RELIABILITY OF LEG MUSCLE SIZE AND QUALITY ESTIMATES FROM ULTRASOUND IN CHILDREN WITH CEREBRAL PALSY

By

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(Under the Direction of Christopher Modlesky)

ABSTRACT

PURPOSE: The aim of this study was to determine if ultrasound imaging can provide reliable estimates of the size and quality of leg muscles in children with spastic cerebral palsy (CP). **METHODS:** Ambulatory children with spastic CP (n = 7) were recruited. Cross-sectional area (CSA) and echo intensity (EI) of the gastrocnemius at 25, 50 and 75 % of muscle belly length and of the TA at 20, 40 and 60 % of tibia length were assessed (n = 14 legs). The tests were repeated four weeks later. **RESULTS:** There were no test-retest differences in CSA or EI estimates in the gastrocnemius or the TA at specific sites, except for TA CSA at the 20 % site, which was lower at the repeat test ($p = 0.023$). There were also no test-retest differences ($p > 0.05$) in the average CSA and EI of the different sites for the gastrocnemius and the TA). **CONCLUSION**: The reliability of most ultrasound day-to-day estimates of CSA and EI for the gastrocnemius and TA are considered good to excellent in children with spastic CP.

INDEX WORDS: cerebral palsy, ultrasound, muscle cross-sectional area, muscle volume, echo intensity, muscle belly

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CHAPTER 1

INTRODUCTION

Cerebral palsy is a disorder of movement and posture that results from abnormal brain development in utero or from a brain injury occurring before, during or shortly after birth. Cerebral palsy is very prevalent in children with an incidence of 3.6 per 1000 children born per year in the United States (Yeargin-Allsopp et al. 2008). There are different ways in which CP can be classified. One classification approach is to separate children based on the limbs affected (Novak et al. 2014). In monoplegic CP, one limb is affected. In diplegic CP, the lower extremities are primarily affected. The upper extremities can also be affected, but degree of the affect is typically to a lesser extent than the lower extremities. In hemiplegic CP, one side of the body is affected. In quadriplegic CP, all four limbs as well as the trunk and face are affected; therefore, it is considered the most severe form of CP (O'Shea T. M. et al. 2008). Children with CP can also be classified as spastic, dyskinetic, or ataxic. Children with spastic CP, which is the most common type of CP, experience muscle stiffness, as well as involuntary movements or twitching. Dyskinetic CP represents a more severe occurrence of involuntary muscle movement and is associated with large twisting or abrupt movements. Ataxic CP is characterized by a decrease in coordination and balance. Children with ataxic CP are often shaky and can have trouble with small precise movements such as writing. It is important to note that it is possible for children with CP to have one or a combination of spasticity, dyskinesia, or ataxia (Cans et al. 2007).

Children with CP have muscles that are small and of poor quality (Elder et al. 2007; Matthews et al. 2005; Johnson et al. 2009; Elder et al. 2003). These muscle deficits, paired with a decrease in coordination and balance, can have large effects on the mobility and ability of children with CP to be involved in physical activities (Beckung et al. 2002). The limitations children with CP face in regard to muscle status can have many negative health consequences, such as an increase in sedentary behavior, body fat, intramuscular fat, and risk for cardiometabolic disease and cancer (Bjorntorp et al. 1992). This creates a negative cycle whereby limited physical activity can contribute to further deficits in muscle size, strength, and quality and lead to further inactivity. This cycle can lead to decreased opportunities for independence, participation in school and recreational activities, and a decreased quality of life. Therefore, it is imperative to properly monitor the size and quality of muscle in children with CP.

Currently, different types of medical imaging are used to examine muscle size and quality in children with CP, with the gold standard being magnetic resonance imaging (MRI) (Mitsiopoulos et al. 1998). Magnetic resonance imaging provides unparalleled in vivo images of skeletal muscle and has been validated for the assessment of muscle cross-sectional area (Mitsiopoulos et al. 1998) and intramuscular fat (Hu et al. 2011). While MRI is the gold standard for assessing muscle and other soft tissues, its use in research and in the clinical setting is not widespread. This is largely due to the expense and availability of MRI equipment. The use of MRI to assess muscle is particularly challenging for children with CP due to the spasticity and attention deficits that are often present in this population. An alternative to MRI is ultrasound imaging. Ultrasound offers a more affordable, time efficient, and tolerable alternative to MRI.

An additional benefit of ultrasound is its greater feasibility within the clinical setting. There is evidence that ultrasound provides accurate estimates of muscle cross-sectional area (CSA) and volume (Scott et al. 2012; Barber et al. 2009). There is also evidence that ultrasound measurements of echo intensity reflect intramuscular fat (Young et al. 2015). The ability to monitor the muscle growth and development of children with CP would make research studies focused on muscle more feasible, especially studies that require return visits to monitor natural growth or the effects of an intervention. Unfortunately, few studies have assessed the reliability of ultrasound measurements of muscle size and echo intensity in children with CP. Furthermore, to our knowledge, no studies have determined their day-to-day reliability. Moreover, no studies have assessed the smallest amount of change in leg muscle size and echo intensity that is detectable by ultrasound in children with CP. Therefore, one aim of this study was to determine if ultrasound imaging can provide reliable estimates of leg muscle size and echo intensity in children with spastic cerebral palsy (CP).

Statement of the Problem

Children with CP have muscles that are small and have poor quality. Ultrasound has been shown to provide accurate measures of muscle CSA and echo intensity, which reflect the size and quality of muscle, respectively. However, the reliability of these measures in children with CP needs to be established because viable options for accessible and tolerable muscle imaging for children with CP are needed.

Specific Aim

To determine if ultrasound provides reliable estimates of leg muscle size and echo intensity in children with CP.

Hypotheses

Hypothesis 1.1: Gastrocnemius CSA, belly length, volume and echo intensity will be reliably estimated using ultrasound in children with CP.

Hypothesis 1.1: Tibialis anterior CSA and echo intensity will be reliably estimated using ultrasound in children with CP.

Significance of Study

Cerebral palsy is a movement and posture disorder that results from abnormal brain development in utero, or from a brain injury that occurred before, during or shortly after birth. The proposed study is significant because children with CP have small and weak muscles that are highly infiltrated with fat, which increases their risk of developing chronic disease. Ultrasound is a noninvasive imaging technique that has been used to assess muscle size and quality; however, studies determining whether it provides reliable estimates are limited and the magnitude of changes that can be detected is not established. To address this gap in the literature, the proposed study determined the day-to-day reliability of muscle size and muscle quality estimates from

ultrasound in children with CP, which can aid in the assessment of natural growth and in planning interventions focused on increasing muscle size and improving muscle quality.

Innovation

The proposed study is innovative because studies evaluating the reliability of ultrasound for the assessment of muscle size and quality in children with CP are lacking. Studies by Noorkoiv M et al. 2019 and Barber et al. 2019 validated and assessed reliability of free-hand ultrasound to assess muscle volume, with Noorkoiv's study comparing 3D ultrasound to MRI for validation. Both studies found ultrasound to be a reliable method; however, the muscle measured, and ultrasound collection protocol differed in each study. Additionally, the reported protocols for the collection of ultrasound images often lack structure and consistency (Modlesky et al. 2019). The proposed study will develop and assess a very specific ultrasound collection protocol for children. Through the comparison of ultrasound assessment on two separate days, it will be possible to determine whether muscle size and quality can reliably be assessed in children with CP. The findings from this study will be relevant to the clinical treatment of children with CP as MRI is not tolerable for many children with CP and MRI accessibility is often limited. If it is found that ultrasound provides reliable estimates of muscle CSA, belly length, volume, and echo intensity, scientists and clinicians will have more options to assess and monitor muscle size and quality in children with CP.

CHAPTER 2
REVIEW OF LITERATURE

Cerebral Palsy

Cerebral palsy is a disorder of movement and posture that is the most common cause of motor disability in children (Kirby et al. 2011). Cerebral palsy is caused by a brain injury or malformation that occurs during fetal development, during birth, or shortly after birth. It is associated with decreased muscular strength, muscle stiffness, and a decreased range of joint motion (Elder et al. 2007). Cerebral palsy is typically diagnosed shortly after birth, with movement delays appearing at around 9 months of age. By 30 months of age, most movement delays are present (Novak et al. 2017). Cerebral palsy is often accompanied by comorbidities such as seizures, intellectual disability, and hearing, vison, or speech disabilities (National Institute of Neurological Disorders and Stroke, *Cerebral palsy: Hope through research, 2013*).

Inactivity and decreased fat-free mass have been linked to chronic disease; unfortunately, children with CP struggle with both of these issues (Baumgartner et al. 1995). Overall, lower mobility, resting energy expenditure, and fat-free mass and a higher proportion of body fat are present in children with CP compared to their typically developing peers (Azcue et al. 1996; Bandini et al. 1991). In addition, children with CP have been found to have a decrease in overall BMI and a decrease in fat free tissue in relation to poor nutritional status indicators (Stallings et al. 1995). Children with CP also exhibit elevated adipose tissue infiltration of skeletal muscle, which has been related to their low level of physical activity (Johnson et al. 2009) and is likely related to their low-fat free mass (Oftedal S et al. 2017). These differences in fat free mass can be seen as early as preschool in children with spastic CP and are positively associated with energy

intake and levels of physical activity (Walker JL et al. 2015; Oftedal et al. 2017). Though children with CP commonly report lower levels of fat free mass and an increase in fat mass, the prevalence and status of overweight/obesity in children with CP might be underestimated when using traditional BMI calculations (Whitney et al. 2019).

The primary types of CP include diplegic, hemiplegic and or quadriplegic (Novak et al. 2014). In individuals with diplegic CP, the lower extremities are affected, showing a decrease in motor control. Although the upper extremities can also be affected in diplegic CP, they are generally less affected than the lower extremities or not affected at all. In individuals with hemiplegic CP, one side of the body is primarily affected. In individuals with quadriplegic CP, the most severe form of CP, all four of the extremities are affected, as are the trunk and the face (Cans et al. 2007). Children with CP typically begin working with physicians and therapists at an early age, to monitor and facilitate growth (National Institute of Neurological Disorders and Stroke, *Cerebral palsy: Hope through research,* 2013).

Skeletal Muscle Anatomy

Skeletal muscles are made up of fascicles surrounded by a connective tissue called perimysium, these fascicles bunch together to form the muscle and are encapsulated by epimysium, a connective tissue. Muscles are further broken down into individual muscle fibers, which are covered in endomysium (connective tissue) and make up the fascicle (Järvinen et al. 2002). Each of these individual fibers are made up of bundles of myofibrils which are full of two proteins, actin and myosin. Actin and myosin make up the fundamental unit of force production in the muscle, the sarcomeres. These sarcomeres attached end to end to build up the myofibrils.

The interaction between actin and myosin along with muscle velocity create muscle force, this relationship is explained with the sarcomere length tension curve. Simplified, this curve shows and increase of force production at short sarcomere lengths which increases until reaching a medium length and then begins to plateau as the sarcomere continues to be stretched.

Satellite cells are located below the basal lamina of the myofibers and contribute to muscle growth, muscle recovery, and are important for muscle health and function (Valerie et al. 2002). Each muscle fiber can be described as a different classification or type, these types reflect the neuromuscular input, firing rates, and the metabolic pathways the fiber uses (Schiaffino et al. 2011). Fibers can be classified as slow twitch (type 1) or fast twitch (type 2). Type 1 fibers primarily use oxidative metabolism to fuel the movement, while type 2 fibers primarily use glycolytic metabolism. In other words, type 1 fibers are more aerobic while type 2 fibers are more anerobic. Type 1 fibers are found in muscles that are typically active for long portions of the day or used for endurance activities, distance running for example. While type 2 fibers are typically more prominent in power generating muscles used for explosive movement like jumping or throwing.

In typically developing children, muscle size and volume tend to increase linearly from the age of three until around puberty, this increase in muscle size and volume goes hand in hand with an increase in muscle force output (Beunen et al. 2000). As children continue to grow and develop more full body and complex motor functions, their coordination and muscle endurance increases as does their physical activity levels (Gallahue et al. 2006).

Muscle in CP

A number of studies have demonstrated that CP is associated with smaller and weaker muscles when compared to their typically developing peers (Elder et al. 2007; Matthews et al 2005; Johnson et al. 2009; Elder et al. 2003). Low muscle volume can negatively affect an individual's ability to complete activities of daily living, especially the muscles of the lower limbs (Elder et al. 2007). Size reductions and morphological alterations are often seen in the plantar flexor and dorsiflexor muscles that are important for walking. The plantar flexor muscle tendon has been found to show morphological alterations in children with CP, potentially leading to muscle weakness (Kruse et al. 2018). Additionally, the dorsiflexor muscle group has been found to have decreased CSA and muscle thickness as measured with ultrasound in children with CP (Bandholm et al. 2009).

Although children with CP are generally small in size, they have lower muscle volume when normalized to body mass in comparison to their typically developing peers. In fact, it has been reported that the normalized muscle volume in children with CP is 13% less at 40 kg when compared to their typically developing peers, and 27.4% less at a weight of 80 kg when compared to their typically developing peers (Noble et al. 2017). When looking at the triceps surae (the combination of the gastrocnemius and solus muscles), children with CP demonstrated significantly lower muscle volume, CSA, and muscle length when compared to typically developing children. The decrease in overall size of these plantar flexors could negatively influence the functional capacity of the lower limb in children with CP (Pitcher et al. 2018). This discrepancy in muscle size can be seen early on in children with CP. Children with

CP show decreased muscle size when compared to their typically developing peers as early as 2 years old. In a study examining gastrocnemius muscle volume, CSA, fascicle length, muscle length, and pennation angle in young children with CP, ages 2 to 5, researchers found that children with CP had a muscle volume that was 22% lower when compared to their typically developing peers. The fascicle length was not significantly different between groups, showing that muscle CSA size reductions played the largest role in volume reduction (Barber et al. 2011). Interestingly, when comparing fiber length in children with diplegia to their typically developing peers, no significant differences were found suggesting that muscle fiber diameter may be a better target than muscle fiber length when evaluating the extent of muscle contracture may be better examined by looking at muscle fiber diameter than fiber length (Shortland et al. 2002).

Smaller muscles in the lower limbs can make it challenging for children to move without support. For example, to walk, the legs must produce enough force to propel the body forward. The force generating capacity of the muscle has been found to be directly related to the size of the muscle in question (Handsfield et al. 2014). Important muscles for walking are the gastrocnemius, soleus, and the tibialis anterior. The medial gastrocnemius muscle belly and gastrocnemius volume have been reported to be smaller in children with CP, which is thought to be due to a decrease in CSA growth and not due to a decrease in fascicle length (Malaiya et al. 2007). It has been demonstrated that the tibialis anterior muscle, an essential muscle for walking and balance, has a smaller CSA in children with CP when compared to their typically developing peers (Bland et al. 2011). In addition, the cross-sectional area of the gastrocnemius is also lower in children with CP when compared to their typically developing peers (Schless et

al. 2017). The decrease in muscle volume and muscle CSA in the gastrocnemius and tibialis anterior in children with CP may directly relate to decreased force output and therefore may directly relate to difficulty walking in this population.

In addition to smaller muscle size in the lower limbs, children with CP may struggle to fully activate the muscles of the lower limb when trying to produce movement or force. When compared to their typically developing peers, children with CP were found to have lower activation of their plantar and dorsiflexors during a maximum contraction and to have higher coactivation of antagonists during the movement. These findings suggest that incomplete activation and increased antagonist coactivation lends to dorsiflexor weakness in children with CP (Elder et al. 2003). A study by Lorenzo et al. used ultrasound and 3-dimentional motion capture to asses muscle and tendon length on force production during gait. They found that fascicle length and muscle length were shorter in children with CP, while the Achilles tendon was found to be longer when compared to their typically developing peers. Children with CP had lower force production during gait, this could be attributed their decrease in fascicle and muscle length and to the large tendon to fascicle ratio (Lorenzo et al. 2018). An overall decrease in size of lower limb muscles and the inability to fully activate these muscles could greatly affect the functional ability of children with CP. Muscle monitoring and testing in children with CP could be an important step in the treatment process to improve and sustain functional ability.

Finally, in addition to having smaller and weaker muscles, there is evidence that children with CP have muscles that are highly infiltrated with fat. For example, children with quadriplegic

CP have a 2.3 – fold higher intermuscular adipose tissue when compared to their typically developing peers (Johnson et al. 2011). Adipose tissue infiltration into skeletal muscle has been linked to decreased levels of physical activity as well as decreased resting energy expenditure (Johnson et al. 2009; Manini et al. 2007). Higher infiltration of fat within and around muscle is associated with lower muscle mass, lower muscle strength and an increased risk of developing a chronic disease, such as diabetes (Goodpaster et al. 2000). Adipose tissue infiltration into skeletal muscle has been linked to decreased levels of physical activity as well as decreased resting energy expenditure (Johnson et al. 2009; Manini et al. 2007). With decreased physical activity, increased adipose tissue infiltration, and decreased resting energy expenditure, children with CP are at a greater risk for cardiovascular disease (Baumgartner et al. 1995).

In addition to skeletal muscle and fat-free mass deficits, children with CP also have underdeveloped bone architecture and fat infiltration of bone marrow, both of which are associated with low bone strength (Whitney et al. 2017). Bone strength in children with CP, as indicated by pSSI, reports compromised bone structure, not due to lower cortical bone density by to smaller and thinner bones (T. Binkley et al. 2005). In the distal femur of children with CP, underdeveloped trabecular bone microarchitecture has been detected and becomes more pronounced as the bone extends past the growth plate (Modlesky et al 2007; Modlesky et al 2008; Modlesky et at 2015). The low participation in physical activity and underdeveloped bone architecture that are common in children with CP, are likely tied to their relatively high number of low-energy fractures (McIvor et al 1966; Presedo et al. 1997). Monitoring both

muscle and bone strength and quality is imperative to keep children with CP physically active and safe.

Muscle Assessment Techniques

It is critical that valid and reliable assessment techniques are available to monitor muscle growth and development in children with CP. Advanced imaging techniques offer a powerful way to accurately and reliably monitor muscle size and quality. Magnetic resonance imaging (MRI), currently the gold standard for assessing muscle size *in vivo*, uses a magnetic field and radio waves to create detailed images of skeletal muscle, as well as other tissues, including bone, ligaments and tendons (Mitsiopoulos et al. 1998). The validity of MRI in the assessment of muscle size was demonstrated in a study by Mitsiopoulos et al 1998, in which researchers compared MRI estimates of muscle, interstitial adipose tissue, and subcutaneous adipose tissue CSA to high-resolution, photograph-based estimates in cadavers. Very strong correlations and measures of agreement supported the use of MRI as an accurate measurement tool for muscle and adipose tissue CSA. The recognition that MRI is the gold standard for the assessment of muscle size was also demonstrated in a systematic review by Pons et al (2018), in which thirty articles on MRI skeletal muscle volume were compared for reliability and validity and MRI was found to be good to excellent.

Magnetic resonance imaging is also a valid way to assess aspects of muscle quality, such as the concentration of fat within muscle. Specifically, MRI has been found as a valid tool to estimate absolute fat mass in mammalian muscles, adipose, and lean tissues (Hu et al. 2011) and measurements have been found to be reproducible and accurate across MRI scanners (Kang et al. 2011). Magnetic resonance imaging has been used to demonstrate the substantial deficit in

muscle size and the high concentration of intermuscular (Johnson et al. 2009) and intramuscular fat (Whitney et al. 2018) in children with CP. For example, a study by Johnson et al. 2009 used MRI imaging to estimate CSA and intermuscular fat concentration in children with quadriplegic CP. They reported that when compared to their typically developing peers, children with quadriplegic CP had a 2.3-fold higher intermuscular fat concentration and a 51% lower muscle cross sectional area at midthigh. Additionally, the proportion of intermuscular adipose tissue and subfascial adipose tissue compared to the subcutaneous adipose tissue were 2.5-fold and 1.8-fold higher in children with CP at the midthigh compared to their typically developing peers. This study also found that these results could be related to the 70 % lower physical activity level observed in children with CP when compared to the control group. This trend can be seen as children with CP grow and develop, as young adults (mean age 22) with bilateral spastic CP have also been found to have increased intermuscular and intramuscular fat concentration when compared to their typically developing peers (Nobel et al. 2014). Despite the advantages of MRI, it is expensive, it requires significant technical expertise, it generates a high level of noise, the testing space for participants is small and it is sensitive to movement making it difficult to adopt as a primary method for the assessment of muscle size and quality in children with CP. Therefore, a more cost efficient and tolerable method is needed (Modlesky et al. 2019).

Computed tomography has been shown to have similar accuracy for assessing muscle CSA as MRI (Mitsiopoulos et al. 1998). It can also assess fat concentration of muscle (Goodpaster et al. 2000). The main limitation of using computed tomography to assess muscle size and quality is the associated radiation exposure. Computed tomography is a computerized x-ray procedure used to collect images of the body. It works by passing narrow beams of x-rays through the patient

and is quickly rotated around the body to create cross sectional images. The exposure to x-ray risks ionizing radiation which has the potential to cause biological effects in the tissue and repeated exposure could cause serious damage to healthy tissue or put an individual at risk for cancer (Power et al. 2016).

Dual-energy X-ray absorptiometry (DXA) is another promising technique that may be used to estimate muscle size from fat-free soft tissue mass. The advantage of DXA is that scans are completed within a few minutes and there is little exposure to radiation (Modlesky et al. 2019). Furthermore, a recent study suggests estimates of lower extremity muscle mass can be made in children with CP using DXA (Zhang et al. 2019). Unfortunately, it is not possible to estimate the size of individual muscles using DXA. In addition, the quality of muscle can't be determined. A third imaging technique that has considerable promise to serve as alternative to MRI for the assessment of muscle size and quality in children with CP is ultrasound imaging.

Ultrasound Assessment of Muscle Size and Quality

Ultrasound is a cost effective and tolerable alternative to MRI and has the potential to yield similar estimates of muscle size and quality (Scott et al. 2012; Young et al. 2015). Ultrasound transducers, or probes, create a sound wave that is above 20 *kHz* and undetectable to human ears. These sound waves then bounce back and are detected by the transducer which sends electrical signals to the ultrasound scanner. A two-dimensional image is generated using the speed of sound and the time of each echo's return to calculate the distances from the probe to the tissues. Ultrasound measures include CSA, belly length, volume, and echo intensity of the muscle. A visual description of the general underlying principle of ultrasound is in Figure 1.

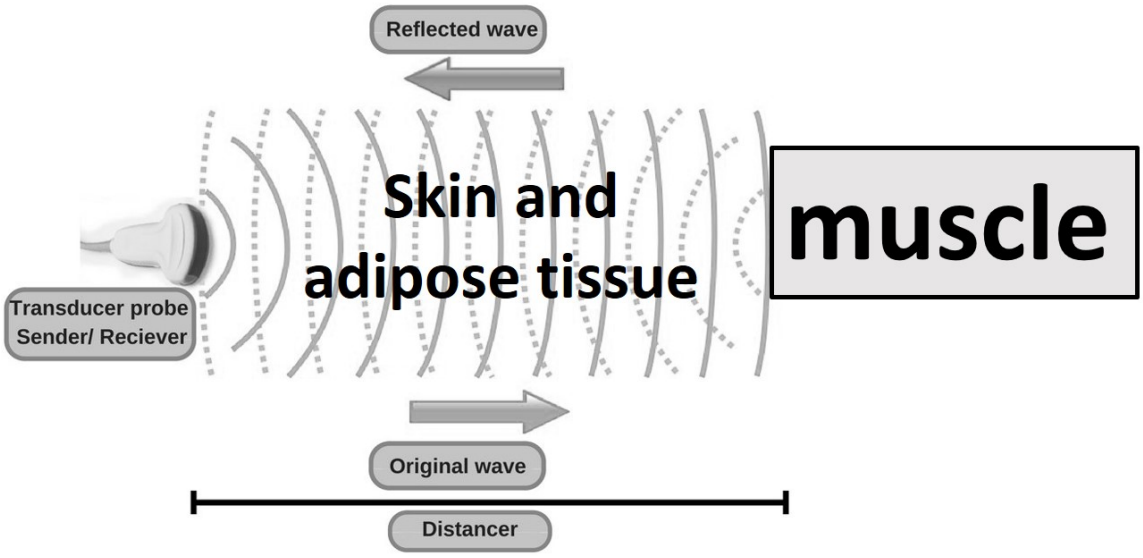

Figure 1. Ultrasound Sound Waves: Transmitted sound waves pass through the skin and other tissues (such as adipose tissue) reflecting the waves back to the transducer creating an image. Figure modified from Ashish et al. 2019.

Ultrasound has been found to reliably measure muscle thickness, pentation angle, and fiber length of the gastrocnemius in healthy young children. In a study by Legerlotz et al. 2010, images of the medial gastrocnemius of healthy young children were taken using two separate ultrasound machines. From these images muscle thickness, pennation angle, and fiber length were collected. The same process was repeated 4-6 weeks later and all measurements were found to be within the same ranges showing that ultrasound can reliably quantify muscle thickness, pennation angle, and fiber length of the medial gastrocnemius in healthy young children Though this study did not focus on children with CP, it does suggest that ultrasound is a reliable method to assess muscle size in young children. Ultrasound assessment has also been used to evaluate fascicle length muscle length, and tendon length in children with CP (Lorenzo et al. 2018). A number of studies have used ultrasound to show that the muscles of children

with CP are substantially smaller than the muscles of typically developing children (Modlesky et al. 2019; Bandholm et al, 2009; Barber et al. 2011; Malaiya et al. 2007; Pitcher et al. 2018).

Ultrasound has been compared to MRI in a handful of studies, one of which found that ultrasound can accurately estimate dorsiflexor thickness and that this measure reflects cross sectional area measurements obtained by MRI (Park et al. 2014). In this study by Park et al 2014, the legs of nine children with spastic CP (age 2-6) were investigated to find tibial length, muscle thickness, and the cross-sectional area of the gastrocnemius muscle. Magnetic resonance imaging scans were also taken of the legs to find muscle volume for comparison. The study found that ultrasound data for CSA, muscle thickness, and tibia length can be accurately collected and used to estimate muscle volume in children with CP. Additionally, use of a 3D ultrasound system was found to reliably estimate muscle volume and muscle belly length of the gastrocnemius in healthy adults compared to muscle volume and muscle belly length data collected from an MRI (Barber et al. 2009). Though this study was done in healthy adults, it is promising for future studies. When quantifying skeletal muscle CSA and volume for individual muscles, ultrasound has been found to be both reliable and valid technique when assessing the vastus lateralis, rectus femoris, medial gastrocnemius, and lateral gastrocnemius when compared to MRI values, although notably MRI values were systematically larger than those produces with ultrasound (Scott et al. 2012).

In order to calculate CSA with ultrasound, panoramic ultrasound images are collected. Panoramic images allow the technician to fully capture the muscle in question and has been found to produce CSA measurements similar to MRI measurements (Scott et al. 2017). Studies

have assessed the accuracy of muscle size estimates from ultrasound imaging in children with CP (Barber et al. 2018; Noorkoiv et al. 2019). One such study by Schless et al. used ultrasound imaging in children with spastic CP to calculate aCSA, muscle volume, and muscle length of the gastrocnemius muscle. The study found that ultrasound can reliably calculate muscle volume using aCSA and muscle length (Schless et al. 2017). Additional studies have also concluded that ultrasound is a valid assessment tool for estimating muscle volume of the gastrocnemius in children with spastic CP (Park et al. 2014). In this study by Park et al 2014, the legs of nine children with spastic CP (age 2-6) were investigated to find tibial length, muscle thickness, and the CSA of the gastrocnemius muscle. Magnetic resonance imaging scans were also taken of the legs to find muscle volume for comparison. The study found that ultrasound data for CSA, muscle thickness, and tibia length can be accurately collected and used to estimate muscle volume in children with CP. Furthermore, three-dimensional ultrasound has been found to be a valid method for measurement of muscle volume and muscle length in adults, typically developing children, and in children with CP when compared to muscle volume data collected with MRI (Noorkoiv et al. 2018; Cenni et al. 2016). When quantifying skeletal muscle CSA and volume for individual muscles, ultrasound has been found to be both reliable and valid technique when assessing the vastus lateralis, rectus femoris, medial gastrocnemius, and lateral gastrocnemius when compared to MRI values, although notably MRI values were systematically larger than those produces with ultrasound (Scott et al. 2012).

Though there is not a specific way to measure intramuscular fat with ultrasound the same way one would with an MRI, echo intensity is believed to reflect muscle quality. Excessive or

abnormal amounts of fat and fibrous tissue appear in the muscle and cause changes to the orientation of reflective surfaces of the muscle. These changes lead to an increase in the echo intensity measure (Heckmatt et al. 1982). Ultrasound measurements of echo intensity reflect skeletal muscle composition, reflect quality of muscle architecture, and could shed light on muscle health and quality in children with CP. A study by Pitcher et al. demonstrated ultrasounds ability to measure altered muscular tissue in spastic CP. Children with CP, GMFCS III, consistently show elevated levels of muscular echo intensity using ultrasound compared to their typically developing peers (Pitcher et al. 2015). Since echo intensity reflects muscle quality by representing changes in the muscle tissue caused by adipose tissue, high echo intensity values are associated with muscular dystrophies (Heckmatt et al. 1982). In one study comparing ultrasound echo intensity to MRI estimates of intramuscular fat in healthy adults, ultrasound echo intensity strongly correlated to MRI percent fat calculations (Young et al. 2015). Another study compared ultrasound measurements of skeletal muscle echo intensity to muscle attenuation as assessed by computer tomography in both healthy young and healthy older adults. Both significant and moderate correlations were found between echo intensity and muscle attenuation values (Watanabe et al. 2018). This study gives additional support for echo intensity from ultrasound as an indicator of intramuscular fat, especially in younger individuals.

Summary

This review of the literature shows that CP, the most common cause of motor disability in children (Kirby et al. 2011), is associated with considerable deficits in muscle size and quality. Although there are several methods that can be used to assess these muscle features, ultrasound has considerable promise because it is a valid method that is cost effective and tolerable.

However, whether it provides reliable estimates of muscle size and echo intensity in children with CP requires further investigation.

CHAPTER 3

RELIABILITY OF LEG MUSCLE SIZE AND QUALITY ESTIMATES FROM ULTRASOUND IN CHILDREN WITH CEREBRAL PALSY

Introduction

Cerebral palsy (CP) is a movement disorder that results from abnormal brain development in utero, or from a brain injury that occurred before, during or shortly after birth. Cerebral palsy is one of the most prevalent disabilities affecting children in the United States with an incidence of 3.6 per 1000 children (Yeargin-Allsopp et al. 2008). The ability to properly monitor muscle development and function is imperative to identifying appropriate treatments in this population.

Children with CP experience motor control deficits, increased muscle tone (i.e., hypertonicity) (Damiano et al. 2009), and an increased concentration of fat within and around muscle. They also experience a decrease in muscle size and strength (Damiano et al. 2001). These issues can be seen in both the upper and lower extremities in children with CP; however, the effects on the lower extremities may be more profound than what is observed in the upper extremities (Zhang et al. 2019). These impairments coupled with poor balance and poor muscle coordination lead to limited participation in physical activities, such as walking (Kramer et al. 1194; Bjornson et al. 2007). The decreased ability of children with CP to use their muscles can lead to activity limitations and restricted participation in both recreational activities and activities of daily living (Beckung et al. 2002). A decrease in physical activity is linked to increased risk for obesity, diabetes, cardiovascular disease (Hubert et al. 1983; Despres et al. 1990; Bjorntorp et al.), psycho-social concerns (Neumark-Aztainer et al. 1997), and increased risk for some forms of cancer (Ballard et al. 1994; Huang et al. 1997). Though children with CP are more likely to

exhibit sedentary behaviors, increased levels of obesity, and increased risk for cardiovascular diseases, physical activity has been shown to greatly reduce these risks. (Goran et al. 1999). Due to the activity limitations that children with CP face, it is imperative to find ways to monitor muscle strength and health in order to improve treatment and increase physical activity.

One method that has been used to examine muscle development in children with CP is magnetic resonance imaging (MRI) (Modlesky et al. 2008). While MRI provides unparalleled in vivo imaging of human muscle (Mitsiopoulos et al. 1998), it has drawbacks that limit its widespread use in children such as its high cost, and limited availability of the equipment and technological expertise. Additionally, MRI testing requires that the patient is very still throughout the testing session, which can be challenging for children with CP because spasticity and attention deficits are often present. On the other hand, ultrasound offers a more affordable, attainable, and time efficient alternative to MRI. Furthermore, studies have demonstrated the excellent accuracy of ultrasound in the assessment of muscle size (Scott et al. 2012). Unfortunately, few studies have assessed the reliability of muscle measurements from ultrasound in children with CP. Therefore, one aim of this study was to determine whether ultrasound can be used to reliably assess muscle size, as reflected by muscle cross-sectional area (CSA) and quality, as reflected by echo intensity, in the lower extremity muscles of children with CP. To assess the test-retest reliability of leg muscle CSA and echo intensity estimates from ultrasound images, muscle measurements taken twice, on separate days, in children with CP were compared.

Methods

To address the aims, this study was conducted as part of an ongoing randomized controlled trial examining the effect of vibration on the muscle properties of children with CP.

Protocol

Children with spastic CP between the ages of 5 and 11 were recruited from local clinics and schools through the use of flyers distributed at local clinics and schools, newspaper advertisements, social media advertisements and word of mouth. Anthropometrics were assessed using standard methods and sexual maturity was assessed using Tanner staging. Muscle size and quality were assessed in the leg muscles twice, four weeks apart, using ultrasound.

Tests

Anthropometrics

Height and body mass were measured while children wore minimal clothing and were without shoes or braces. Height was measured in an erect standing position using a stadiometer to the nearest 0.1 cm. Body mass of all children was determined using a digital scale (Detecto 6550, Cardinal Scale, Webb City, MO) to the nearest 0.1 kg.

Gross motor function

Gross motor function was assessed by a health professional using the Gross Motor Function Classification System (GMFCS) (Wood et al. 2000).The scale ranges from I to V: GMFCS I and II reflect gross motor independence, such as walking, but with limited ability of speed, balance and coordination; GMFCS III reflects the use of assistive walking devices; and GMFCS IV-V reflect wheelchair-empowered mobility). Children classified as GMFCS I or II were included in the study.

Sexual maturity

The participant's sexual maturity was assessed by their parent using the Tanner staging technique (Tanner et al. 1962). Signs of pubic hair growth and testicular/penis development were assessed in boys and signs of pubic hair and breast development were assessed in girls. The rating system ranges from I to V, with I indicating no signs of sexual development, II indicating early sexual development, and V indicating full development.

Ultrasound

Cross-sectional area and echo intensity of the gastrocnemius and the tibialis anterior were determined using ultrasound imaging (ACUSON S2000 Ultrasound System; Siemens Healthcare; Erlangen, GER). Cross-sectional images were collected from the gastrocnemius and the tibialis anterior using a 9L4 linear transducer in the musculoskeletal mode and the panoramic setting. This function allows the technician to start image collection at one side of the muscle and to continue to gather image data as you move across the entire muscle, creating a panoramic image of the entire muscle. The panoramic images were used to estimates CSA and echo intensity of the gastrocnemius and the tibialis anterior muscles. Still images were collected in a subsample of children with CP (n = 12 limbs) to estimate the thickness of the adipose tissue overlaying the gastrocnemius and tibialis anterior.

Before images were collected, the foot was held in place at an ankle angle of 30 degrees of plantarflexion using a holding device constructed in house that consists of wooden plates with an adjustable metal hinge and Velcro straps. A goniometer was used to the check the ankle angle. Holding the participant's ankle joint at a set angle ensured that the region of the muscle belly scanned was consistent for each participant and at each test session. Panoramic and still images

of the gastrocnemius were collected at 25, 50, and 75 % of muscle belly length (2 sets of images per site), which was measured from the most proximal edge of the medial tibial condyle to the distal insertion of the medial gastrocnemius. The medial tibial condyle was located by palpating the knee, while the distal insertion of the medial gastrocnemius was located using ultrasound imaging. To find the distal insertion of the medial gastrocnemius, the ultrasound 9L4 probe was placed at the origin of the gastrocnemius. The connection of the medial and lateral gastrocnemius was used to identify the medial gastrocnemius muscle belly. The probe was then slowly moved down the muscle until the muscle belly receded into the Achilles tendon. This area was then marked as the insertion point. Using a soft tape measure, the length of the gastrocnemius was measured from the palpated medial tibial condyle to the medial gastrocnemius muscle belly insertion, and the 25, 50, and 75 % measurements were marked. To ensure that muscle measurements were made in the correct locations, a guide system created in-house was placed on the gastrocnemius to outline each specific region of interest (25, 50, and 75 %). The guide system was comprised of a cushioned hard plate that rested on the shin. The plate was equipped with Velcro along the edges so that adjustable straps made from flexible plastic could be secured. A visual depiction of the setup for collecting ultrasound images of the gastrocnemius is presented in Figure 1A.

The tibialis anterior measurements were taken at 20, 40, and 60 % of tibia length (2 sets of images per site). Using a soft tape measure, tibia length was measured from the medial tibial condyle to the most distal aspect of the medial malleolus. The same guiding system as described for the gastrocnemius was used to ensure that the scans were all collected in the appropriate location. A visual depiction of the setup for collecting ultrasound images of the tibialis anterior is presented in Figure 1B.

All images where transferred from the ultrasound machine, uploaded to a desktop computer, and then processed using ImageJ (https://imagej.nih.gov) and the following procedures. Each image was calibrated to reflect the 4 cm depth at which it was taken using the line function on ImageJ. For analysis of the panoramic images, the polygon function was used to outline each individual muscle. After the muscles were fully outlined, ImageJ analysis functions were used to calculate the CSA and the echo intensity. The CSA was determined by multiplying pixel size by the number of pixels within the outlined muscle region. The echo intensity was reflected by the calculated mean value. For analysis of the still images, the rule function in ImageJ was used to estimate adipose tissue thickness.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics (version 24). Paired t-tests were used to determine if there were significant differences in muscle CSA and echo intensity estimated during the two different tests. Test-retest reliability of muscle estimates by ultrasound was determined using the intraclass correlation coefficient (ICC) and the within-participant coefficient of variation (CV). An ICC < 0.50 indicates poor reliability, between 0.50 and 0.75 indicates moderate reliability, between 0.75 and 0.90 indicates good reliability and > 0.90 indicates excellent reliability (Koo et al. 2016). Scatter plots were used to visualize the relationship between the test and retest data.

Results

Seven children with CP participated in the study. Of these children, four were male, three were Caucasian and four were African American. Their physical characteristics are reported in Table 1. Ultrasound estimates of muscle CSA and echo intensity are presented in Table 2. There were

no differences in CSA or echo intensity at any site or in either muscle, except for tibialis anterior CSA at the 20 % site, which was lower at the repeat test ($p = 0.023$).

The ICC values comparing test-retest muscle estimates are also reported in Table 2. The ICC was good-to-excellent for CSA and echo intensity estimates for the gastrocnemius at individual sites, ranging from 0.84 to 0.99 (all $p < 0.01$), and excellent for the average CSA and echo intensity of the individual sites (0.98 for CSA and 0.95 for echo intensity, both $p < 0.001$). The ICC was good-to-excellent for CSA and echo intensity estimates for the tibialis anterior individual sites, ranging from 0.90 to 0.99 (all $p < 0.001$) and excellent for the average CSA and echo intensity of the individual sites (0.98 for CSA and 0.97 for echo intensity, all $p < 0.001$). In addition, the ICC was also excellent for gastrocnemius belly length and volume (0.97 and 0.99, respectively, $p < 0.001$), as well as tibia length (0.99, $p < 0.001$). The good-to-excellent relationships for the test-retest muscle estimates are confirmed by the scatter plots presented in Figures 2-6.

The CV values reported in Table 2 ranged from 3.6 to 16.5 % for CSA and echo intensity estimates for the gastrocnemius and tibialis anterior individual sites and were at the lower end of the range when the values at individual sites were averaged, with all averaged values < 8 % (range = 3.5 % to 7.4 %) . The CV was lowest for gastrocnemius muscle belly length (3.3 %) and volume (2.8%).

Subcutaneous adipose tissue thickness measurements are reported in Table 3. Gastrocnemius subcutaneous adipose tissue thickness was similar across sites with the mean ranging from 0.40 to 0.43 and the standard deviation ranging from .07 to 1.0. The mean tibialis anterior

subcutaneous adipose tissue thickness ranged from .33 to .43 with a standard deviation ranging from .05 to .09.

Discussion

To our knowledge, the current study is the first to determine the day-to-day, test-retest reliability of ultrasound estimates of leg muscle size and echo intensity in children with CP. The ICC findings suggest that all estimates are good-to-excellent. Together, the generally higher ICC and lower CV values when the estimates of muscle size and echo intensity from individual sites were averaged compared to estimates from the individual sites suggest that a multi-site approach is beneficial. However, ICC values > 0.8 for all sites suggest that even single site protocols may yield reliable estimates of muscle size and echo intensity. The findings are important because children with CP have considerable muscle deficits, irrespective of the level of CP. Moreover, the results will aid in the planning of future studies aimed at determining the effect of different interventions on muscle in children with CP.

Previous studies have evaluated different aspects of reliability for assessment of muscle size and quality in children with CP using ultrasound. For example, one study reported excellent intra-rater and inter-rater reliability (ICC $\geq$ 0.98) for medial gastrocnemius, lateral gastrocnemius and soleus volume estimated from the same images segmented twice by the same rater and by different raters in children with CP (n = 18 children) (Barber et al. 2019). A limited number of studies have evaluated the reliability of muscle size estimates in children with CP that included the reliability of image acquisition. One study found excellent within session reliability for estimating medial gastrocnemius muscle CSA at 40, 50 and 60 % of muscle length in children

with CP using 3D ultrasound (ICC ≥ 0.98), which included evaluation of the acquisition (2 image acquisitions per participant), as well as processing (2 different processors) (Schless et al. 2017). However, the authors noted that a limitation of their study was that between-session (i.e., between-day) reliability was not assessed. Excellent reliability of muscle size estimates by ultrasound has also been demonstrated using inanimate objects (Cenni et al. 2016). The novel results from the present study confirm that ultrasound also yields estimates of muscle size that have good-to-excellent reliability when values acquired on separate days are compared. This is important as, to our knowledge, no previous studies have assessed the day-to-day reliability of ultrasound estimates of CSA in children with CP.

Few previous studies have assessed the reliability of muscle echo intensity in children with CP. The good-to-excellent reliability of echo intensity measurements from ultrasound observed in the present study suggests that ultrasound can also be used to reliably assess this marker of intramuscular fat. The finding is important because a high proportion of intramuscular fat in the legs (Whitney et al. 2017) and intermuscular fat in the thighs (Johnson et al. 2009) has been reported in children with CP and a high proportion of intramuscular fat has been reported in the gastrocnemius and tibialis anterior of young adults with CP (Noble et al. 2014). The advantage of ultrasound vs. MRI is that the measurements are acquired in a more tolerable environment. Furthermore, the expense is considerably less, and the availability is greater. A study by Battisti et al. (2018) used ultrasound to evaluate echo intensity of the gastrocnemius and soleus muscles in children with CP. This study used the Heckmatt scale grade, this is a four grade qualitative scale that evaluates echo intensity: grade I normal, grade II increase in echo intensity while bone echo is still distinct, grade III increase in muscle echo intensity with reduced bone echo, and grade IV very high muscle echo intensity with complete loss of bone echo (Heckmatt et al.

1982). The study reported a score of I and II as most common for the gastrocnemius and a score of III or I for the soleus. A moderate inter-rater relationship was observed for the gastrocnemius, as indicated by a K values between 0.60 and 0.73. However, it should be noted that echo intensity measures using a grading scale are susceptible to subjectivity. In the present study, muscle echo intensity was evaluated objectively based on estimates from grey-scale analysis of ultrasound images. Hence, the stronger reliability in the present study is not surprising.

The current study used ultrasound to measured CSA and echo intensity of the gastrocnemius and tibialis anterior at three separate locations on the muscle 25%, 50%, and 75% for the gastrocnemius; 20% 40%, and 60% for the tibialis anterior. This is similar to previous studies that have collected ultrasound images at multiple locations of skeletal muscle to assess muscle size and quality in children with CP and in healthy populations (Schless et al. 2017, Scott et al. 2017). The collection procedure in the current study can be completed in 30 to 45 minutes by a trained technician and depending on the patient's ability to tolerate ultrasound imaging. When three sites were averaged for CSA or echo intensity in the gastrocnemius and tibialis anterior all measurements of reliability improved when compared to the individual sites. The collection of three measurements for the gastrocnemius and the tibialis anterior reported increased reliability when the three locations where averaged, with reliability increasing to r^2 of .87 to .96 a significant increase from the reliability of the individual sites r^2 of .63 to .78. The reliability of echo intensity also increased when the three sites where averaged, increasing to r^2 of 7.2 for gastrocnemius and r^2 of 4.2 for tibialis anterior. Values for ICC also improved when the three sites were averaged, increasing from .84 to .98. The collection of multiple images for each muscle might increase the total collection time, but as is seen in the current study, with a collection of three images the reliability increases.

It is plausible that subcutaneous adipose tissue thickness had an influence on muscle echo intensity measures from ultrasound in the present study. A study by Young et al. (2015) found that adjustment for subcutaneous adipose tissue thickness improved the relationship between muscle echo intensity measures from ultrasound and the intramuscular fat measures from MRI in adults. If there was an influence of subcutaneous adipose tissue thickness on echo intensity measures in the present study, the effect was likely much smaller than reported in the Young study due to less variability in adipose tissue thickness in adults than in children with CP. This notion is supported by a comparison of adipose tissue thickness data from adults in a study by Wang et al. 2018 and the present study of children with CP. Wang et al. (2018) found that the average adipose tissue thickness at the gastrocnemius in adults with normal muscle mass (.46 cm) and in a adults with low muscle mass (0.43 cm) was similar to that observed in the present study of children with CP (.42 cm). However, the standard deviation of the subcutaneous adipose tissue thickness measurements in the present study (.07 cm) was less than half the standard deviation of the measurements in the study by Wang et al. (2018) (0.17 cm and 0.17 cm, respectively). Nevertheless, future studies that examine the potential influence of subcutaneous adipose tissue thickness on echo intensity measurements from ultrasound in children with CP are warranted.

Limitations of the present study, not already mentioned, must be considered. First, only ambulatory children with CP were included in the study. Therefore, whether the findings are applicable to nonambulatory children with CP is unknown. Second, the age range of the study participants was limited to 5 to 11 years. Whether a similar level of reliability would be present in younger children with CP requires additional study. Third, in addition to the narrow type of

CP and age range of the study participants, the sample size was small. Nevertheless, despite the limited range of the sample, good-to-excellent reliability was observed.

In conclusion, the findings from the present study suggest that ultrasound imaging can reliably estimate the size and echo intensity of the gastrocnemius and tibialis anterior muscles day-to-day in children with CP. The best reliability and detection levels are observed when estimates from multiple sites are averaged; although even a single ultrasound image can provide reasonable estimates of muscle size and quality.

Acknowledgements

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Figure Legends

Figure 1. Set up demonstrating how the foot and leg were position for ultrasound imaging of the gastrocnemius (A) and the tibialis anterior (B). The foot was held in a position at 30 degrees of plantarflexion using a foot plate built in-house. The gastrocnemius was imaged at 25, 50 and 75 % of gastrocnemius belly length while the participant was lying face-down on a plinth. The tibialis anterior was imaged at 20, 40 and 60 % of tibia length while the participant was lying supine. The reliability of the measurements was facilitated by using plastic guides at the regions of interest.

Figure 2. Test-retest gastrocnemius cross-sectional area (CSA) estimates from ultrasound at 25 % (A), 50 % (B) and 75 % (C) of gastrocnemius belly length and the average of the 3 sites (D). All relationships are statistically significant ($p < 0.05$).

Figure 3. Test-retest gastrocnemius echo intensity (EI) estimates from ultrasound at 25 % (A), 50 % (B) and 75 % (C) of gastrocnemius belly length and the average of the 3 sites (D). All relationships are statistically significant ($p < 0.05$).

Figure 4. Test-retest tibialis anterior cross-sectional area (CSA) estimates from ultrasound at 20 % (A), 40 % (B) and 60 % (C) of tibia length and the average of the 3 sites (D). All relationships are statistically significant ($p < 0.05$).

Figure 5. Test-retest tibialis anterior echo intensity (EI) estimates from ultrasound at 20 % (A), 40 % (B) and 60 % (C) of tibia length and the average of the 3 sites (D). All relationships are statistically significant ($p < 0.05$).

Figure 6. Test-retest gastrocnemius belly length (A) and volume (B) estimates from ultrasound. All relationships are statistically significant ($p < 0.05$).

Table 1. Physical characteristics of children with cerebral palsy

	CP (n = 7)
Age (y)	9.9 ± 1.1
Tanner stage (I/II/III/IV/V)	
Pubic hair	4/1/1/1/0
Breast/testicular	4/2/1/0/0
Height (m)	1.40 ± 0.11
Height (%)	57 ± 35
Body mass (kg)	32.1 ± 5.5
Body mass (%)	48 ± 28
BMI (kg/m²)	16.3 ± 1.3
BMI (%)	41 ± 25
GMFCS (I/II)	6/1

Values are mean ± SD; % for height, body mass and BMI reflect the percentile relative to age- and sex-based norms; GMFCS = gross motor function classification system.

Table 2. Test-retest muscle estimates from ultrasound

Measure	Test 1	Test 2	ICC (95 %CI)	CV (%)
Gastrocnemius				
25 % CSA (cm^2)	7.1 ± 2.1	7.4 ± 1.9	.96 (.88, .99)	5.5
50 % CSA (cm^2)	7.4 ± 0.4	7.7 ± 0.5	.97 (.88, .99)	4.6
75 % CSA (cm^2)	2.9 ± 0.3	2.8 ± 0.3	.84 (.49, .95)	16.4
Average CSA (cm^2)	5.8 ± 0.3	5.9 ± 0.4	.98 (.93, .99)	3.5
25 % EI (cm^2)	32.4 ± 2.6	33.7 ± 4.1	.90 (.68, .97)	8.4
50 % EI (cm^2)	36.1 ± 12.7	38.3 ± 11.3	.84 (.52, .95)	12.0
75 % EI (cm^2)	53.6 ± 17.1	58.9 ± 16.4	.85 (.55, .95)	12.5
Average EI (cm^2)	40.7 ± 12.2	43.6 ± 11.7	.95 (.80, .99)	7.4
Muscle Length (cm)	16.7 ± 3.1	16.6 ± 2.8	.97 (.92, .99)	2.8
Volume (cm^2)	97.5 ± 27.5	99.2 ± 29.0	.99 (.97, .99)	3.3
Tibialis anterior				
20 % CSA (cm^2)	2.5 ± 0.7*	2.3 ± 0.6	.90 (.59, .97)	10.0
40 % CSA (cm^2)	3.2 ± 0.9	3.3 ± 0.9	.99 (.96, .99)	3.6
60 % CSA (cm^2)	1.8 ± 1.0	1.9 ± 1.1	.99 (.97, .99)	7.5
Average CSA (cm^2)	2.5 ± 0.8	2.5 ± 0.8	.98 (.97, .99)	4.1
20 % EI (cm^2)	32.2 ± 13.2	32.6 ± 12.0	.95 (.81, .98)	7.2
40 % EI (cm^2)	27.1 ± 10.2	28.6 ± 9.1	.95 (.81, .98)	11.8
60 % EI (cm^2)	25 ± 8.1	25.4 ± 6.9	.92 (.75, .97)	10.7
Average EI (cm^2)	28.1 ± 9.3	28.9 ± 8.8	.97 (.92, .99)	6.6
Tibia length (cm)	31.1 ± 4.3	31 ± 4.6	.99 (.99, .99)	0.9

Test values are reported as means ± SD. *Difference between tests, $p < 0.05$.

Table 3. Subcutaneous adipose tissue thickness

Measure	Mean ± SD
Gastrocnemius	
25 % CSA (cm)	.40 ± .07
50 % CSA (cm)	.44 ± .07
75 % CSA (cm)	.44 ± 1.0
Average 25-75% (cm)	.42 ± .07
Tibialis anterior	
20 % CSA (cm^2)	.40 ± .07
40 % CSA (cm^2)	.33 ± .07
60 % CSA (cm^2)	.43 ± .09
Average 20-60% (cm)	.39 ± .05

CSA = cross-sectional area

Figure 1

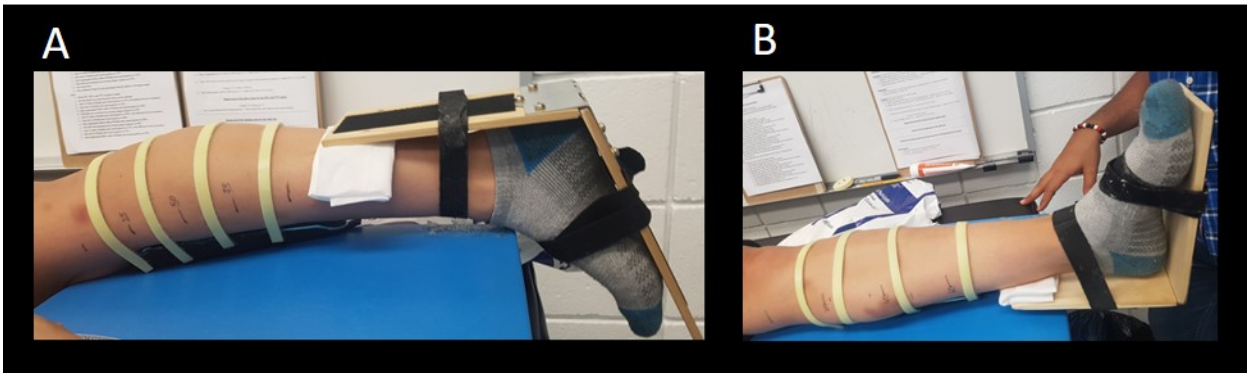


Figure 2.

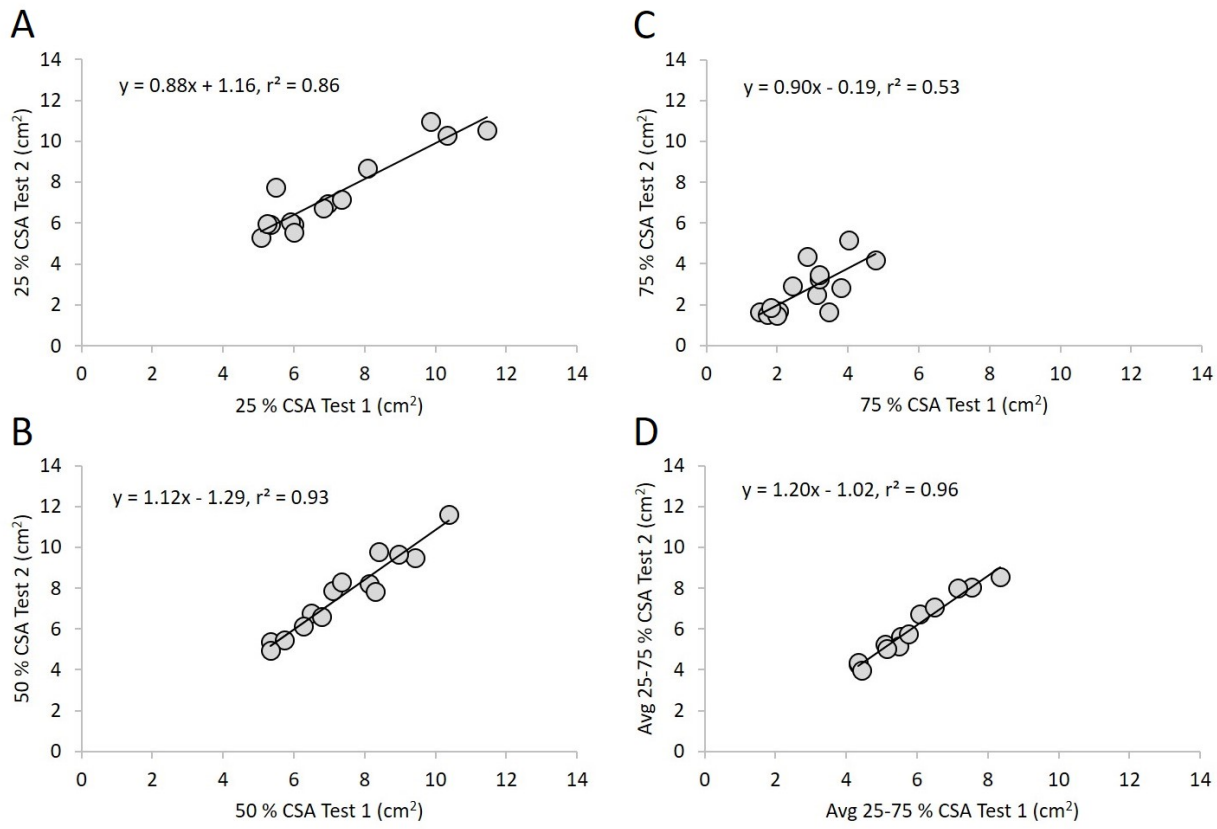

A
y = 0.88x + 1.16, r² = 0.86
25 % CSA Test 2 (cm²)
25 % CSA Test 1 (cm²)
B
y = 1.12x - 1.29, r² = 0.93
50 % CSA Test 2 (cm²)
50 % CSA Test 1 (cm²)
C
y = 0.90x - 0.19, r² = 0.53
75 % CSA Test 2 (cm²)
75 % CSA Test 1 (cm²)
D
y = 1.20x - 1.02, r² = 0.96
Avg 25-75 % CSA Test 2 (cm²)
Avg 25-75 % CSA Test 1 (cm²)

Figure 3.

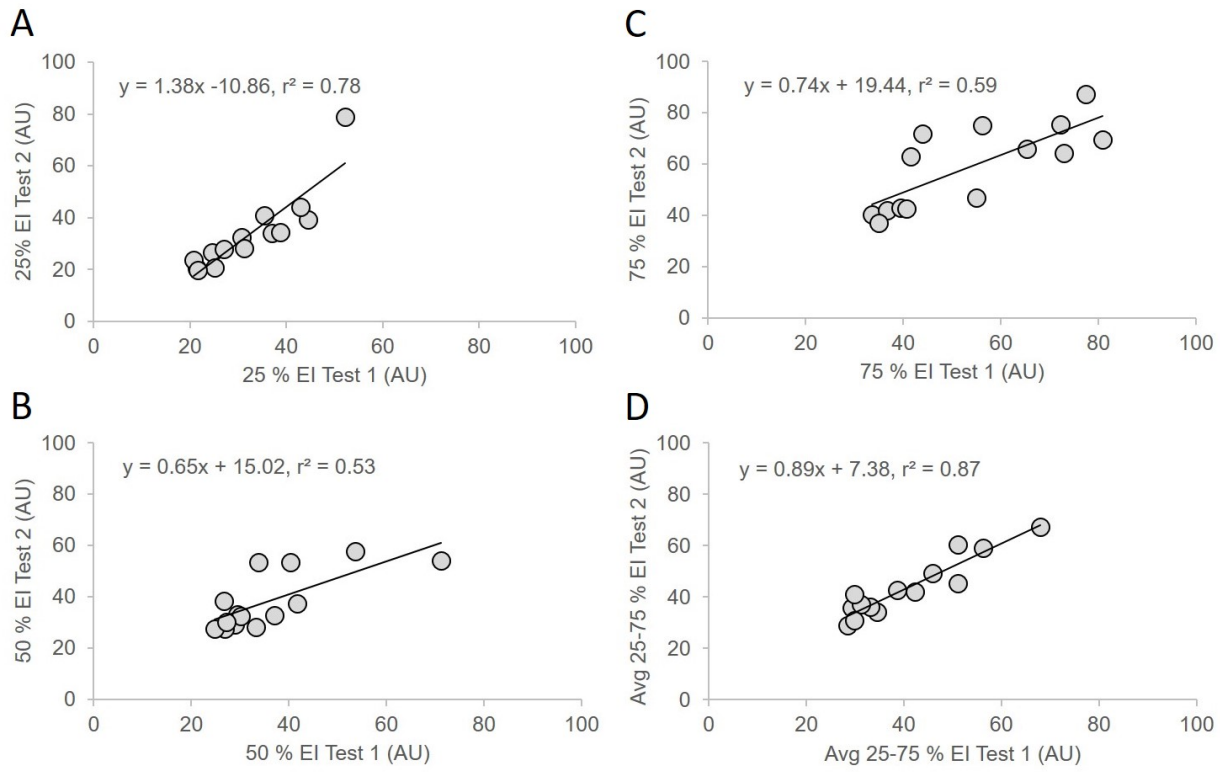

A
y = 1.38x -10.86, r² = 0.78
25% EI Test 2 (AU)
25 % EI Test 1 (AU)
B
y = 0.65x + 15.02, r² = 0.53
50 % EI Test 2 (AU)
50 % EI Test 1 (AU)
C
y = 0.74x + 19.44, r² = 0.59
75 % EI Test 2 (AU)
75 % EI Test 1 (AU)
D
y = 0.89x + 7.38, r² = 0.87
Avg 25-75 % EI Test 2 (AU)
Avg 25-75 % EI Test 1 (AU)

Figure 4

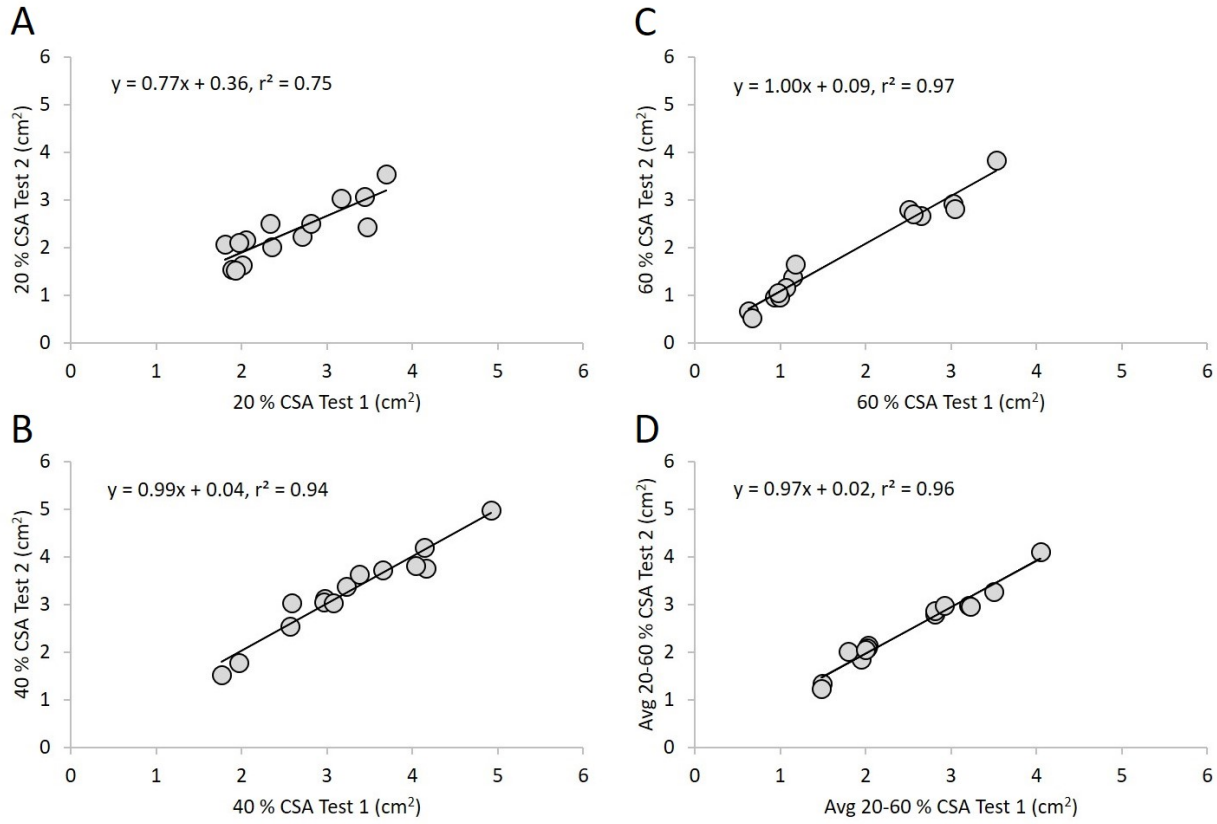

A
y = 0.77x + 0.36, r² = 0.75
20 % CSA Test 2 (cm²)
20 % CSA Test 1 (cm²)
C
y = 1.00x + 0.09, r² = 0.97
60 % CSA Test 2 (cm²)
60 % CSA Test 1 (cm²)
B
y = 0.99x + 0.04, r² = 0.94
40 % CSA Test 2 (cm²)
40 % CSA Test 1 (cm²)
D
y = 0.97x + 0.02, r² = 0.96
Avg 20-60 % CSA Test 2 (cm²)
Avg 20-60 % CSA Test 1 (cm²)

Figure 5.

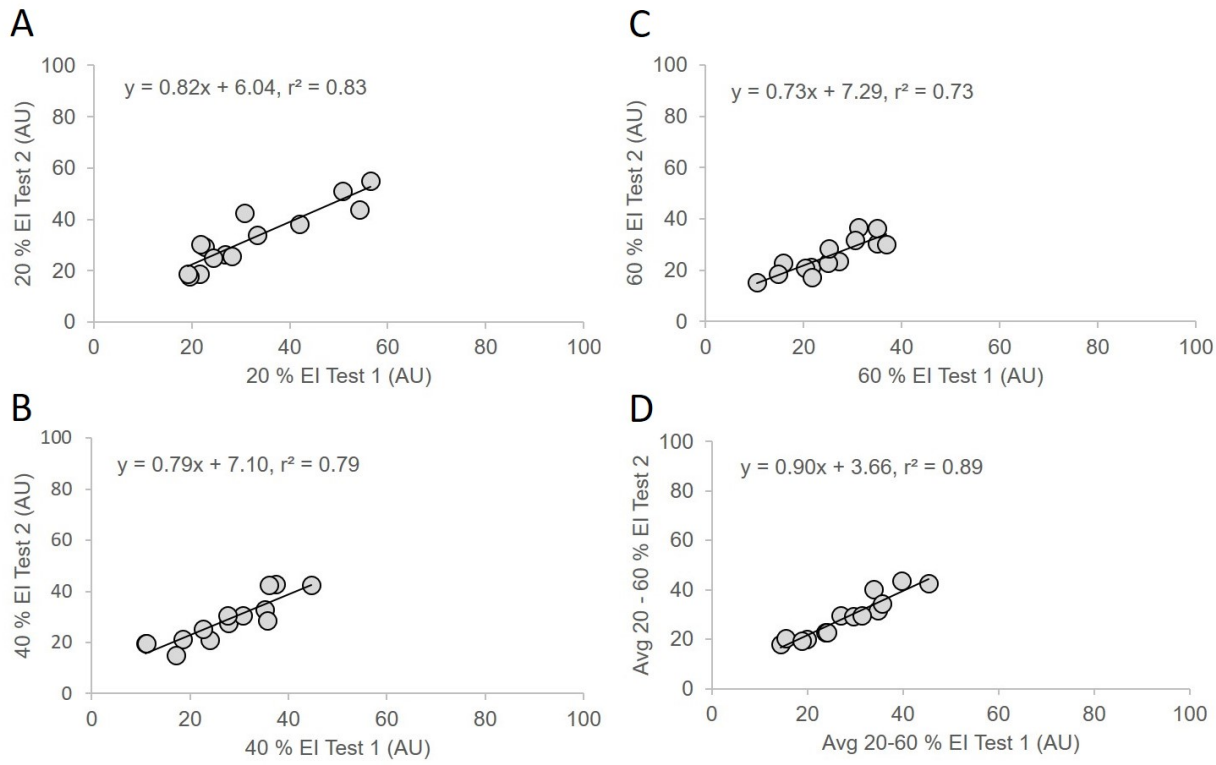
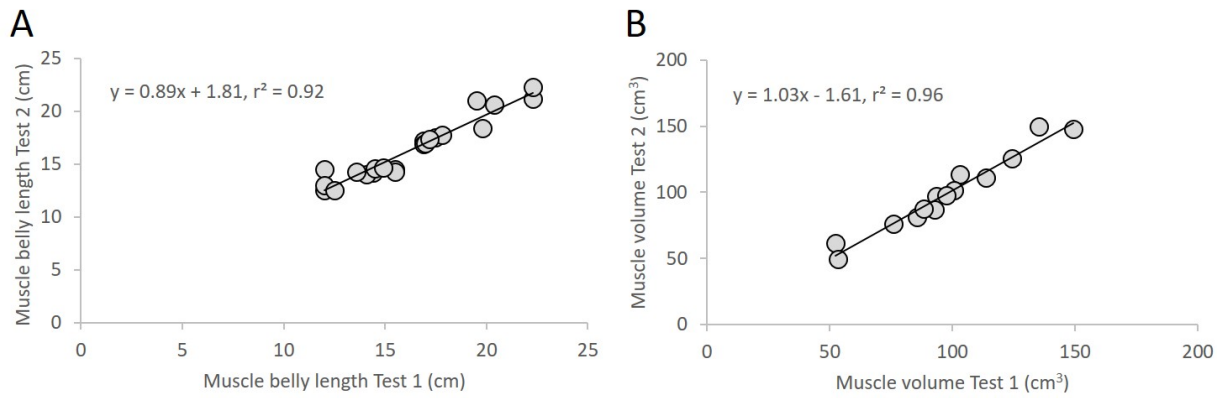

A
y = 0.82x + 6.04, r² = 0.83
20 % EI Test 2 (AU)
20 % EI Test 1 (AU)
C
y = 0.73x + 7.29, r² = 0.73
60 % EI Test 2 (AU)
60 % EI Test 1 (AU)
B
y = 0.79x + 7.10, r² = 0.79
40 % EI Test 2 (AU)
40 % EI Test 1 (AU)
D
y = 0.90x + 3.66, r² = 0.89
Avg 20 - 60 % EI Test 2
Avg 20-60 % EI Test 1 (AU)

Figure 6.


A
y = 0.89x + 1.81, r² = 0.92
Muscle belly length Test 2 (cm)
Muscle belly length Test 1 (cm)
0
5
10
15
20
25
B
y = 1.03x - 1.61, r² = 0.96
Muscle volume Test 2 (cm³)
Muscle volume Test 1 (cm³)
0
50
100
150
200

CHAPTER 4

Conclusions and Summary

The overall objective of this study was to determine if ultrasound provides reliable estimates of leg muscle CSA and leg muscle echo intensity in children with CP. Ultrasound imaging was used to collect cross sectional panoramic images of both the gastrocnemius and tibialis anterior muscles of both legs in children with CP on two separate occasions, one month apart, to determine if ultrasound could provide a reliable estimates of leg muscle size and echo intensity. The study determined that ultrasound provides reliable day-to-day size estimates for the gastrocnemius and tibialis anterior muscles in children with spastic CP, as indicated by very high ICC values. Although the tibialis anterior CSA at the 20% site was reported lower at the retest ($p = .023$), the discrepancy was small and no other test-retest differences were observed. The reliability of the estimates was greatest when data from multiple sites were combined. However, high ICC values indicate that even single site protocols may yield reliable estimates of muscle size and echo intensity.

Echo intensity reflects muscle quality and is related to the amount of adipose tissue located within the muscle that is being scanned. The findings from the present study that echo intensity can be reliably be assessed in the muscles of children with CP is important. Future studies that determine whether echo intensity measurements from ultrasound are related to muscle function in children with CP can be pursued. Furthermore, future studies can determine the effect of different interventions on muscle quality. Although the reliability of muscle size and echo intensity estimates from ultrasound was determined for the gastrocnemius and tibialis anterior,

further studies are needed to determine the reliability of ultrasound estimates of the echo intensity (and size) for other skeletal muscles in children with CP.

In conclusion, ultrasound provides reliable day-to-day estimates of the size and echo intensity of the gastrocnemius and tibialis anterior muscles in children with spastic CP. The findings are important because children with CP have considerable muscle deficits, irrespective of the level of CP. Moreover, the results will aid in the planning of future studies aimed at determining the importance of muscle size and quality on muscle function and the effect of different interventions on muscle in children with CP.

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