_f^0 – optimal fiber length (of a muscle)

L_m – muscle length

MRI – Magnetic Resonance Imaging

PCSA – Physiological Cross Sectional Area

TD – Typically Developing

TE – Time to Echo

TR – Time to Repetition

α – flip angle

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TABLES

Subject	1	2	3	4	5
GMFCS	I	I	II	II	II
Gender	m	m	m	m	m
Age	14	15	15	12	13
Ethnicity	white	white	white	white	white
Mass (kg)	41.5	54.6	65.1	63.1	60
Height (m)	1.535	1.753	1.625	1.534	1.544
BMI(kg/m^2)	17.6	17.8	24.7	26.8	25.2
CP Etiology	placenta previa	stroke	prematurity	stroke	stroke
CP subtype	diplegia	hemiplegia	diplegia	hemiplegia	hemiplegia
Most Affected Side	left	right	right	right	right
Surgical History	none	none	hamstring release (semitend), previous botox	gastroc-soleus-achilles lengthening	none
Gait	foot drag	equinovarus	crouch	equinus, crouch	equinus
Orthosis Use	over-the-counter arch supports	none	over-the-counter arch supports	posterior shell ankle-foot-orthosis	articulated ankle-foot-orthosis

Table 1a: Subjects 1-5 Characteristics

Subject	**6**	**7**	**8**	**9**	**10**
GMFCS	II	II	II	II	III
Gender	m	f	m	f	m
Age	17	13	16	11	13
Ethnicity	white	other	white	white	white
Mass (kg)	96.1	53.6	52.2	36.4	71.5
Height (m)	1.748	1.499	1.698	1.345	1.632
BMI(kg/m^2)	31.5	23.9	18.1	20.1	26.8
CP Etiology	unknown	prematurity	hemorrhage/ infarct	prematurity	stroke / hemorrhage
CP subtype	diplegia	hemiplegia	hemiplegia	hemiplegia	diplegia
Most Affected Side	right	right	right	left	left
Surgical History	gastroc-soleus-achilles lengthening	femoral derotation osteotomy, gastroc-soleus-achilles lengthening	gastroc-soleus-achilles lengthening	gastroc-soleus-achilles lengthening	hamstring lengthening (semitend.), dorsal rhizotomy
Gait	claw toe, bilateral cavus feet	in-toeing, equinus	equinovalgus	equinus	crouch
Orthosis Use	none	ankle-foot-orthosis	Right articulated ankle-foot-orthosis	over-the-counter arch supports	right Loftstrand crutch, bilateral floor reaction ankle-foot-orthoses, left thigh cuff

Table 1b: Subjects 6-10 Characteristics

Muscles Ranked by Severity of Volume Deficit		Muscles Ranked by Heterogeneity Across Subjects	
muscle	**average Z-score**	**muscle**	**variance of Z-score**
soleus	-4.0	gluteus minimus	4.0
digital extensors	-3.4	quadratus femoris	3.9
medial gastrocnemius	-3.0	iliacus	3.7
tibialis anterior	-2.9	semitendinosus	3.2
semimembranosus	-2.6	vastus medialis	3.2
rectus femoris	-2.1	semimembranosus	2.7
vastus medialis	-2.1	vastus lateralis	2.7
semitendinosus	-2.1	adductor magnus	2.6
adductor magnus	-2.0	gemelli	2.5
psoas major	-1.9	digital extensors	2.2
vastus lateralis	-1.9	obturator internus	2.1
fibularis muscles	-1.8	flexor digitorum longus	2.1
lateral gastrocnemius	-1.8	vastus intermedius	2.0
adductor brevis	-1.4	tibialis posterior	2.0
biceps femoris: l.h.	-1.3	adductor brevis	1.9
flexor hallucis longus	-1.3	gracilis	1.7
adductor longus	-1.2	piriformis	1.6
gluteus maximus	-1.1	popliteus	1.6
biceps femoris: s.h.	-1.0	fibularis muscles	1.5
obturator externus	-1.0	sartorius	1.5
gluteus minimus	-0.8	soleus	1.3
tibialis posterior	-0.8	medial gastrocnemius	1.1
popliteus	-0.8	psoas major	1.1
gracilis	-0.6	flexor hallucis longus	1.0
sartorius	-0.6	rectus femoris	1.0
pectineus	-0.4	biceps femoris: l.h.	0.9
tensor fasciae latae	-0.4	gluteus maximus	0.8
flexor digitorum longus	-0.4	obturator externus	0.8
vastus intermedius	-0.4	adductor longus	0.7
gemelli	-0.3	lateral gastrocnemius	0.7
obturator internus	-0.3	tensor fasciae latae	0.7
quadratus femoris	0.0	gluteus medius	0.7
iliacus	0.0	biceps femoris: s.h.	0.5
piriformis	0.1	tibialis anterior	0.4
gluteus medius	0.1	pectineus	0.4

Table 2: Muscles ranked by severity (left) and heterogeneity (right) of volume Z-scores. Average Z-scores: means computed across 10 CP subjects for each muscle. Variance of Z-scores: variances computed across 10 CP subjects for each muscle.

lower limb muscle	**% volume$_{norm}$ difference** of CP group (* *P*<0.05 ** *P*<0.01 *** *P*<0.001)	**%CSA$_{norm}$ difference** of CP group (* *P*<0.05 ** *P*<0.01 *** *P*<0.001)	**%length$_{norm}$ difference** of CP group (* *P*<0.05 ** *P*<0.01 *** *P*<0.001)	# of CP muscles small in volume (Z_V < -2)	# of CP muscles small in CSA (Z_{CSA} < -2)	# of CP muscles short in length (Z_L < -2)	# of CP muscles small in CSA and short in length
tibialis anterior	**-43.4*** **	**-40.1*** **	-6.0	9	7	4	3
medial gastrocnemius	**-42.2*** **	**-32.2*** **	**-15.5** **	8	5	9	5
soleus	**-39.3*** **	**-34.0*** **	**-7.5***	9	9	1	1
lateral gastrocnemius	**-35.8*** **	**-24.8** **	**-14.4***	4	4	5	2
semitendinosus	**-35.3** **	**-23.5***	**-19.5***	4	3	4	2
semimembranosus	**-30.5** **	**-25.2***	-7.2	8	7	0	0
flexor hallucis longus	**-30.5** **	**-33.5** **	3.7	3	3	2	1
rectus femoris	**-29.2*** **	**-28.9*** **	-0.9	5	4	1	1
digital ext. (EDL&EHL)	**-28.6*** **	**-24.2** **	**-5.6** **	8	6	1	0
psoas	**-27.4** **	**-23.7** **	-4.9	6	4	1	0
fibularis muscles	**-27.3** **	**-24.1***	**-4.9***	5	3	4	2
biceps femoris: l.head	**-24.6***	**-22.9** **	-3.5	3	1	0	0
adductor longus	**-22.3***	-17.4	-5.5	2	2	3	0
biceps femoris: s.head	-22.2	**-16.9***	-5.4	1	2	2	1
vastus medialis	**-21.4***	**-19.3***	-3.0	6	6	2	2
adductor magnus	**-20.8***	-15.1	-6.3	5	6	3	1
vastus lateralis	**-20.6***	**-20.2***	-0.8	5	5	3	2
obturator externus	-20.4	-14.5	-12.7	2	0	0	0
adductor brevis	**-19.2***	-16.7	-2.3	4	3	1	0
gracilis	-16.0	0.8	**-17.7** **	1	0	7	0
tibialis posterior	-14.3	-12.3	-3.4	3	1	1	1
pectineus	-14.1	-5.9	-6.8	0	0	1	0
gluteus maximus	**-11.5***	-9.3	-2.1	1	3	1	0
tensor fasciae latae	-11.3	-11.9	2.5	0	3	0	0
flex. digitorum longus	-10.5	-6.1	-6.2	1	3	0	0
popliteus	-9.8	-9.6	-0.2	1	1	0	0
gluteus minimus	-9.2	-8.1	-2.2	3	3	0	0
sartorius	-6.7	-3.3	-4.3	2	0	1	0
vastus intermedius	-6.6	-10.4	3.5	2	2	0	0
obturator internus	-6.2	-6.2	-1.5	1	4	0	0
gemelli	-6.0	-10.5	0.8	2	0	0	0
quadratus femoris	-0.7	-11.8	10.0	2	0	1	0
iliacus	-0.1	0.4	-0.8	0	0	1	0
gluteus medius	1.4	-2.1	3.8	0	0	0	0
piriformis	2.0	6.4	4.0	0	0	0	0

Table 3: Mean deficits in muscle volume, anatomical cross sectional area (CSA), and length, and number of muscles with Z-scores outside of the 2σ confidence interval for subjects with CP. Volumes, CSAs, and lengths were normalized to height, mass, and leg length according to

Equations 1, 3, and 5. Significant CP-TD differences are shown in bold and levels of significance are denoted by asterisks.

Comparison of % Volume Deficit using Height-Mass vs. Mass Normalization		
muscle	**%Deficit in V/H-M**	**%Deficit in V/M**
tibialis anterior	-43.3	-45.8
medial gastrocnemius	-42.2	-44.3
soleus	-39.3	-41.5
lateral gastrocnemius	-35.8	-37.9
semitendinosus	-35.3	-37.6
semimembranosus	-30.5	-34.9
flexor hallucis longus	-30.5	-32.6
rectus femoris	-29.2	-32.1
digital extensors (EDL&EHL)	-28.6	-32.2
psoas	-27.4	-30.8
fibularis muscles	-27.3	-30.3
biceps femoris: long head	-24.6	-27.2
adductor longus	-22.3	-26.0
biceps femoris: short head	-22.2	-25.6
vastus medialis	-21.4	-25.3
adductor magnus	-20.8	-24.1
vastus lateralis	-20.6	-24.2
obturator externus	-20.4	-23.8
adductor brevis	-19.2	-22.2
gracilis	-16.0	-19.1
tibialis posterior	-14.3	-19.4
pectineus	-14.1	-17.8
gluteus maximus	-11.5	-15.3
tensor fasciae latae	-11.3	-16.3
flexor digitorum longus	-10.5	-16.1
popliteus	-9.8	-13.8
gluteus minimus	-9.2	-13.5
sartorius	-6.7	-11.1
vastus intermedius	-6.6	-10.7
obturator internus	-6.2	-10.6
gemelli	-6.0	-8.9
quadratus femoris	-0.7	-3.8
iliacus	-0.1	-4.4
gluteus medius	1.4	-3.1
piriformis	2.0	-2.2

Table 4: Normalization of muscle volume by height-mass yields slightly smaller deficits between CP and TD subjects than normalization by mass. Average percent differences between CP and TD populations for muscle volume per height-mass (See Equation 1) are shown in Column 2.

Average percent differences between CP and TD populations for muscle volume per mass are shown in Column 3. Column 2 data is duplicated from Figure 4.

FIGURE LEGENDS

Fig.1: Total muscle volume in the lower limb scales with height·mass in typically developing adolescent controls, typically developing adults (from Handsfield et al.[48]), and subjects with cerebral palsy. The slope of muscle volume per height-mass is significantly reduced for CP subjects (A). Lower limb normalized muscle volume is 20% lower in subjects with cerebral palsy and is stratified by GMFCS level (B). Error bar displays standard deviation; individual data points are overlaid.

Fig. 2: Grid display of Z-scores allows visual comparisons across subjects for a given muscle or across muscles for a given subject (A). Z-scores between ±2 represent the 99% confidence interval for TD muscles. CP muscles with a Z-score less than -2 are considered significantly small. Muscle volumes are normalized by height·mass to reduce the effects of body size differences between TD and CP subjects. Tabulation of significantly small Z-scores ($Z < -2$) across subjects indicates muscles that are commonly significantly small in the CP cohort (B).

Fig. 3: Z-scores are mapped onto subject-specific limb reconstructions. Profiles of muscle size deficits differ within and across subjects. Distal muscles, especially the soleus, were commonly and severely small as evidenced by very low Z-scores in 9 of 10 subjects. Color map from Fig. 2 is duplicated here; 3D visualization effects (e.g. shadowing) may slightly alter perceived color.

Fig. 4: Height-mass normalized muscle volumes are reduced in subjects with cerebral palsy for muscles and muscle groups crossing the ankle (A), knee (B), hip sagittal plane (C), and hip frontal plane (D). Antagonist volume ratios for muscle groups are only significantly different for

the Abduction/Adduction (D) ratio. Muscle groups are underlined, and muscles displaying significant differences are shown in bold.