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PhD thesis

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What can we learn about muscle contractures in children and adults with cerebral palsy from biomechanics, electrophysiology, and imaging?

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Content

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Preface

The experimental work presented in this thesis was carried out at the Department of Neuroscience at the University of Copenhagen, the Elsass Foundation, and the Department of Diagnostic Radiology at Copenhagen University Hospital. The thesis is based on four original studies, three of which have already been published in international peer-reviewed journals. The fourth study is still in manuscript. The complete manuscripts are available in the thesis appendix.

The thesis starts with a general introduction to cerebral palsy and muscle contractures. The introduction is followed by three separate chapters focusing on treatment, characterization, and the underlying causes of muscle contractures in cerebral palsy. In these chapters, the studies conducted as part of this project are presented and discussed separately. The final part of the thesis is a common discussion and conclusion.

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List of manuscripts in thesis

This thesis is based on the four studies listed below, which are referred to by their respective roman numerals throughout the thesis.

Study I

Nonsurgical Treatment Options for Muscle Contractures in Individuals With Neurologic Disorders: A Systematic Review With Meta-Analysis.
Svane C, Nielsen JB, Lorentzen J.
Archives of Rehabilitation Research and Clinical Translation, 2021; 3 (1).

Study II

Muscle Contractures in Adults With Cerebral Palsy Characterized by Combined Ultrasound-Derived Echo Intensity and Handheld Dynamometry Measures.
Svane C, Forman CR, Rasul A, Nielsen JB, Lorentzen J.
Ultrasound in Medicine and Biology, 2022; 48 (4).

Study III

Quantitative MRI and clinical assessment of muscle function in adults with Cerebral Palsy.
Svane C, Forman CR, Rasul A, Nielsen CH, Nielsen JB, Lorentzen J.
Frontiers in Neurology, 2021; 12.

Study IV

Impaired muscle growth and early signs of muscle contractures in children with cerebral palsy: a longitudinal study.
Svane C, Willerslev-Olsen M, Ritterband-Rosenbaum A, Tholin MW, Justiniano MD, Lorentzen, J, Nielsen JB.
Preliminary manuscript. Will be submitted to the journal 'Developmental Medicine & Child Neurology'.

English summary

Muscle contractures are a common complication of cerebral palsy. They limit movement and in severe cause fixated joints in shortened positions. As a consequence, muscle contractures can be very debilitating and considerably reduce both the functional abilities and the quality of life of those affected. It was the purpose of this PhD project to use biomechanical, electrophysiological, and imaging techniques to characterize and quantify muscle contractures in children and adults with cerebral palsy as a foundation for future intervention studies.

In the first study, we systematically reviewed current nonsurgical treatment options for muscle contractures. In this review, we showed that there was insufficient evidence to support the effectiveness of existing nonsurgical treatment options and found only few studies that used objective measures to quantify muscle contractures. Objective measures are necessary to accurately determine the effectiveness of therapies and to investigate the development and functional significance of muscle contractures. In studies two and three, we used imaging techniques, hand-held dynamometry, and tests of muscle function to explore the muscle composition and functional abilities of adults with cerebral palsy. We found that ultrasound-derived echo intensity accurately predicted the presence of muscle contractures and therefore propose using echo intensity as a screening tool for muscle contractures. Furthermore, as muscle composition, but not muscle volume, measured using magnetic resonance imaging was closely related to functional abilities in adults with cerebral palsy, it could constitute an important anatomical marker of muscle function. In the fourth study, these objective measures were used to obtain new evidence of the pathophysiological processes leading to muscle contractures. Using a longitudinal approach, we showed that impaired muscle growth in early childhood is implicated in the development of muscle contractures in children with cerebral palsy.

The present thesis discusses the importance of quantitatively and objectively assessing the development, characteristics, and functional consequences of muscle contractures. Such assessment is essential in order to detect early development of muscle contractures and evaluate the effectiveness of new preventive measures or treatments.

The thesis argues that prevention of muscle contractures could require the implementation of interventions aimed at stimulating muscle growth during a specific period in early childhood.

Dansk resumé

Mange personer med cerebral parese lider af muskelkontrakturer. Da muskelkontrakturer begrænser bevægelighed og i svære tilfælde forårsager fikserede led, kan de være meget invaliderende og påvirke både funktionsevne og livskvalitet betydeligt. Formålet med dette ph.d.-projekt var at anvende biomekaniske, elektrofysiologiske og billeddannende teknikker til at karakterisere og kvantificere muskelkontrakturer hos børn og voksne med cerebral parese som grundlag for fremtidige interventionsstudier.

I projektets første studie gennemgik vi systematisk nuværende ikke-kirurgiske behandlingsmuligheder for muskelkontrakturer. Her viste vi, at der ikke er tilstrækkelig evidens til at understøtte anvendelsen af de eksisterende ikke-kirurgiske behandlingsmuligheder, og fandt at kun få studier kvantificerede muskelkontrakturer ved brug af objektive målemetoder. Objektive målemetoder er nødvendige for præcist at kunne bestemme effekten af behandling samt undersøge udviklingen og den funktionelle betydning af muskelkontrakturer. I studie to og tre brugte vi ultralyd og magnetisk resonans, håndholdt dynamometri og test af muskelfunktion til at kvantificere muskelsammensætning og funktionelle evner hos voksne med cerebral parese. Vi fandt at ekkointensitet målt med ultralyd nøjagtigt forudsagde tilstedeværelsen af muskelkontrakturer og foreslår derfor at ekkointensitet kan bruges som et screeningsværktøj for muskelkontrakturer. Derudover fandt vi, at muskelsammensætning, men ikke muskelvolumen, målt ved magnetisk resonans var tæt relateret til funktionelle evner hos voksne med cerebral parese og derfor kan udgøre en potentielt vigtig anatomisk markør for muskelfunktion. I det fjerde studie brugte vi disse objektive mål til at undersøge de patofysiologiske mekanismer, der fører til muskelkontrakturer. Her viste vi, at nedsat muskelvækst i en specifik periode i den tidlige barndom er involveret i den senere udvikling af muskelkontrakturer hos børn med cerebral parese.

I afhandlingen diskuteres vigtigheden af objektivt at kvantificere udvikling, karakteristika og funktionelle konsekvenser af muskelkontrakturer. En sådan kvantifikation er afgørende for tidligt at kunne opdage udvikling af muskelkontrakturer samt evaluere

effektiviteten af nye forebyggende interventioner og behandlinger. I afhandlingen argumenteres der for, at forebyggelse af muskelkontrakturer kan kræve implementering af interventioner rettet mod at stimulere muskelvækst i en specifik periode i den tidlige barndom.

Introduction

Cerebral palsy

Cerebral palsy is the most common cause of physical disability in children. Based on registry data from European countries, including Denmark, 2-3 children per 1000 live births are later diagnosed with cerebral palsy (Cans 2000; Oskoui et al. 2013; Graham et al. 2016). Premature birth is the most significant risk factor; the estimated prevalence of cerebral palsy in children born below 28 weeks of gestation is approximately fifty times higher than in full-term births (Cans 2000; Reid et al. 2016).

Although the exact definition of cerebral palsy has changed several times during the past century, all proposed definitions have described it as a movement and posture disorder caused by a non-progressive disturbance of the developing brain. The most recent definition was proposed by an international panel in 2005 and has since been widely accepted in the scientific community: “Cerebral palsy describes a group of permanent disorders of the development of movement and posture, causing activity-limitation, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are accompanied by disturbances of sensation, perception, cognition, communication and behaviour, by epilepsy and by secondary musculoskeletal problems.” (Bax et al. 2005). Thus, the most recent definition of cerebral palsy allows appreciation of more than just movement and posture disorders. As cerebral palsy per definition refers to a rather broad group of conditions (with certain key features in common), rather than a specific disease entity, subclassification is necessary to allow adequate description of individuals, comparison of similar groups, and targeted rehabilitation (Graham et al. 2016).

Classification of cerebral palsy

Most commonly, cerebral palsy is subclassified according to motor disorder, anatomical distribution, and gross motor function level, but the disorder can also be subclassified according to e.g., communicative skills or eating and drinking abilities (Paulson and Vargus-Adams 2017).

The type of motor disorder is classified as either spastic, ataxic, dyskinetic, or mix-type (Cans 2000; Cans et al. 2002). To be classified with spastic cerebral palsy, individuals must exhibit at least two of three symptoms: abnormal pattern of posture/movement, increased muscle tone, and/or pathological reflexes. In ataxic cerebral palsy, individuals exhibit an abnormal pattern of posture/movement and loss of muscular coordination, causing movements to be performed with abnormal force, rhythm, and accuracy. In dyskinetic cerebral palsy, individuals exhibit abnormal patterns of posture/movements in addition to involuntary, uncontrolled, and recurring movements. When no single movement disorder pre-dominates, individuals are classified with mixed type cerebral palsy. The most frequent mixed type is a combination of spastic and dyskinetic cerebral palsy. With a prevalence of ~85%, spastic cerebral palsy is by far the most common subtype (Cans et al. 2002; Wimalasundera and Stevenson 2016).

The anatomical distribution of limb involvement in cerebral palsy is classified simply as unilateral or bilateral. In unilateral cerebral palsy only the limbs on one side of the body are affected, whereas the limbs on both sides of the body are affected in bilateral cerebral palsy (Cans et al. 2007).

The Gross Motor Function Classification System (GMFCS) was developed to classify the gross motor function of infants, children, and adolescents with cerebral palsy (Palisano et al. 1997). Although originally developed to be used only until the age of 18, the GMFCS is often used to classify the gross motor function of adults with cerebral palsy too. The GMFCS is scored from level I-V based on the severity of the functional impairments. Individuals aged six or older classified as GMFCS level I walk independently without aid or adaptive equipment, level II walk with limitations but without the need for adaptive equipment, level III walk with the use of hand-held adaptive equipment, level IV are self-mobile only with significant limitations, and level V have limitations impairing all voluntary movement and rely heavily on adaptive equipment, assistive technology, and other people (Palisano et al. 1997, 2008). Generally speaking, levels I-II reflect minor functional impairments, level III reflects moderate functional impairments, and levels IV-V reflect severe functional impairments. Minor functional impairments (GMFCS levels I-II) are more prevalent than severe functional impairments (GMFCS levels IV-V) (Carnahan et al. 2007; Opheim et al. 2009;

Burgess et al. 2021). Whereas children with mild functional impairments increase their functional abilities (gross motor function measure (GMFM-66) score) throughout childhood, the functional abilities of children with severe impairments seem to stabilize – or even deteriorate – after the age of 2-3 years (Rosenbaum et al. 2002; Burgess et al. 2021).

Muscle contractures

Although the underlying neural disturbance underlying cerebral palsy is non-progressive, the secondary functional complications may progress over time. As these progress, functional abilities, such as walking, deteriorate at a much faster rate than in typically developing peers (Opheim et al. 2009). Muscle contractures – one such functional complication – are often linked to the deterioration of walking abilities (Eek and Beckung 2008; Hanna et al. 2009; Theologis 2013). A recent simulation study showed that normal gait may in fact be biomechanically difficult to obtain for individuals with severe muscle contracture development (Fox et al. 2018).

Muscle contractures represent a distinct muscle adaptation characterized by increased passive muscle stiffness and limited joint mobility with little or no active force production (Smith et al., 2011). As muscle contractures cause issues such as fixated joints in shortened positions, limited use of affected limbs, strength loss, muscle atrophy, and rapid fatigue, the complication can be very debilitating and considerably reduce both the functional abilities and the quality of life of affected individuals (Lindsay, 2016; Smith et al., 2011).

With an estimated prevalence of more than 30%, muscle contractures are a very common complication in individuals with cerebral palsy (Makki et al. 2014; Horsch et al. 2019; Krarup et al. 2021). Muscle contractures are more prevalent in children with severe functional impairments (GMFCS levels IV-V) than in children with mild functional impairments (GMFCS levels I-II) (Hägglund and Wagner 2011; Krarup et al. 2021). A study by Krarup et al. found no age-related differences in the prevalence of muscle contractures in children between the age of 6 and 14 years (Krarup et al. 2021), suggesting that muscle contractures might develop early in childhood. This idea is

supported by studies reporting early signs of muscle contractures in children as young as ~2 years (Barber et al. 2011; Willerslev-Olsen et al. 2018; Horsch et al. 2019). In addition to cerebral palsy, the development of muscle contractures is common in other neurological disorders such as stroke, spinal cord injury, and multiple sclerosis, and in muscle diseases such as muscular dystrophy and spinal muscular atrophy (Sackley et al. 2008; Diong et al. 2012; Hoang et al. 2014; Baranello et al. 2021; Houwen-van Opstal et al. 2021).

As the consequences of muscle contractures can be immense, and as they become increasingly debilitating and common throughout the life of those affected, the initiation of early and effective preventive measures or treatments are of the upmost importance.

Aim of the PhD project

The purpose of this PhD project was to characterize and quantify muscle contractures in children and adults with cerebral palsy using biomechanical, electrophysiological, and imaging techniques. Specifically, we systematically reviewed current nonsurgical treatment options for muscle contractures (study I) and used a range of objective measures to characterize and quantify the presence (study II), functional significance (study III), and development (study IV) of muscle contractures. We hope that the knowledge gained from this project will serve as a foundation for future intervention studies.

Treatment of muscle contractures

Muscle contractures are primarily treated using surgical procedures in which the range motion (ROM) of the affected joint is effectively increased through either tendon lengthening or aponeurotic recession (Dreher et al. 2012; Li et al. 2017). Although these procedures can be used to effectively increase the ROM for some time, the recurrence rate is high and many individuals therefore need surgeries on a regular basis in order to maintain the ROM of the contractured joints – especially during periods of growth (Dietz et al. 2006; Joo et al. 2011; Krupiński et al. 2015; Kläusler et al. 2017). In addition to the mental and physical strain of repeated surgery, the procedures cause biomechanical changes to muscles and joints that require the operated individuals to 'relearn' motor tasks with every surgery. Effective nonsurgical treatment of muscle contractures would therefore be an advantageous alternative.

A wide range of nonsurgical treatment options – including different types of muscle stretching, physical activity of all sorts, electrical stimulation, and shockwave treatment – have been proposed within the past ~20 years. The effect of most of these is however yet to be critically and systematically evaluated (Mori et al. 2014; Harvey et al. 2017). This was the aim of study I of this project.

Study I - The effect of nonsurgical treatment options for muscle contractures

In study I, we investigated the effect of all currently available nonsurgical treatment options for muscle contractures in individuals with neurologic disorders through a large-scale systematic review. We hope that by investigating the effect of all nonsurgical treatment options in a single, comprehensive review, clinicians will gain a better understanding of the field.

Through systematic searches in the scientific databases Embase, MEDLINE, Cumulative Index to Nursing (CINAHL), and Allied Health and Physiotherapy Evidence Database (PEDro), we identified ~8,000 trials. Of these, 70 studies fulfilled our PICO (participants, interventions, comparisons, outcomes)-criteria and were included in the

review. The studies covered six main types of interventions: stretching (22 studies), shockwave (6 studies), botulinum toxin (8 studies), electrical stimulation (9 studies), physical activity (10 studies), and robot-assisted rehabilitation (5 studies). Using the Downs and Black checklist, we evaluated the evidence quality of all included studies (Downs and Black 1998). We chose this checklist because it is applicable to both randomized and non-randomized studies. The treatment effects of all main types of interventions were quantified using meta-analyses. As the majority of the included studies quantified muscle contractures using passive ROM measures, the meta-analyses were performed based on these results. The pooled passive ROM from the randomized controlled studies was chosen as the primary outcome measure. All meta-analyses were performed using a random-effects model. We considered treatment effects of > 5° as clinically important.

The meta-analyses revealed no short-term (up to one week after intervention) effects of either shock wave therapy (2°, confidence interval (CI) = -5°, 10°; p = 0.56), physical activity (3°, CI = -2°, 8°; p = 0.28), electrical stimulation (3°, CI = -1°, 6°; p = 0.13), or botulinum toxin treatment (4°, CI = -1°, 8°; p = 0.13), and showed only small, clinically nonimportant effects of stretch (3°, CI = 1, 4°; p < 0.001) and robot-assisted rehabilitation (1, CI = 0, 2; p = 0.03). This was in agreement with previous reviews investigating the effect of stretch and shockwave therapy (Mori et al. 2014; Harvey et al. 2017). The evidence quality of the included studies was moderate to high according to the Downs and Black checklist. However, and importantly, only 18 of 70 trials quantified muscle contractures using proper objective measures. Most of the trials quantified muscle contractures using a simple, manual goniometer. In 23 studies, the goniometric measurements were obtained without assessor-blinding, increasing the risk of bias.

The findings of the first study of this PhD project do not support the use of any of the reviewed nonsurgical treatment options. Furthermore, this study showed that only a few studies used proper objective measures to quantify muscle contractures. We believe that the field will greatly benefit from the implementation of proper objective measures of muscle contractures in future intervention studies. The use of such measures could decrease the variability and risk of bias, aid the detection of early signs of muscle contracture development, and lead to better targeted treatment.

Characterization and quantification of muscle contractures

Generally, the severity of muscle contractures is quantified by using either biomechanical or anatomical measures. Biomechanical measures are used to quantify either the passive ROM of affected joints or the resistance against passive movement of a joint (often referred to as passive muscle stiffness), whereas anatomical measures are used to quantify the intramuscular accumulation of fibrous tissue, which has been found to be increased in contractured muscle (Booth et al. 2001; Mathewson and Lieber 2015; Smith et al. 2021).

Biomechanically, the easiest method of quantifying muscle contractures is to use a simple (often plastic) goniometer to measure the passive ROM of contractured joints. In study I, we found this to be the most common method. There are several issues with this approach to muscle contracture quantification. Although this method is sufficient when wanting to identify the presence of severe muscle contractures in which the passive ROM is substantially decreased, the precision does not allow identification of less severe muscle contractures or the detection of smaller changes in the severity of muscle contractures (i.e., changes of 0-10 degrees in joint mobility). Further, as the assessor must directly interpret the result of the goniometric measurement, the use of this method introduces a risk of both bias and human error. Assessor blinding is therefore always preferred. However, as observed in study I, this is often not the case. Simple goniometric measurements might be useful as a rough screening tool but should not be used to quantify treatment effects or the development in muscle contracture severity over time. Instead, goniometric measurements obtained using digital, and preferably torque-controlled, measures should be used. These measures can be obtained using either hand-held or stationary devices (Boiteau et al. 1995; Lamontagne et al. 1998; Lee et al. 2002; Bénard et al. 2010; Bar-On et al. 2013; Yamaguchi et al. 2018). Many of these devices – hand-held or stationary – double in function as they can also be used to quantify the resistance during goniometric measurements, i.e., the passive stiffness of a muscle. Increased passive muscle stiffness is an essential characteristic of muscle contractures and has been proposed to develop before the

onset of measurable limitations to the passive ROM of affected joints (Willerslev-Olsen et al. 2018). Thus, measurements of passive muscle stiffness could be especially important in order to detect early signs of muscle contractures.

As contractured muscles are characterized by an increase in fibrous tissue (Booth et al. 2001; Smith et al. 2011), the presence of muscle contractures can anatomically be measured using muscle biopsies. The advantage of this approach is the precision by which the fibrous tissue content, indicative of muscle contracture formation, can be quantified. The method does however come with several disadvantages. Firstly, the method is invasive and therefore not possible (or at least not desirable) to do on a regular basis. Secondly, although it is possible to precisely quantify the amount of fibrous tissue in a muscle biopsy, it only represents a very small section of the muscle. Elaborating on the general fibrous tissue content of the muscle in question based on a single muscle biopsy is therefore associated with some uncertainty. More general imaging methods such as magnetic resonance imaging (MRI) and ultrasound can also be used to quantify the composition of skeletal muscle. These methods might not have the precision of muscle biopsy measurements, but as they are non-invasive and can be used to quantify the composition of entire muscles (or muscle groups) they might be an advantageous alternative.

In study II and study III, we used ultrasound and MRI-derived measures of muscle composition to perform functionally relevant anatomical examinations of contractured muscles.

Study II – Using ultrasound-derived echo intensity to characterize muscle contractures

In study II, we investigated the use of ultrasound-derived echo intensity to characterize muscle contractures. We included 11 adults with cerebral palsy (GMFCS level I-II; mean age = 41 years, SD = 12 years; 7 female) and 11 neurologically intact adults (mean age = 35 years, SD = 10 years; 5 female) in the study.

In recent years, echo intensity has been adopted as a measure of skeletal muscle composition across a wide span of research areas and in individuals both with and without neurological disorders (Stock and Thompson 2021). The idea behind echo intensity is that contractile tissue (muscle tissue) appears black in obtained images while non-contractile tissue (fat and fibrous tissue) appears white when observed using selected MRI protocols, computed tomography, or, as in this study, brightness mode ultrasound (Stock and Thompson 2021). This color difference allows quantification of echo intensity on a grayscale reflecting the general composition of a specific muscle or specific area of a muscle. Low (dark) echo intensity values are thus indicative of large relative amounts of muscle tissue, whereas higher (lighter) echo intensity values are indicative of larger amounts of fat and fibrous tissue. Echo intensity measurements can be used to compare the muscle composition within and between subjects. Here, it should however be noted that the echo intensity is heavily affected by device parameter settings and that these therefore cannot differ. Although echo intensity has been quickly adopted across a wide range of research areas and used in an ever-rising number of studies, the extent to which different tissue types affect the echo intensity is still debated. Some suggest that the echo intensity value primarily reflects accumulation of fatty tissue (Young et al. 2015; Akima et al. 2016) while others argue that also the fibrous tissue content makes a significant contribution (Pillen et al. 2009; Arts et al. 2012). This discrepancy could be explained by the fact that the amount of fibrous tissue is relatively homogeneous in the neurologically intact population, meaning that the variability in echo intensity therefore could be largely explained by the variability in fatty tissue content. In populations with muscle contractures and presumably increased fibrous tissue content, such as the cerebral palsy population, it seems more likely that fibrous tissue could significantly affect the echo intensity.

In this study, we obtained echo intensity measurements of the medial gastrocnemius muscle using brightness mode ultrasound and used the hand-held dynamometer PSAD (portable spasticity assessment device) (Yamaguchi et al. 2018) to obtain measurements of ankle joint passive ROM and plantar flexor passive muscle stiffness – biomechanical measures of muscle contractures. In individuals with cerebral palsy, all measurements were obtained from the most affected side. In neurologically intact adults, measurements were obtained from the right side.

Although unpaired t-tests and Fisher's tests showed no significant differences in age, weight, height, body mass index, or sex distribution between groups ($p = 0.3$), the echo intensity ($p = 0.02$) and passive muscle stiffness ($p < 0.001$) was significantly higher, and the passive ROM ($p < 0.001$) significantly lower, in adults with cerebral palsy compared to neurologically intact adults. This was expected and indicates that the adults with cerebral palsy who were included in this study did in fact have muscle contracture development and increased intramuscular infiltration of fat and fibrous tissue. Interestingly, we also found echo intensity to correlate with both passive muscle stiffness ($r = 0.57$, $p = 0.006$) and passive ROM ($r = 0.56$, $p = 0.006$) – more established biomechanical measures of muscle contractures. Thus, ultrasound-derived echo intensity seems to be related to the presence of muscle contractures and therefore presumably fibrous tissue content (Smith et al. 2011; Mathewson and Lieber 2015).

The use of echo intensity to quantify fibrous tissue content in populations with increased levels of fibrous tissue has been the focus of two previous studies on animals (Pillen et al. 2009) and humans (Arts et al. 2012) respectively. Pillen et al. (Pillen et al. 2009) reported a strong correlation ($r = 0.87$, $p < 0.01$) between measurements of ultrasound-derived echo intensity and fibrous tissue content in dogs with muscular dystrophy, a condition that causes a large increase in intramuscular fibrous tissue content. Arts et al. (Arts et al. 2012) used a similar approach in a subject with multiple sclerosis and also found a strong correlation ($r = 0.86$, $p = 0.007$) between ultrasound-derived echo intensity and fibrous tissue content. Interestingly, as this correlation remained ($r = 0.81$, $p = 0.026$) after correcting for fat content and as a significant correlation was not found between echo intensity and fat content, the authors argue that fibrous tissue content seems to be the most important determinant of echo intensity in multiple sclerosis. As

individuals with multiple sclerosis have a similar development of muscle contractures, this might be transferable to the cerebral palsy population.

Study II showed that contractured muscle is both anatomically and biomechanically different from muscle not affected by muscle contractures. These findings add to an increasing body of evidence suggesting that fibrous tissue significantly (and critically) affects echo intensity in populations with heterogeneous amounts of intramuscular fibrous tissue content such as the muscular dystrophy, multiple sclerosis, and cerebral palsy populations. As the effect of different tissue types is not yet fully clarified and must be at least partly affected by intramuscular fat content even in populations with increased intramuscular fibrous tissue content, echo intensity should not stand alone as a measure of muscle contractures. However, we see a great potential in using ultrasound-derived echo intensity as either a general measure of muscle composition, in combination with an objective biomechanical measure, or as a screening tool for muscle contractures. As ultrasound devices are both portable, affordable, and already widely used in the clinic, the use of ultrasound-derived echo intensity should be easily implementable in both the clinic and in research studies.

Study III – The functional transferability of MRI-obtained measures of muscle volume and composition

In study III, we investigated the functional transferability of quantitative MRI measures in the cerebral palsy population. This study was based on a separate set of experiments performed in the same group of participants that were included in study II. Thus, 11 adults with cerebral palsy (GMFCS level I-II; mean age = 41 years, SD = 12 years; 7 female) and 11 neurologically intact adults (mean age = 35 years, SD = 10 years; 5 female) also participated in study III.

Functional abilities and muscle strength are closely related to the volume of the muscle in neurologically intact children and adults (Lieber and Fridén 2000). In cerebral palsy however, this relationship appears to be less clear (Reid et al. 2015). This might partly be attributed to a difference in muscle composition in the two populations. Individuals with cerebral palsy have a greater accumulation and variability in the amount of fat and fibrous tissue due to factors such as sedentary lifestyle and muscle contracture development (Smith et al. 2011; Makki et al. 2014; Noble, Charles-Edwards, et al. 2014). As noncontractile tissue accumulation complicates the relationship between muscle size and function, measures of muscle composition may be a better predictor of functional abilities in cerebral palsy.

In this study, we used a 3.0 Tesla MRI scanner to measure the muscle volume and composition of the ankle plantar flexors on both the left and right side of all participants. We obtained measures of muscle volume by drawing the region of interest (medial gastrocnemius) on T1-weighted axial image slices of both legs. The region of interest drawings were subsequently overlaid images acquired using the Dixon two-point technique (Dixon 1984). The Dixon two-point technique generates two images from a modified spin-echo pulse sequence (Dixon 1984). Water and fat are in phase in one of these images, while there is a 180° phase difference in the other. These images were then used to calculate the fat-water ratio (fat fraction) of the plantar flexor muscles as a measure of muscle composition (Dixon 1984; Noble, Keevil, et al. 2014). We quantified lower body functional abilities as the torque elicited by a maximal voluntary plantarflexion (MVC) and as the jump height of a countermovement jump (CMJ). Thus,

we obtained a rather isolated measure (MVC) and a more general (CMJ) measure of functional abilities.

We found that the medial gastrocnemius muscle volume was significantly decreased ($p < 0.001$) and the fat fraction significantly increased ($p = 0.002$) in adults with cerebral palsy compared to neurologically intact adults. Furthermore, both the isolated ($p < 0.001$) and general measure ($p < 0.001$) of functional abilities were significantly decreased in adults with cerebral palsy. In neurologically intact adults, the muscle volume significantly correlated with functional abilities (MVC: $r = 0.66$, $p = 0.02$; CMJ: $r = 0.69$, $p = 0.02$). In adults with cerebral palsy however, we were unable to find this relation (MVC: $r = 0.36$, $p = 0.31$; CMJ: $r = 0.06$, $p = 0.9$). We found, on the other hand, that muscle composition was closely related to general functional abilities (CMJ: $r = 0.75$, $p < 0.01$) in adults with cerebral palsy.

In line with previous research (Lieber and Fridén 2000) and the general notion that a larger muscle is a stronger muscle, we found that muscle volume was closely related to functional abilities in neurologically intact adults. Similarly to the findings of Reid et al. on children with cerebral palsy however (Reid et al. 2015), we did not find a similar relation in adults with cerebral palsy. This could be explained by the difference in muscle composition between the groups. In the adults with cerebral palsy, the accumulation of noncontractile tissue was both larger and more variable than in neurologically intact adults. In support of this, we found MRI measures of muscle composition to be more closely related to functional abilities than MRI measures of muscle volume in adults with cerebral palsy. The findings of study III therefore suggest that muscle composition measured using MRI could represent an anatomical marker for reduced functional abilities in adults with cerebral palsy. Future studies should seek to explore this relation further in both children and adults with cerebral palsy.

The development of muscle contractures

Although the underlying pathophysiological mechanisms are yet to be fully understood, most research indicates that the passive muscle stiffness and reduced joint mobility associated with muscle contractures are related to structural muscle sarcomere alterations and a change in the extracellular matrix structure.

Muscle sarcomeres, defined as the region between two Z-discs, are the smallest functional contractile units in human skeletal muscle (Howard and Herzog 2021; Howard et al. 2022). Muscle sarcomeres are arranged in series and consist of actin and myosin filaments. According to the sliding filament theory, which was first proposed in 1954 by Huxley and Niedergerke (Huxley and Niedergerke 1954), muscle contraction is imposed by the binding and sliding of these myosin and actin filaments. The myosin and actin filaments overlap to different degrees depending on the length of the sarcomere (Howard and Herzog 2021). Muscle sarcomeres have consistently been found to be highly stretched in the contractured muscles of individuals with cerebral palsy (Lieber and Fridén 2002, 2019; Lieber et al. 2005; Smith et al. 2011). This stretch of muscle sarcomeres causes a decrease in actin-myosin overlap, which skews the muscle length-tension curve and results in low force production capabilities for a large part of the ROM (Gordon et al. 1966; Lieber and Ward 2011) – and partly explains why contractured muscles are generally weak. Muscle sarcomeres stretched to the degree reported by Lieber et al. (Lieber and Fridén 2002; Lieber et al. 2005) and Smith et al. (Smith et al. 2011) (3.5-4.0 μm as compared to ~2.5 μm in typically developing muscle (Gordon et al. 1966)) would result in only ~20% of normal force production (Lieber and Fridén 2019).

Muscle sarcomeres are serially arranged in myofibrils, the contractile organelles of muscle fibers (Howard and Herzog 2021). As muscles affected by contractures are generally shorter than typically developed muscles, the finding of highly stretched sarcomeres initially appears counterintuitive. This does however seem to be explained by the presence of fewer sarcomeres in series in contractured muscles (Smith et al. 2011; Mathewson and Lieber 2015). The fewer sarcomeres in series are thus stretched to a higher degree. When the reduced number of sarcomeres in series cannot be

compensated by increased sarcomere stretch, the myofibrils are effectively shortened, resulting in shortened muscle fibers and reduced total muscle length. The deviations in the number and length of muscle sarcomeres in contractured muscle compared to typically developing muscle have been suggested to represent a change in tissue homeostasis, likely involving signals of both cellular, molecular, and neural origin (Pingel et al. 2017; Lieber and Fridén 2019).

A complex network of collagens, glycoproteins, proteoglycans, and elastin make up the extracellular matrix of human skeletal muscles (Csapo et al. 2020). The collagen-based part of this network is generally thought to be divided into three different layers: the endomysium, the perimysium, and the epimysium (Csapo et al. 2020). The endomysium encloses individual muscle fibers, the perimysium contains muscle fiber bundles, and the epimysium encloses the entire muscle. The stiffness of muscle fiber *bundles*, including the associated collagen-based parts of the extracellular matrix, seems to be increased in contractured muscle, despite the fact that *single* muscle fiber stiffness, not including the extracellular matrix, is comparable to that of typically developed muscle (Smith et al. 2011). This finding has led to the idea that the extracellular matrix is involved in the passive stiffness associated with muscle contractures. As the general collagen content of the extracellular matrix is drastically increased in contractured muscle, most studies report a three to five-fold increase, collagen is suspected to be implicated in this increased stiffness (Mathewson and Lieber 2015; Lieber and Fridén 2019; Smith et al. 2021). Because the extracellular matrix is made up of many different types of collagen isotypes as well as other structural proteins, a general measure of collagen accumulation may not adequately describe the variation in muscle stiffness in a population (Csapo et al. 2020; Pingel, Kampmann, et al. 2021; Smith et al. 2021). In a recent publication on this matter, Smith et al. (Smith et al. 2021) described how muscle stiffness can be rather precisely predicted through a complex model using concentrations of multiple collagen isotypes and other structural proteins.

Two opposing theories

There are two prevailing and opposing theories on the cause of muscle contractures. One theory argues that muscle contractures develop because of neural *hyper*-activity in

the form of spasticity-related muscle-overactivity, while the other argues that muscle contractures develop because of neural *hypo*-activity in the form of paresis/paralysis-induced disuse.

The theory that muscle contractures are caused by spasticity-related muscle-overactivity is based partly on clinical observations and partly on the idea that spasticity-related muscle-overactivity maintains muscles in a shortened position, ultimately causing permanent length adaptations and thus muscle contractures (Botte et al. 1988; Hof 2001; Pingel, Harrison, et al. 2021). As this was the dominating theory for many years, a lot of individuals affected by muscle contractures have been treated with various anti-spastic measures. However, as muscle contractures develop in individuals treated with anti-spastic medication and in individuals who have received selective dorsal rhizotomy – a neurosurgical procedure effectively reducing spasticity by cutting afferent nerve fibers where they enter the posterior root of the spine – spasticity does not seem to explain the development of muscle contractures (Tedroff et al. 2011, 2015).

The other prevailing theory is that muscle contractures develop because of paresis/paralysis-related disuse. This theory is based on both animal (Trudel et al. 1999; Nagai et al. 2014; Minamimoto et al. 2021) and human (Clavet et al. 2008) immobilization studies. In animal models, knee (Trudel et al. 1999; Nagai et al. 2014) and hip joint (Minamimoto et al. 2021) contractures have consistently been found to develop after only 1-2 weeks of immobilization. The same pattern has been observed in patients hospitalized (and therefore immobilized) at an intensive care unit due to critical illness (Clavet et al. 2008). Clavet et al. found that 34% of patients staying at an intensive care unit for an average of 3.1 weeks developed at least one contracture of functional significance. However, as not all paretic/paralytic individuals develop muscle contractures, and as those that do seem to only do so in distinct paretic/paralytic limbs, disuse does not appear to be the sole explanation of muscle contracture development (Nas et al. 2015).

Ultimately, neither the theory on neural *hyper*-activity (spasticity) or the theory on neural *hypo*-activity (paresis/paralysis) sufficiently explains the development of muscle contractures.

An alternative explanation

The many failed attempts at finding effective treatment options and the lack of clear pathophysiological explanations seem to suggest that muscle contractures are a complex and perhaps heterogeneous disorder. It seems that the initiation of muscle contractures is triggered by the interaction of multiple factors and that the relative importance of these factors could depend on age, underlying injury, genetics etc. (Smith et al. 2009; Gough and Shortland 2012; Pingel et al. 2017; Lieber and Fridén 2019; Pingel, Harrison, et al. 2021; Howard et al. 2022).

In cerebral palsy, impaired muscle growth during early childhood has been proposed as such a contributing factor in the development of muscle contractures (Barber et al. 2011; Gough and Shortland 2012; Barber and Boyd 2016; Herskind et al. 2016; Willerslev-Olsen et al. 2018). Muscle volume has consistently been found to be reduced in children with cerebral palsy (Barrett and Lichtwark 2010; Barber et al. 2011; Herskind et al. 2016; Willerslev-Olsen et al. 2018). As most muscles have a pennate structure, impaired muscle growth cause reductions in both muscle volume and length (Gough and Shortland 2012). With bone length increasing rapidly in early childhood, the impaired muscle growth may cause muscles that are short in relation to bone length. This constant stretching and muscle stress is what has been proposed to be implicated in the development of muscle contractures in cerebral palsy (Gough and Shortland 2012).

The muscle volume of children with cerebral palsy has been found to deviate from that of typically developing children at only 12-15 months of age (Barber et al. 2011, 2016; Herskind et al. 2016; Willerslev-Olsen et al. 2018) – corresponding roughly to the development of functional gait in typically developing children (Jeng et al. 2008; Størvold et al. 2013). A cross-sectional study by Willerslev-Olsen et al. (Willerslev-Olsen et al. 2018) suggests that the reduced muscle volume is followed by the development of measurable signs of early contracture development. While cross-sectional studies can provide indications, longitudinal studies with measurements at multiple time points are required to determine the pathophysiological mechanisms underlying the development of muscle contractures in cerebral palsy. Such longitudinal studies should ideally be initiated during early development (Howard et al. 2022). Using such a longitudinal study

design, we aimed to further investigate the contribution of impaired muscle growth to the development of muscle contractures in study IV.

Study IV - Impaired muscle growth and early signs of muscle contractures in children with cerebral palsy

In study IV, we investigated the relation between impaired muscle growth and early muscle contracture development in cerebral palsy. Using a longitudinal study design, we followed a large group of infants at high risk of cerebral palsy from birth until the age of four. Of these children, 13 were diagnosed with cerebral palsy. The children diagnosed with cerebral palsy were compared to data from 95 typically developing children; 50 who were followed longitudinally in the same manner as the children with cerebral palsy and 45 who were assessed only once.

We assessed the children using freehand three-dimensional ultrasound and the hand-held dynamometer PSAD. Ultrasound was used to quantify medial gastrocnemius muscle volume and echo intensity. The PSAD was used to quantify ankle joint passive ROM and passive muscle torque of the ankle plantar flexors. Ultrasound measurements from 199 individual time points in the typically developing population (74 children, 2.7 per child) and 70 individual time points in the cerebral palsy population (13 children, 5.4 per child) were included. Dynamometric measurements from 126 individual time points (from 54 children, 2.3 per child) in the typically developing population and 70 individual time points in the cerebral palsy population (13 children, 5.4 per child) were included.

Using linear regression analysis, we initially plotted individual data points, regression lines, 95% CI intervals, and 95% prediction intervals (PI) for the age-dependent development in muscle volume (Figure 1A), echo intensity (Figure 1B), passive muscle stiffness (Figure 1C), and passive ROM (Figure 1D) in typically developing children. We did this to enable comparison of longitudinal data from individual children with cerebral palsy to the typically developing population. In figure 2, we plotted the longitudinal data from children with cerebral palsy together with the regression line, 95% CI, and 95% PI from the typically developing population (Figure 2A-D).

Looking at the muscle growth (Figure 2A), it appears that the children with cerebral palsy were divided into two subgroups: one that followed the muscle growth trajectory of typically developing peers (seven children) and another that had reduced muscle growth, deviating from the PI of typically developing children at ~20 months of age. This

finding is roughly in accordance with previous cross-sectional studies that have reported significant differences in medial gastrocnemius muscle volume from the age of 12-15 months (Herskind et al. 2016; Willerslev-Olsen et al. 2018). Of the six children with reduced muscle growth, three later developed increased plantar flexor passive muscle torque, while none of the children that followed the muscle growth trajectory of typically developing peers developed increased passive torque (Figure 2C). We found passive ROM to clearly decrease before the age of four years in both typically developing children and in children with cerebral palsy (Figure 1D and figure 2D). With a single exception however, children with cerebral palsy did not clearly decrease more than typically developing children. The variability of the echo intensity was high in both typically developing children and children with cerebral palsy: two children with cerebral palsy deviated from the PI of typically developing children. The high variability in the echo intensity measure might be attributed to a combination of small muscles and movement during measurements. Further, a recent study found that ultrasound images obtained using near maximal pressure improves the reliability of the echo intensity measure (Pigula-Tresansky et al. 2018). Due to the young age of the children in this study, we were unable to use near-maximal pressure when obtaining ultrasound measurements, which may have contributed to the variability in the echo intensity measure.

As increased passive muscle torque exclusively developed in children with prior muscle growth impairments, we believe that study IV supports the theory that impaired muscle growth is implicated in the subsequent development of muscle contractures (Barber et al. 2011; Gough and Shortland 2012; Herskind et al. 2016; Willerslev-Olsen et al. 2018). Future studies should therefore seek to investigate whether the implementation of preventive measures stimulating muscle growth during early childhood can reduce muscle contracture development. When compared to measures of passive ROM and echo intensity, dynamometric measurements of passive muscle stiffness appeared to be more sensitive in detecting early signs of muscle contracture development.

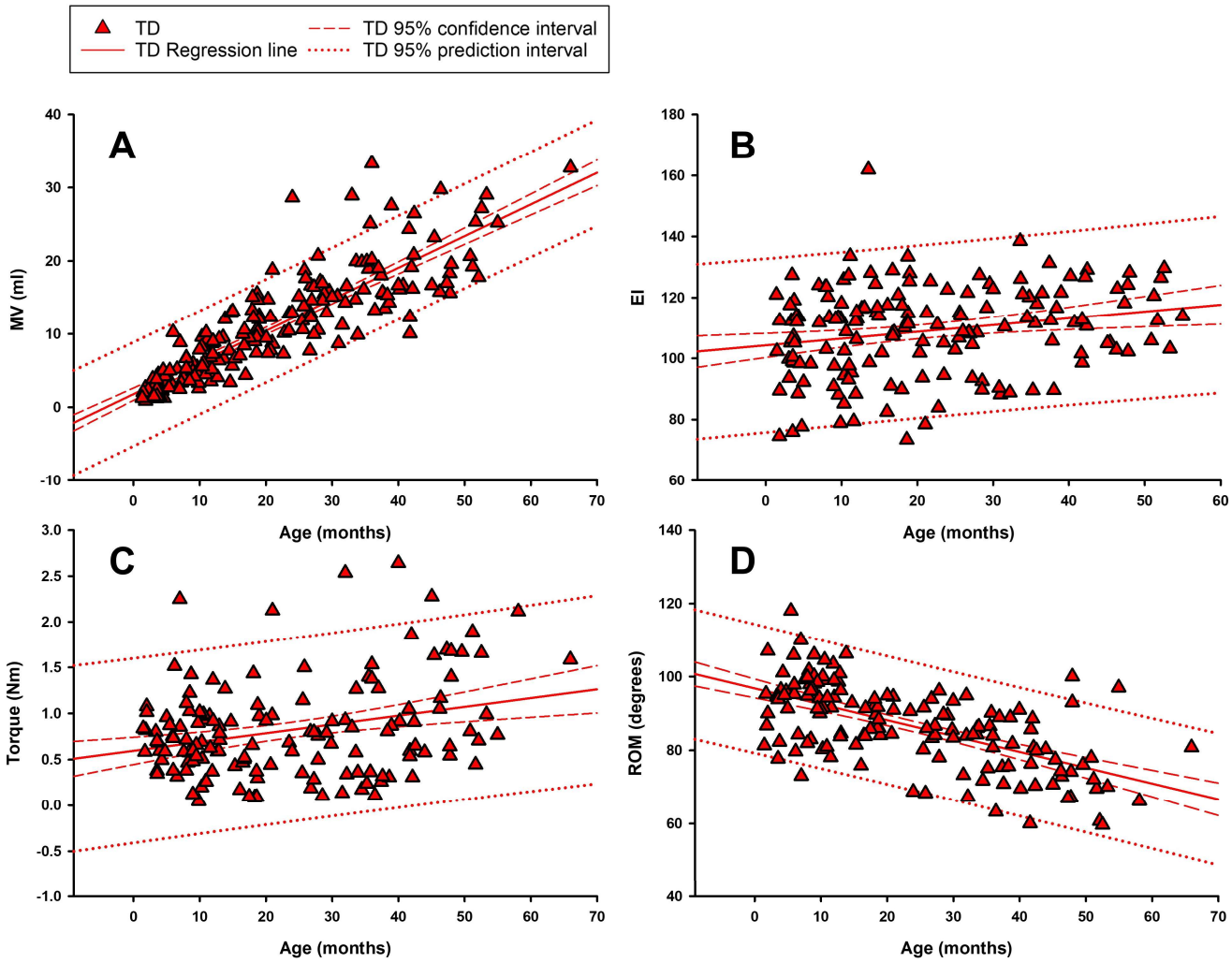

Figure 1: Depiction of the development in muscle volume (A), echo intensity (B), passive torque (C), and passive range of motion (ROM) (D) with age in typically developing children (Red triangles). The regression line, 95% confidence interval, and 95% prediction are shown by solid, dashed, and dotted red lines respectively. TD=typically developing. This figure can also be found in the article manuscript.

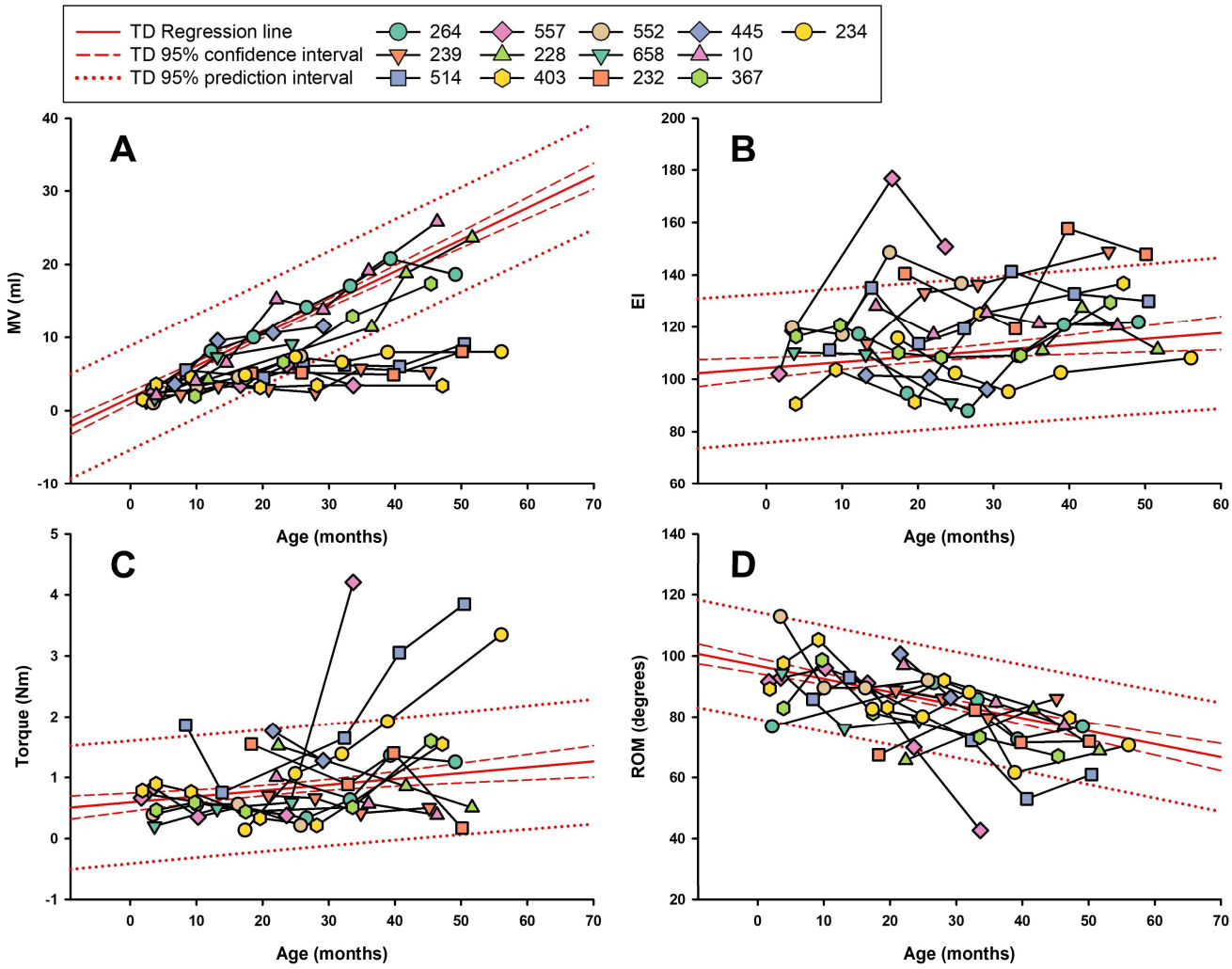

Figure 2: Depiction of the development in muscle volume (A), echo intensity (B), passive torque (C), and passive range of motion (ROM) (D) with age in children with cerebral palsy. Individual children with cerebral palsy are marked by the same color and symbol in A-D and measurements from the same child are connected by solid black lines. The regression line, 95% confidence interval, and 95% prediction interval for typically developing individuals are shown by solid, dashed, and dotted red lines respectively. TD=typically developing. This figure can also be found in the article manuscript.

General discussion

It was the purpose of this PhD project to use biomechanical, electrophysiological, and imaging techniques to characterize and quantify muscle contractures in children and adults with cerebral palsy as a foundation for future intervention studies. The findings presented in this thesis highlight the importance of using quantitative and objective measures when characterizing and assessing the functional significance of muscle contractures, identifying children at risk of developing muscle contractures, and evaluating the effectiveness of therapies. Furthermore, the findings add further evidence to the suggestion that muscle contractures are complex and seem to develop due to the interaction of many factors (Smith et al. 2009; Gough and Shortland 2012; Pingel et al. 2017; Lieber and Fridén 2019; Pingel, Harrison, et al. 2021; Howard et al. 2022). In cerebral palsy, there seem to be a close relation between impaired muscle growth and subsequent signs of early muscle contracture development during a specific period in early childhood. This relation may hold the key to effective prevention and treatment of muscle contractures.

It is well established that children and adults with cerebral palsy generally have smaller muscles than typically developed peers (Barrett and Lichtwark 2010; Barber et al. 2011; Herskind et al. 2016; Willerslev-Olsen et al. 2018). For the ankle plantar flexors, the difference in muscle size seem to consistently appear between the age of 1 and 2 years. In accordance with cross-sectional indications (Willerslev-Olsen et al. 2018), we showed that impairments in muscle growth were often followed by early signs of muscle contractures, indicating that preventive measures should preferably be initiated before the age of one year and focus on stimulating muscle growth. An upcoming intervention study in our research group will assess the effect of such preventive measures (Willerslev-Olsen et al. 2021). The study will seek to induce muscle growth through a combination of home-based training, diet, and electrical muscle stimulation.

Although the implementation of preventive measures focusing on muscle growth in early childhood might reduce muscle contracture development, it is naïve to believe that future generations of adults with cerebral palsy will be free of muscle contractures. As the nonsurgical treatment options available today are seemingly unable to effectively

treat existing muscle contractures, surgical measures such as tendon-lengthening procedures currently seem as the only clinically effective option (at least temporarily). As such surgeries cause biomechanical changes and can be the cause of both physical and mental strain, they should however be used with caution.

Epigenetics describe changes in gene expression that do not involve alterations of the underlying DNA sequence. Recent studies have both hypothesized and indicated that such epigenetic changes could play a significant role in the onset and severity of muscle contractures (Pingel et al. 2017; von Walden et al. 2020; Howard and Herzog 2021; Romero et al. 2021; Sibley et al. 2021). In continuation of this, one could argue that the consistency of the period in which impaired muscle volume and subsequent early signs of muscle contractures has been found may indicate the existence of a sensitive period in the development of muscle contractures (Hensch 2004, 2005; Knudsen 2004). As epigenetic changes are implicated in marking the end of these periods, and as sensitive periods have successfully been re-opened pharmacologically (Putignano et al. 2007; Silingardi et al. 2010; Yang et al. 2012; Gervain et al. 2013), the existence of a sensitive period could represent a target for future treatment of muscle contractures. One of the epigenetic changes acting as a ‘brake’ on sensitive periods involves the actions of histone deacetylase (HDAC) (Gervain et al. 2013). Using pharmacological HDAC inhibitors, the actions of HDAC can be blocked. This results in hyperacetylation of histones and a subsequent change in gene expression (Thiagalinham et al. 2003). Studies on mice and humans have shown that HDAC inhibitors can be used to reopen sensitive and critical periods, enabling recovery from amblyopia (Putignano et al. 2007; Silingardi et al. 2010) and facilitating new types of auditory learning (Yang et al. 2012; Gervain et al. 2013). Furthermore, HDAC inhibitors have been shown to effectively reduce collagen content and thus reduce the amount of muscle contractures in mice (Gurpur et al. 2009; Seet et al. 2016). This may further indicate that a sensitive period for the development of muscle contractures exists. Treatment utilizing the existence of such a sensitive period has the potential to effectively treat muscle contractures without the use of surgical procedures.

Conclusion

This thesis has demonstrated that existing nonsurgical options for treating muscle contractures are ineffective, emphasized the importance of assessing muscle contractures using proper objective measures, and added longitudinal evidence to the suggestion that impaired muscle growth is implicated in the development of muscle contractures in cerebral palsy. Preventive measures aimed at stimulating muscle growth in early childhood may thus reduce the development of muscle contractures. Furthermore, the thesis argues that the close relation between impaired muscle growth during a specific period in early childhood and subsequent signs of early muscle contracture development may point to the existence of a sensitive period in muscle contracture development. The pharmacological reopening of such a period later in life may hold the key to effectively treating existing muscle contractures without surgery.

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