From Dept. of Women´s and Children´s Health
Karolinska Institutet, Stockholm, Sweden

MUSCLE COMPOSITION, REACH, PHYSICAL ACTIVITY AND BOTULINUM TOXIN TREATMENT IN CHILDREN WITH CEREBRAL PALSY

Stefan Gantelius

Karolinska Institutet

Stockholm 2018

Published by Karolinska Institutet.
Printed by E-Print AB 2018.

ISBN 978-91-7831-257-3

Muscle composition, reach, physical activity and botulinum toxin treatment in children with cerebral palsy

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Stefan Gantelius

Principal Supervisor:
Eva Pontén, M.D., Ph.D.
Karolinska Institutet
Department of Women´s and Children´s health
Division of Pediatric Neurology

Co-supervisors:
Professor Elena M Gutierrez-Farewik
Royal Institute of Technology
KTH Mechanics

Associate Professor Gustavo A Nader
The Pennsylvania State University
Department of Kinesiology and
Huck Institute of the Life Sciences

Ferdinand von Walden, M.D., Ph.D.
Karolinska Institutet
Department of Women´s and Children´s health
Division of Pediatric Neurology

Opponent:
Tim Theologis, M.D., MSc, Ph.D., FRCS
University of Oxford
Nuffield Department of Orthopaedics,
Rheumatology and Musculoskeletal Sciences
Medical Sciences Division

Examination Board:
Professor Gunnar Hägglund
Lund University
Department of Clinical Sciences

Associate Professor Anders Holsgaard Larsen
University of Southern Denmark
Orthopaedic Research Unit
Department of Clinical Research

Professor Thomas Sejersen
Karolinska Institutet
Department of Women´s and Children´s Health
Division of Pediatric Neurology

Till Ebba, Edvin, Liv och Tom

och till min egen stora förvåning.

ABSTRACT

Cerebral palsy (CP), the most common cause of movement disorders and physical disability in children all over the world, is caused by an injury to the developing brain. Although this injury is not progressive, the manifestations of the disorder change as the child ages. Spasticity and altered viscoelastic properties of the muscle often enhance muscle resistance to stretch and the peak spasticity of the calf muscles in children with CP observed at four years of age declines thereafter until twelve years of age. The cause of muscle contractures, which appear early and progress throughout childhood and adolescence is unclear and needs to be elucidated further to help design more effective preventive measures. Therefore, the first aim of this thesis was to further characterize muscle pathophysiology in children with CP with fixed contractures.

Treatment of patients with CP involves a wide variety of efforts. Standardized and objective follow-up, as well as cost effectiveness are of considerable importance. Therefore, the second aim here focused on novel tools for clinical evaluation, as well as assessment of the economics of switching between two botulinum toxins (BoNT-A) in treatment of children with CP. We developed further a three-dimensional test of arm reach in typically developing (TD) children and tested the feasibility of this test in young adults with CP. We also developed a new approach evaluating the effects of BoNT-A treatment involving monitoring the child´s home and school environments with four accelerometers. Moreover, we made a controlled switch from one brand of BoNT-A to another in attempt to reduce the drug cost without reducing efficacy or duration or exacerbating side-effects.

The major novel findings with respect to muscle pathophysiology are that impaired production of ribosomes probably explains, at least in part, the deficient growth of skeletal muscle observed in children with CP and that levels of pro-inflammatory cytokines are elevated in their muscles. The latter alteration contributes to both inhibition of growth and expansion of the extracellular matrix and perhaps to the development of muscle contractures as well in children with CP. In addition, in the muscles of these children the number of satellite cells was reduced, the amount of intramuscular collagen situated around bundles of muscle fibers elevated and expression of the fatigable and fast Myosin Heavy Chain IIx isoform in wrist flexors higher in comparison to TD children.

The major findings with respect to the development of novel tools for assessment and cost-effectiveness are that the 3D Reach Test for the arms exhibits excellent inter- and intra-session reliability in TD children, as well as excellent feasibility and reliability in a pilot study on young adults with CP. Moreover, monitoring children with CP in their own environment with four accelerometers following BoNT-A treatment reveals previously unknown effects on physical activity, effects not detected by routine clinical follow-up, such as a decline in ambulatory activity after injection into the legs. When the BoNT-A preparation used previously was replaced by another commercial BoNT-A product, the parents reported the treatment of their children with CP to be equally effective, while the cost was 41% lower with few, similar and transient side-effects.

In conclusion, the present investigations provide new information on the pathophysiology of muscle in children with cerebral palsy that can help improve our understanding of contracture formation. The 3D Reach Test and post treatment monitoring with accelerometers are promising new tools for evaluation of the effects of treatment on reach and everyday movement. The cost of BoNT-A treatment can be reduced considerably by switching to another brand without compromising either the effect or patient safety.

LIST OF SCIENTIFIC PAPERS

I. **Gantelius S**, Hedström Y, Pontén E.
Higher expression of myosin heavy chain IIx in wrist flexors in cerebral palsy.
Clin Orthop Relat Res 2012, 470(5):1272-1277.

II. von Walden F, **Gantelius S**, Liu C, Borgström H, Björk L, Gremark O, Stål P, Nader GA, Pontén E.
Muscle contractures in patients with cerebral palsy and acquired brain injury are associated with extracellular matrix expansion, pro-inflammatory gene expression, and reduced rRNA synthesis.
Muscle Nerve 2018, 58(2):277-285.

III. **Gantelius S**, Borgström H, Mao H, Pontén E, Gutierrez-Farewik EM.
A new method of three-dimensional analysis of upper extremity reach suitable for cerebral palsy.
In manuscript.

IV. **Gantelius S**, Vikerfors S, Jansson Edqvist J, von Walden F, Hagströmer M, Pontén E.
New and unexpected insights to the effects of treating children with cerebral palsy with botulinum toxin based on accelerometer monitoring in their own environment.
Submitted.

V. Tedroff K, Befrits G, Tedroff CJ, **Gantelius S**.
To switch from Botox to Dysport in children with CP, a real world, dose conversion, cost-effectiveness study.
Eur J Paediatr Neurol, 2018;22(3):412-8

CONTENTS

LIST OF ABBREVIATIONS

ABI	acquired brain injury
ACPR	Australian Cerebral Palsy Register
AHA	Assisting Hand Assessment
ANOVA	analysis of variance
BoNT-A	Botulinum NeuroToxin Type A
CDC	Centers for Disease Control and Prevention
cDNA	complementary deoxyribonucleic acid
COPM	Canadian Occupational Performance Measure
CP	cerebral palsy
cps	counts per second
CPUP	Cerebral Pares UppföljningsProgram (Follow-Up Program)
DAPI	4´,6-diamidino-2-fenylindole
DNA	deoxyribonucleic acid
ECM	extracellular matrix
ECRB	extensor carpi radialis brevis
ECRL	extensor carpi radialis longus
ECU	extensor carpi ulnaris
EMG	electromyography
FCR	flexor carpi radialis
FCU	flexor carpi ulnaris
GMFCS	Gross Motor Function Classification System
ICC	interclass correlation coefficient
IL-6	Interleukin-6
IL-1b	Interleukin-1b
JIA	juvenile idiopathic arthritis
MACS	Manual Ability Classification System
MAS	Modified Ashworth Scale
MA2	Melbourne Assessment 2
MCP3	third metacarpophalangeal joint
MRI	Magnetic Resonance Imaging

MSTN	myostatin
mTOR	Mammalian Target of Rapamycin
MyHC	myosin heavy chain
NCAM	Neural Cell Adhesion Molecule
PEDI	Pediatric Evaluation of Disability Inventory
PIP	proximal interphalangeal joint
POL 1	DNA polymerase 1
pROM	passive range of motion
qRT-PCR	quantitative Real Time-Polymerase Chain Reaction
QUEST	Quality of Upper Extremity Skills Test
rDNA	ribosomal deoxyribonucleic acid
RNA	ribonucleic acid
ROM	range of motion
rRNA	ribosomal ribonucleic acid
SC	satellite cell
SCPE	Surveillance of Cerebral Palsy in Europe
SD	standard deviation
SDC	smallest detectable change
SDR	Selective Dorsal Rhizotomy
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEK	Swedish crown
SEM	standard error of measurement
SHUEE	Shriners Hospital Upper Extremity Evaluation
SLL	Stockholms Läns Landsting (Stockholm County Council)
TD	typically developing
TGF-beta	transforming growth factor-beta
TIF-1A	transcription initiation factor-1A
TNF	tumor necrosis factor
U	units
UBF	upstream binding factor
3D	three-dimensional

1 INTRODUCTION

Today, 17 million people worldwide suffer from cerebral palsy (CP). Most children with CP will survive until adulthood. Despite an improved survival rate, life expectancy is still lower than in typically developing (TD) children [1]. Childhood CP is the most common cause of physical disability and movement disorders, with a prevalence of 2-3 per 1000 live births [2], more common in boys than girls [3-5]. Despite major advances in neonatal care and obstetrics during the last four decades the overall prevalence of CP has not declined [1], perhaps due, at least in part to the increased survival of children born at lower gestational age [6]. The prevalence of CP among children born preterm is particularly high [7].

Over the years a number of attempts to define and classify CP have been made. Spastic CP was first described by the orthopedic surgeon William Little in 1862 as the result of an injury to the nervous system acquired during a difficult birth. The initial term "Little´s disease" [8] was replaced thirty years later by the term cerebral palsy when Sir William Osler wrote the book "The Cerebral Palsies of Children" [9]. Sigmund Freud was the first to propose that cerebral palsy could also be caused by intra-uterine events [10]. During the second half of the 20th century, Lesley Mutch and colleagues [11] defined CP as "an umbrella term covering a group of non-progressive, but often changing, motor impairment syndromes secondary to lesions or anomalies of the brain arising in the early stages of development".

The present definition of CP focuses not only on this motor disorder *per se*, but includes accompanying disturbances: "Cerebral Palsy describes a group of disorders of the development of movement and posture, causing activity limitations, which are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbance of sensation, perception, cognition, communication, and behavior, by epilepsy, and secondary musculoskeletal problems" [12]. The etiology of the accompanying brain lesion is multifactorial and may involve genetic disorders and malformation, disturbance of cerebral circulation, infection, inflammation and trauma [13-16]. The brain injury is acquired during antenatal, perinatal or early postnatal period [1]. In 2005, Bax and collaborators (the Executive Committee for the Definition of Cerebral Palsy) [17] proposed that the first two or three years of life may be concerned rather than specified time limits meaning that the disturbance resulting in CP is assumed to happen before the affected function, e.g. walking or manipulation of objects, has developed. Since it is sometimes difficult to identify a neurological abnormality in a very young child and brain lesions in pre-school children are associated with similar sequelae, the Australian Cerebral Palsy Register (ACPR) has chosen five years as the upper age limit for occurrence of the brain lesion [18]. Although the brain injury itself is non-progressive, the manifestations of the disorder change with time [12].

1.1 ETIOLOGY AND BRAIN LESION CHARACTERISTICS

Depending on when, rather than how or why, during this time span the injury occurred different areas will be susceptible to injury, corresponding to the concept of selective vulnerability. A wide range of etiological risk factors, maternal, pregnancy or child related, has been identified. However, only rarely, such as in severe perinatal or postnatal asphyxia, will CP be caused by a single factor. More commonly is the etiology multifactorial and several causative pathways has been identified. Some, identified risk factors for the development of CP are maternal obesity, infections occurring during pregnancy or

postnatal, multiple pregnancies, low birth weight, injury, immunization i.e. Rh or A-B-O immunization, placental dysfunction and neonatal stroke [19-22]. The Apgar score [23] has been shown to inversely correlate to risk of CP in children born at term [24].

As an effect of the selective vulnerability of the growing brain, lesions that occur early in pregnancy, during the first and second trimester, frequently result in brain malformation irrespective of the etiological factors. In late second, and early third trimester, white matter damage of immaturity (WMDI), including periventricular lesions, are common. In late pregnancy and around the time of birth cortical, subcortical, and basal ganglia gray matter lesions are more frequent. Bax and co-workers [19] found brain malformations in about 10%, white-matter damage in about 40% and gray matter lesions in about 10% of children with CP. White matter damage of immaturity is the characteristic lesion seen in cerebral palsy associated with preterm birth [25], preterm birth being an important risk factor for cerebral palsy [1]. Dyskinetic CP is associated with basal ganglia lesions seen in full-term children with severe birth asphyxia or postnatally acquired CP [19, 26]. In no less than 10% of children with CP a normal MRI is seen [19].

1.2 CLASSIFICATION

The classification of CP by the Surveillance of Cerebral Palsy in Europe (SCPE) [27], now utilized worldwide, states that the predominant motor disorder should be categorized as spastic, dyskinetic or ataxic and the anatomical distribution determined to be uni- or bilateral. In three Swedish studies, unilateral spastic CP accounted for 35-45% and bilateral spastic CP for 35-40%. Dyskinetic CP was found in 12-15% and 3-8% were diagnosed with ataxia [28-30].

The Gross Motor Function Classification System (GMFCS) [31] and Manual Ability Classification System (MACS) [32] classify the functional abilities of children with CP in daily life which is useful clinically. In both of these systems, level I designates the children least affected and level V those most severely affected. Children classified as GMFCS level I can walk independently while those in GMFCS level III use wheelchairs (manual or electric) themselves for longer distances, but can walk with a hand-held walking aid. Children classified as GMFCS level V are severely limited in mobility and must be transported in a wheelchair by others [31].

Those at MACS level I handle objects easily and successfully, while children at MACS level III handle objects with difficulty and need help to prepare and/or modify activities. At MACS level V children either cannot handle objects at all or have a severely limited ability to perform even simple actions and therefore require total assistance [32]. Arner and co-workers [30] report 64% of children with CP independent in age-relevant manual activities (MACS I-II) and 14% with no active hand function or as total dependent on others in daily activities (MACS V). MACS classifies the overall ability to handle objects, not the function of each hand separately [32]. For children one to four years of age, the Mini-MACS classification is used in a similar manner to that for older children [33]. Hand function in individuals with CP can also be assessed according to the House classification system, where 0 reflects no use of the hand, 1-3 different levels of passive helper hand, 4-6 different levels of active helper hand, and 7 and 8 the ability of the hand to manipulate objects [34-36].

Motor dysfunctions associated with CP can be divided into two main categories: Positive dysfunctions involve pathology added to normal motor behavior, e.g., spasticity and musculoskeletal malformations; while with negative motor dysfunctions, the motor repertoire fails to develop normally, resulting in e.g., muscle weakness, paresis and central

dyscoordination as well as co-contraction and mirror movements indicating deficiencies in sensorimotor control [37].

In addition, the Surveillance of Cerebral Palsy in Europe allows categorization of impairments accompanying CP, e.g., cognitive impairment, attention deficit, problems with vision and hearing, and emotional and behavioral disorders [27]. The lifestyle of children with CP is more sedentary with lower levels of physical activity [38, 39], a potential cause of secondary health problems. Physical activity has been associated with positive health outcomes in children both with and without disabilities [39]. Peterson and co-workers [40, 41] observed a significantly elevated prevalence of diabetes, hypertension, coronary heart disease, stroke, joint pain and arthritis among adults with CP.

1.3 MUSCLE PHYSIOLOGY AND PATHOLOGY

Skeletal muscle of children with CP differs from that of TD children in a variety of ways. Their total muscle mass is reduced and the muscles in their affected arms and legs are weak, short and thin [42-44]. Furthermore, there is more variability in the size of muscle fibers [45, 46] and a lower capillary density in muscles of children with CP [47]. Moreover, magnetic resonance imaging (MRI) reveals more intramuscular fat [48], and reduction in muscle volume, cross-sectional area and length [49].

Increased resistance to passive muscle stretch is common in children with CP. This phenomenon involves both a neuronal component, spasticity, and a mechanical or non-neuronal component, consisting of changes in elasticity and viscosity [50]. One definition of spasticity commonly used has been formulated by Lance: "a motor disorder characterized by a velocity dependent increase in tonic stretch reflexes ('muscle tone') with exaggerated tendon jerks, resulting from hyper excitability of the stretch reflex" [51]. Sanger and colleagues [52] define spasticity as "hypertonia in which one or both of the following signs are present: 1) resistance to externally imposed movement increases with increasing speed of stretch and varies with the direction of joint movement, and/or 2) resistance to externally imposed movement rises rapidly above the threshold speed or joint angle".

Spasticity of the calf muscles peaks at four years of age and declines thereafter until the age of twelve [53]. However, the reduced passive range of motion (pROM) involving alterations in the visco-elastic properties of muscles, that many children with CP develop worsens continuously throughout childhood and adolescence, becoming more evident during puberty [54, 55], a process referred to as contracture development [56, 57]. Approximately one third of children with CP develop contractures in the arms [57]. The passive stiffnes, secondary to an upper motor neuron lesion [58-60] is altered in both plantar flexors and forearm muscles [44].

The sarcomere is the smallest contractile unit of skeletal muscle. Tandem repeats of sarcomeres form myofibrils and parallel myofibrils form muscle fibers that are held together in bundles surrounded by the perimysium, part of the extracellular matrix (ECM). The sarcomere is defined as the segment between two Z-lines where the actin filaments are anchored. The force in a contracting muscle is generated when the motor protein myosin interdigitates, between actin filaments, shortening the sarcomere [61]. Conversely, when the muscle stretches, the sarcomere lengthens.

With increasing age and progressive contracture development, the sarcomeres in the muscles of children with CP become longer [62]. For example, the sarcomeres in the flexor carpi ulnaris (FCU) is extremely long when a wrist with a flexion contracture is held in a

neutral position [63]. A reduction in the number of sarcomere in series might explain to this phenomenon [64-66].

Myosin is composed of two heavy (MyHCs) and two light chains. The isoform of myosin present determines the velocity of contraction and endurance of the muscle fibers. Muscle fibers with much MyHC I, typically found in muscles responsible for postural control, are slow with extensive endurance, those with much MyHC IIx contract rapidly but are easily fatigued, whereas fibers with a high content of MyHC IIa contract rapidly and exhibit intermediate endurance [67].

Various stimuli influence the expression of MyHC. In general, voluntary use of a muscle, irrespective of intensity, down-regulates MyHC IIx [68] and electrical stimulation can also alter expression of MyHC. Paretic skeletal muscle resulting from a spinal cord injury expresses equal amounts of MyHC IIa and MyHC IIx, but following electrical stimulation, there is an almost complete dominance of MyHC IIa [69]. These findings indicate that neuronal signaling exerts an important impact on the expression of myosin and, thus, on the type of muscle fiber. In the muscles of children with CP, the muscle fibers shift from the slow to fast phenotype [70, 71], with a higher proportion of fibers containing MyHC IIx [47], which has been proposed to be due to both inactivity and altered neuronal signaling.

Furthermore, the content and quality of the extracellular matrix (ECM) in the muscles in individuals with CP are altered. The ECM within skeletal muscle is composed primarily of collagen [72] and the area fraction of ECM in individuals with CP is enhanced [73]. Booth and co-workers [74] showed that children with CP, involving severe spasticity and contractures demonstrate more collagen in the knee extensor vastus lateralis than both those with less severe CP and TD children. de Bruin and collaborators [75] found this increased amount of collagen mainly around the bundle of muscle fibers in the form of a thickening of the perimysial ECM. Examination by ultrasound reveals an increased echo intensity in the skeletal muscle of children with CP suggestive of an increased content of collagen [76]. Laminin and collagen IV, components of the basal membrane, are up-regulated in muscles with fixed contractures, suggesting that changes in the ECM are neither limited to classical isoforms of collagen nor restricted to the perimysium [77] [78].

Skeletal muscle grows primarily through hypertrophy of existing muscle fibers [79, 80] and involves increased synthesis of proteins, mainly actin and myosin, which depends on the status and number of the ribosomes [81]. Thus, a growth stimulus elevates the biogenesis of ribosomes before any elevation in protein content can be detected [82-85]. The rate-limiting step for ribosome production and cell growth [86-88] is thought to be the transcription of genes encoding ribosomal (r)DNA by RNA polymerase I.

Satellite cells (SC) are stem cells belonging to a heterogeneous lineage of myogenic progenitor cells responsible for the postnatal growth and regeneration of skeletal muscle. Since they are terminally differentiated and mature myofibers cannot regenerate, the pool of SC is thus essential for muscle repair and regeneration [89, 90]. Hamstrings muscles with fixed contractures in children with CP contain fewer SCs than these same muscles in TD children [91]. A reduced number or dysfunction of SCs has been suggested to underlie the impaired growth of muscles in cases of CP, potentially contributing to contracture development [91].

This proposal is supported by recent experiments indicating that in adult mice satellite cells are not required for hypertrophy, whereas young mice do require satellite cell-mediated myonuclear accretion to undergo hypertrophy [92]. In addition, a reduction in the number

of satellite cells does not impede the addition of sarcomeres in series, but instead reduces the number of myofibers and decreases the muscle area fraction [93]. On the other hand, chronic loading of a muscle with a reduced number of satellite cells triggers excessive ECM production [93, 94].

Children with CP often display characteristic positioning of the affected arm, with flexion of the elbow, wrist, and fingers and pronation of the forearm [95]. This typical pattern is deleterious to hand usage, with such improper positioning of the elbow and wrist impairing both reach and grip function [96]. Wrist flexors and extensors work as agonists to stabilize the wrist during grip [97]. The poor selective motor control leads to inadequate adjustment of muscle tone in children with CP. Their wrist flexors demonstrate a larger cross-sectional area and are stronger than wrist extensors, pulling the wrist into the flexed position often observed [98]. One arm is often more affected than the other, since the brain injury is usually asymmetric, making the arm less affected dominant. Other deviant postures typically observed in children with CP include ankle equinus, deficient knee extension, hip adduction (with or without scissoring of the legs), deficient hip extension, and, on occasion, hip dislocation [99]. Contracture, co-contraction, spasticity, weakness and poor selective muscle control influence the positioning of the limbs of these children, both during activity and at rest.

1.4 CONTRACTURE DEVELOPMENT

One-third of children with CP will develop an arm contracture [57]. Muscle contractures develop continuously throughout childhood and adolescence in these children, although more rapidly during puberty [54, 55]. The MACS level is the strongest predictor of contracture formation in the arm, with children at MACS level V exhibiting a 17-fold higher risk of such development than those of MACS level I [57]. The various explanations proposed include a mismatch between bone and muscle growth, alterations in neural activation, mechanotransduction, tensional homeostasis, micro-vascularization, genetics and epigenetics [100]. A flexed joint position in itself [101], with a resultant loss of sarcomeres [62] has also been suggested to result in contractures.

Spasticity has been proposed to cause shortening of joint flexors [102], even though the stretch reflex is often enhanced in both the joint flexors and extensors of children with CP. However, even with successful elimination of spasticity (e.g. by selective dorsal rhizotomy), range of motion continues to decrease [103]. Furthermore, after BoNT-A treatment and successful reduction of spasticity, contractures continue to develop [104]. If spasticity is the primary cause of contracture development, elimination of spasticity should slow down or stop this process but this is not the case.

A primary growth defect has been proposed to contribute to contracture development in CP muscle [100, 105] since the muscles of very young children with CP grow more slowly prior to such development [56]. Herskind and colleagues [56] detected a reduced volume of gastrocnemius muscle in children with CP already at 15 months of age, with no concomitant reduction in the rate of bone growth. Furthermore, the GMFCS level and thus, the degree of walking and weight bearing was correlated with the volume of the gastrocnemius muscle [56].

Depending on the muscle architecture, post-natal growth of skeletal muscle could involve an increase in the cross-sectional area rather than the length [79, 80]. Therefore, a failure to increase muscle fiber thickness rather than longitudinal growth of the fiber could also be of relevance to contracture formation. The hypothesis that a reduced growth rate is one cause of contracture formation, is supported by the lower numbers of satellite cells and therefore,

reduced potential for growth, in the hamstring muscle of children with CP. Potentially, the associated increases in laminin and collagen contents could function as a mechanical barrier that blocks the migration of satellite cells during activation [78].

The skeletal muscle of adult patients with myositis and muscular dystrophies are fibrotic, i.e., contain an elevated amount of ECM around individual fibers, at the expense of functional tissue [106]. With these muscular disorders elevated levels of cytokines commonly precede fibrosis [107]. The existence of similar pro-inflammatory and pro-fibrotic networks has been suggested by transcriptional studies of the skeletal muscle of children with CP [78, 108]. Pro-inflammatory cytokines influence the size of skeletal muscles negatively, promoting atrophy and hampering growth [109]. Thus, attenuated growth of skeletal muscle and elevated collagen content result from elevated levels of pro-inflammatory cytokines and might explain, at least in part, contracture formation in children with CP.

It should be noted that most biochemical and histological investigations on muscle from individuals with CP have been performed on biopsy material obtained during surgical procedures intended to alleviate fixed contractures. Thus, it remains unclear whether the changes described above in muscles from children with CP are the causes or consequences of the contracture.

1.5 REDUCED REACH AND EVALUATION OF THE ARMS

The active range of motion (ROM) of the shoulder, elbow, wrist and fingers in combination with the total length of the arm determines the three-dimensional (3D) reach of the arm, which is important for many daily activities, i.e., eating and moving the hand towards the axilla and perineum to care for personal hygiene. The ability to reach out for objects is also an important requirement for independent living. The arms of children with CP often have a limited 3D reach due to the brain injury and resultant typical positioning of the arm and hand [110].

To facilitate and standardize clinical evaluation of the arms of children with CP, e.g., following BoNT-A treatment, a number of tools have been developed.

1.5.1 The Melbourne assessment of unilateral upper limb function

The Melbourne assessment of unilateral upper limb function [111] involves video-based evaluation and was initially developed for 5-15-year-old children with neurological impairments. Four major aspects of movement are assessed: 1) the pROM of all upper limb joints, 2) accuracy of reach, 3) dexterity of finger movements, and 4) the fluency and smoothness of movement. These four aspects are observed during completion of 16 tasks, using one hand at a time, selected to mimic everyday activities [111]. After the development into The Melbourne Assessment 2 (MA2) [112] assessment of children 2.5-15 are possible. After removal of two test items 14 items are left. The MA2 also offers a possibility to use the four sub-scales for range, accuracy, fluency and dexterity separately.

1.5.2 The Assisting Hand Assessment

The Assisting Hand Assessment (AHA), developed for children with unilateral arm disabilities, measures how effectively the affected hand is used for bimanual activities, which, for these children, might be the most important aspect of hand function. First created as a valid instrument for children between the ages of 18 months and 12 years [113], subsequent development of the Assisting Hand Assessment for Adolescents (Ad-AHA)

[114] and the mini-AHA [115] now allow scoring between range 8 months to 18 years of age.

1.5.3 Shriners Hospital for Children Upper Extremity Evaluation

The Shriners Hospital for Children Upper Extremity Evaluation (SHUEE), a video-based tool for children with hemiplegic cerebral palsy, includes spontaneous functional and dynamic positional analysis and assesses the ability to perform grasp and release [116].

1.5.4 The Modified Ashworth Scale

The Modified Ashworth Scale (MAS) assesses resistance during passive stretch and is easily used in clinical practice as a measure of spasticity. The scale is ordinal, ranging from 0 = no increase in muscle tone to 4 = the joint is rigid [117]. Bohannon and Smith [117] reported good reliability whereas Mutlu and colleagues recommend great caution when interpreting assessments with the MAS [118]. Ansari et al [119] claim that the MAS does not assess spasticity reliably.

1.6 MOTION ANALYSIS

In children with CP, several methods for evaluation of the arms are available, although some of these are to some extent subjective and time-consuming [120-123]. Analysis of arm movement is in many ways more challenging than gait analysis, since arm movements are voluntary and more varied than walking which is more automatic and consistent. Protocols for 3D movement analysis of arm movements in children with cerebral palsy have been developed, although more than one method is required to obtain full functional assessment of upper extremity function [124-127], since no test covers all abilities and difficulties and none provide quantitative data for use in connection with follow-up during childhood. Moreover, only kinematics (the motion of body segments in relation to each other) can be obtained from the analysis of arm movements. Gait analysis has been used as a tool in the evaluation of children with CP since the 1990´s [128, 129].

1.7 ACCELEROMETRY

Accelerometers measure the rate of change in velocity with respect to time, usually expressed in SI units (Système international (d'unités), International System of Units) as acceleration is meters per second squared (m/s^2). Today´s clinical accelerometers are the size of a wristwatch and allows portable monitoring of body movements in one, two or three axes [130], capturing large amounts of data for storage or transmission [131]. Although, accelerometers do not measure all physical activity, such as isometric exercises, most energy is consumed during dynamic physical activity, such as walking and running, which they do measure reliably [132]. Accelerometers enable a reliable and valid measurement of physical activity in walking children with CP [133, 134] and have been employed to quantify both the duration and intensity of unilateral and bilateral use of the arms by adult stroke patients [135]. Four accelerometers placed on both wrists, around the waist and around one ankle can monitor changes in both walking and bimanual activity and have been utilized to evaluate stroke rehabilitation [136].

1.8 TREATMENT OPTIONS TARGETING MUSCLE

Treatment often targets primarily one component of the resistance to stretch: Spasticity can be reduced by intramuscular injection of botulinum toxin type A (BoNT-A) [137], oral or intrathecal baclofen [138] or selective dorsal rhizotomy (SDR) [103]; while splinting and serial casting, for example, targets the viscoelastic component [139]. When non-invasive treatment or injection of BoNT-A proves inadequate, a variety of different surgical treatments can be offered to the child [101]. Surgery alters the viscoelastic component of resistance and the range of motion, indirectly alleviating spasticity by changing the tension

of the muscle spindles. Surgical correction of contractures involves, for example, tendon lengthening, release or lengthening of the affected muscle–tendon unit, myofascial lengthening, tenotomies, recession of the aponeurosis, or tendon transfers that restore the balance around the joint [140, 141]. The common Green transfer involves transfer of a tendon from an overactive wrist flexor to weaker wrist extensors in order to balance the wrist [142]. In severe cases of wrist flexion contracture, resection of the proximal carpal bones in combination with a wrist arthrodesis may be the more effective solution [143]. All treatment options involves some risk of permanent loss of force and/or movement [144] or with only temporary effect [145]. Co-contraction and poor selective motor control are difficult, if not impossible, to treat.

1.9 TREATMENT WITH BOTULINUM TOXIN

The short-term reduction in spasticity of both the arms [146] and legs [147] following intramuscular injections of BoNT-A is well documented [148]. Indeed, this is one of the few interventions that reduce spasticity effectively [137]. In a Norwegian population-based study, almost 70% of all children with spastic CP underwent BoNT-A treatment [149]. The CPUP (Cerebral Pares Uppföljnings-Program), the Swedish CP follow-up program and healthcare registry, reveals that 26% of children with CP received BoNT-A treatment during a two-year period [150], with similar proportions in the years 2010 and 2015 [150].

When BoNT-A is injected into the spastic muscle, precision is commonly ensured by electrical stimulation or guidance by electromyography (EMG) or ultrasound. BoNT-A is a polypeptide that cleaves a protein required for vesicle fusion in the motor nerve ending thereby preventing the neurosecretory vesicle from fusing with the synaptic plasma membrane and inhibiting the release of acetylcholine. This interferes with nerve impulses and reduces muscle activity in a reversible manner. The effect is observed days after injection and maximal after approximately three weeks, lasting typically for a total of 3-6 months [151]. The magnitude of this chemical denervation is dependent on the nature of the disorder and dose [151-153]. Recovery of muscle function following injection of BoNT-A has been attributed to axonal sprouting and subsequent re-innervation of the muscle fibers [154].

BoNT-A enhances the passive range of motion of the knee joint, although this improvement is sometime lost with time [155]. Furthermore, in combination with physiotherapy and the use of orthoses, BoNT-A improves gait [156]. In case of arms, evidence in favor of improved function and enhanced usage is inconclusive [157], and post-injection training is necessary for functional improvement [146]. The side-effects of BoNT-A treatment are few and transient. Pain and weakness have been reported following treatment of the legs of a pediatric population [158, 159].

Mainly because of co-contraction, arm swing is often reduced in children with CP [160], which slows down walking speed. Typically, the arms become more active and arm movements become larger as walking speed increases, indicating arm involvement in gaining walking speed [160]. Therefore, improving arm swing in ambulatory children might improve their general ability to participate in physical activities. Moreover, more symmetrical gait is desirable in terms of appearance for many teenagers. BoNT-A treatment can also relieve pain and ease of the care of non-ambulatory children most affected with no manual ability (GMFCS V, MACS V). Attenuation of pain through intramuscular injections of BoNT-A is believed to act both via reduction of spasticity and through alternative pharmacological pathways [161].

There is no evidence that therapeutic doses of BoNT-A injected into the muscle cross the blood-brain barrier [162]. However, BoNT-A might exert an indirect effect on the central nervous system through interference with function of intrafusal fibers in the injected muscles. Subsequent sensory afference to the spinal cord may influence the brainstem and cortical areas and the resulting feedback changes might then, in turn, affect muscles other than the ones initially injected [162]. In addition, BoNT-A appears to act directly on the spinal cord, probably influencing retrograde axonal trafficking of active neurotoxin. Thus, BoNT-A probably alters the excitability of spinal pathways, causing central chemo-denervation via retrograde axonal transport to the spinal cord [163].

BoNT-A treatment requires co-operation between different professionals, with a team often consisting of pediatric orthopedic surgeons, child neurologists, physiotherapists, occupational therapists, specialized pediatric nurses and anesthesiologists. The injections are commonly administrated by the pediatric orthopedic surgeons or pediatric neurologists. Intra-muscular injections are painful and anesthesiologists may provide anesthesia or analgesia with nitrous oxide. In the clinical follow-up, physiotherapists and occupational therapists focus on the child's attainment of goals, everyday activities, pROM and spasticity. In addition, a physiotherapist evaluates gross motor activity and an occupational therapist manual ability. Individual goals for the treatment are set by the team together with the child and his/her parents. In a 24-months follow-up after injection, attainment of goals increased gradually up to 12 months and was thereafter maintained [164]. Consensus documents concerning doses and which muscles to treat are available [151, 165]. The team must also plan for possible future interventions, e.g., additional BoNT-A treatment, serial casting, orthoses, training and/or surgery.

Clinical follow-up typically takes place in a hospital or habilitation center, i.e., an environment with which the child is somewhat unfamiliar, so it is somewhat unclear to what extent the findings reflect performance, level of physical activity (PA) and usage of the arms in a day-to-day context. Indeed, children and their parents sometimes describe features not revealed by the clinical evaluation. Furthermore, it remains unknown whether the extent of PA or arm movements are affected by injecting BoNT-A into the legs. In addition, it is of interest to know whether the arms swing more actively only when walking or during other manual activities as well.

1.10 THE ECONOMICS OF BOTULINUM TOXIN TREATMENT

The Centers for Disease Control and Prevention (CDC) in the USA [166] has estimated the medical costs for a child with CP to be 10 times higher than those for a TD child. For the 50% of all children with CP who also demonstrate an intellectual impairment [167], these costs were 26 times higher [166]. However, when comparing the cost of different drugs (e.g. biosimilars), the price is not the only factor that needs to be taken into consideration. From a clinical and socioeconomic perspective, the effect and duration are equally important and of course, any adverse effects must also be taken into account. Since BoNT-A treatment involves a large professional team, the total cost includes much more than simply that of the drug. The time parents take off from work also impacts the total socioeconomic cost, making the duration of treatment of importance. Few studies on the health economics on this topic have been reported [168] and only two studies involved switching between BoNT-A products [169, 170].

The BoNT-A preparations currently available for clinical use differ with respect to price and potency [165, 171] and, as a consequence, each has its own dosing recommendations and they cannot be simply replaced by one another [172]. Two of these products, Botox® (Allergan, Irvine, CA, USA) and Dysport® (Ipsen Limited, Slough, Berkshire, UK) are

more commonly used in children with CP. To date, there is no generally accepted factor for translating one unit of Botox® into a unit of Dysport®, with ratios of 1:2-1:11 having been proposed [173]. Obviously, this conversion factor influences the total cost.

In Sweden, pediatric health care is funded fully by taxes and free of charge to the individual patient. County Councils procure drugs for a specific indication at the best possible price. Health care providers choice on what drug to use is based on recommendations or decisions associated with this procurement process. In 2014, the Stockholm County Council (SLL), which provides health care for about 2.5 million inhabitants, procured Botox® and Dysport® utilizing a conversion factor of 1:3 at the cost of 1332 SEK for 100 U (in packages of 10 vials) and 1099 SEK for a single 300 U vial, respectively.

2 AIMS

The general aims of this thesis were to explore muscle composition to improve our understanding of the mechanisms of contracture formation in connection with CP and to investigate two aspects of BoNT-A treatment of children with CP, i.e., whether another follow-up approach can provide information that traditional post-injection follow-ups do not and to compare the efficacy, duration of improvement, side-effects and cost of switching from Botox® to Dysport®. Another aim was to develop further the analysis of arm movement for monitoring the effect of treatment.

The specific aims were as follows:

2.1 PAPER I

To compare the MyHC composition of the forearm muscles of children with and without CP and to relate this composition to functional classifications and clinical findings.

2.2 PAPER II

To compare gene expression data and histological findings in muscle from children with CP to those of TD children in an attempt to explain contracture formation. We focused specifically on factors related to growth and size homeostasis, i.e. expression of genes related to ribosome biogenesis, negative regulators of muscle mass and the numbers of satellite cells in skeletal muscle. We also examined the levels of mRNAs encoding pro-inflammatory cytokines and factors involved in collagen synthesis as well as the intramuscular content of collagen.

2.3 PAPER III

To develop a three-dimensional reach test and evaluate its intra- and inter-session reliability in TD children. A secondary aim was to compare the 3D reach of the dominant and non-dominant hand of young adults with CP as a test of the utility of this method and its feasibility and reliability in this group.

2.4 PAPER IV

To determine whether monitoring of children with CP following BoNT-A injection with accelerometers at home and at school can provide information that traditional follow-ups do not. We were specifically interested in whether other effects of BoNT-A would be detected and whether injection elevated the duration of ambulatory activity and/or diminished the differences in movement of the arms more and less affected.

2.5 PAPER V

To evaluate switching from one brand of BoNT-A (Botox®) to another (Dysport®), with a conversion factor of 1:2, for treatment of children with CP, with special focus on cost per treatment and whether parents perceived the treatments as equivalent with respect to efficacy, duration and side-effects.

3 MATERIALS AND METHODS

3.1 PARTICIPANTS

3.1.1 Paper I

Nine children with CP (age 8–17 years, mean 14; 2 girls, 7 boys) scheduled for tendon transfer of the forearm were recruited. Five TD children (age 7–13 years, mean 10; 2 girls, 3 boys) in need of surgery for open reduction of a forearm fracture served as the control group.

3.1.2 Paper II

Twenty children and adolescents (age 9-18 years, mean 15.5 years; 3 girls, 17 boys) with spasticity (18 with CP, 2 with an ABI at 7 and 8 years of age) scheduled for surgical lengthening of the biceps tendon were included. Muscle samples from ten TD children and adolescents (age 7-21 years, mean 15.2 years; 2 girls, 8 boys) and who had suffered an accidental death were obtained post-mortem.

3.1.3 Paper III

A convenience population of 34 TD children (age 5-18 years, mean 11; 17 girls, 17 boys) was utilized. Complete data on 32 and 30 children was collected for evaluation of intra-session and inter-session reliability respectively. For four children data from one session was incomplete and therefore excluded from the corresponding reliability evaluation. To evaluate the utility of the 3D Reach Test in a clinical setting, a convenience sample of five young adults with unilateral spastic CP (age 18-24 years, mean 20.5; 1 girl, 4 boys,) was examined.

3.1.4 Paper IV

Twelve ambulatory children (age 4-13 years, mean 7.9; 5 girls, 7 boys) with CP (GMFCS I-III) scheduled to receive a BoNT-A injection in the leg muscles with or without injection in the arm most affected were included. All were injected in the legs and five in one arm as well.

3.1.5 Paper V

A total of 170 children, 159 with CP (age 5-14 years, mean 9, 65 girls, 94 boys) who received BoNT-A treatment to reduce increased muscle tone due to spasticity or dystonia (at the Department of Pediatric Orthopedic Surgery, Astrid Lindgren Children's Hospital, at Karolinska University Hospital between September 1, 2014, and December 31, 2015) were evaluated. During this period, the children with CP visited the hospital 341 times for treatment. A total of 278 (2014) and 301 (2015) treatments administered for CP, post-traumatic brain injury, hereditary dystonia or stroke were used to calculate the cost/treatment.

3.2 METHODS

3.2.1 Clinical examination, surgical procedures and injection procedure

In Paper I, the hand function of individuals with CP was classified according to House [34-36], ambulatory ability assessed according to the GMFCS [31] and the ability to handle objects in daily life classified according to the MACS [32]. The maximal passive extension of the wrist was measured with the fingers flexed.

Fracture surgery in TD children was performed under general anesthesia within 24 hours of the trauma. These patients had had their arm immobilized in the emergency room. Biopsies were taken from exposed muscles in the middle 1/3 of the forearm, one flexor and one extensor in all cases. Specifically, the flexor carpi ulnaris (FCU, 4 cases), the flexor carpi radialis (FCR, 1 case); the extensor carpi radialis brevis (ECRB, 3 cases), the extensor carpi ulnaris (ECU, 2 cases) were sampled. All muscles were sampled with good visibility and no visible injury at the site of biopsy. Biopsies from wrist extensor and flexor muscles of the children with CP were obtained during surgery under general anesthesia, after exposure, but prior to the tendon transfer. All surgery was performed following fasting for 6-8 hours.

In Paper II, the maximal extension of the elbow prior to surgery was measured with a goniometer by an occupational therapist with all patients included exhibiting an extension deficit of >10°. Samples of the biceps brachii muscle were obtained intra-operatively under general anesthesia, after exposure but before tendon lengthening. Control samples of the biceps brachii were obtained from TD children and adolescents during autopsy within 24 hours of death.

In Paper III, bilateral arm length, the dominant arm, sex and age were recorded. Arm length was determined from the acromion to the metacarpophalangeal joint 3 (MCP3) and to the proximal interphalangeal joint 3 (PIP3) with a measuring tape.

In Paper IV, the BoNT-A (Botox®, Allergan, Irvine, CA, USA) injections were given by pediatric orthopedic surgeons employing EMG guidance and/or electrical stimulation with the child under nitrous oxide analgesia. Treatment goals were set by physiotherapists and occupational therapists together with the child and his/her parents and the pediatric orthopedic surgeon chose which muscles to inject on the basis of these specific goals. In the case of the arms, this decision-making was also supported by the results of the AHA [113], the SHUEE [116] or the Melbourne Assessment [111]. When treating the legs, Botox® was diluted to 50 IU/ml and for the smaller muscles in the arm, to 100 IU/ml. The dose per individual muscle and patient was determined in accordance with prevailing guidelines, with maximal doses of 12 IU/kg body weight and 50 IU/injection site [174]. Prior to, and as well as three weeks and three months after injections a physiotherapist and an occupational therapist assessed, among other parameters, passive range of motion (pROM) and spasticity according to the Modified Ashworth Scale (MAS) [117].

In Paper V, all children were assessed by a team consisting of pediatric orthopedic surgeons, child neurologists, physiotherapists, occupational therapists both prior to treatment, and three weeks and three months post-injection. Treatment goals were set and followed up as described for *Paper IV*. Additional follow-up measures included spasticity according to the Modified Ashworth Scale (MAS) [117], joint range of motion (ROM), the AHA [113], the Melbourne Assessment [111, 112], SHUEE [116] and assessment of pain for patients with pain reduction as goal. The majority of injections were administered with nitrous oxide (N_2O) analgesia, a few under general anesthesia, and an even smaller number with only local or oral analgesia. All injections were administrated with EMG or ultrasound guidance.

3.2.2 Muscle biopsies

All muscle biopsies were frozen immediately in either isopropane (*Paper I*) or isopentane (*Paper II*) cooled with liquid nitrogen and stored thereafter at -80° C until being analyzed. For histochemistry, muscle samples from children with CP and TD children were cut into 7μm or 10 μm sections, depending of the staining planned, at -20° C using a cryostat.

3.2.3 Protein separation and quantification

SDS-PAGE was used for protein separation in *Paper I*. The levels of the MyHC I, IIa, and IIx isoforms were determined from silver-stained 6% SDS-PAGE [175] where three distinct bands could be identified as MyHC I (migrating fastest), IIa, and IIx isoforms (slowest). The gels were scanned in a soft laser densitometer to determine the relative proportion of each isoform. Results from this procedure has previously been found to correlate well with MyHC content quantified with immuno-histochemistry [176].

3.2.4 Satellite cells, intramuscular collagen and muscle fiber size

Satellite cells were identified in sections fixed and stained according to Lindström et al. [177] utilizing a light microscope equipped with excitation filters connected to a camera. From each cross-section, the number of satellite cells per number of nuclei was determined. A satellite cell was defined as fulfilling the following criteria: positive staining with CD56/NCAM (Neural Cell Adhesion Molecule); possessing a nucleus stained by DAPI (4´,6-diamidino-2-phenylindole); and in a sublaminar position, as confirmed by the laminin staining.

Intramuscular collagen content was visualized by Sirius Red staining according to Junquiera et al. [178], the sections washed and dehydrated as described by de Bruin et al [75] and the cross-sections quantified with a light microscope and camera. Custom-made software for analysis of collagen content and fiber size was developed. Calibrated areas were measured excluding regions with fibers cut longitudinally or folded sections, as well as those containing larger nerves and blood vessels. For each cross-section, the total tissue area and the area staining specifically for collagen were determined and the percentage area of collagen calculated.

Sections stained with Picro Sirius Red were also used for measuring fiber size. Four areas chosen randomly, each containing 150-200 fibers were examined and fiber size assessed by image analysis. Again, regions containing fibers cut longitudinally or folded sections were excluded.

3.2.5 RNA and protein extraction, cDNA synthesis and qRT-PCR

Skeletal muscle tissue was homogenized and RNA and protein extracted utilizing standard techniques. RNA was quantified spectrophotometrically and its integrity assessed by agarose gel electrophoresis. The protein concentration was determined with a colorimetric assay. RNA was reverse-transcribed to cDNA and gene expression evaluated by quantitative real-time polymerase chain reaction (qRT-PCR). For each target gene, all reactions were run in triplicate. Relative levels of expression were obtained by normalization to the level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA.

3.2.6 Western blotting

In *Paper II*, samples containing equal amounts of protein were separated by SDS-PAGE and Western blotting then performed using standard techniques with primary antibodies targeting Transcription Initiation Factor-1A (TIF-1A), Upstream Binding Factor (UBF) and GAPDH. Secondary antibodies were diluted in blocking buffer. Immuno-reactive bands were visualized using infrared fluorescence and band density determined with the Image Studio software.

3.2.7 Analysis of arm movements

The voluntary ROM of the arms was captured by an 8-camera optoelectronic motion capture system (Vicon Oxford, UK). Nineteen spherical reflective markers were attached to

the hands, arms, head and trunk [179]. All anatomic positions that could easily be palpated. The head markers were attached to a headband.

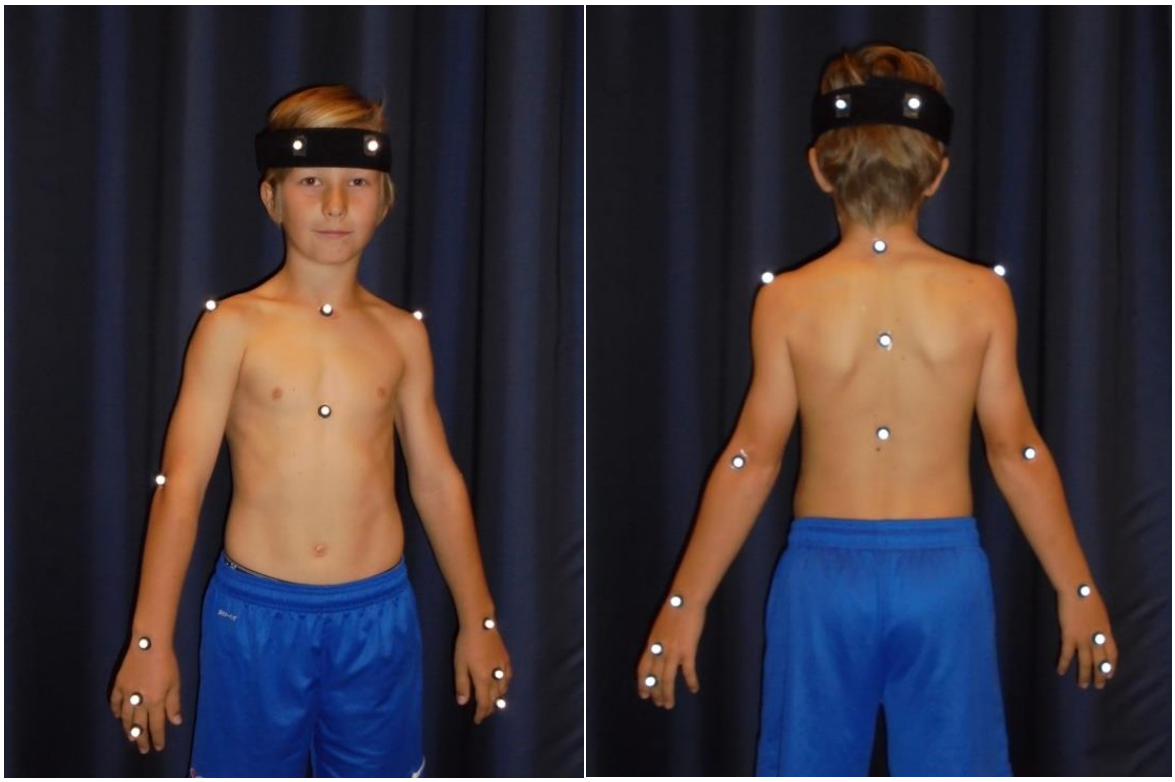

Figure 1. Marker placement (Photo by the author, published with permission from the child and parents)

RFHD = right front head; LFHD = left front head; CLAV = jugular notch; STRN = xiphoid process; LBHD = left back head; RBHD = right back head; C7 = spinous process of the seventh cervical vertebra; T10 = spinous process of the tenth thoracic vertebra; RBAK = one spinous process between the seventh cervical and the tenth thoracic vertebras; LSHO = left acromion; RSHO = right acromion; LELB = left radial epicondyle; RELB = right radial epicondyle; LWRA = dorsal midpoint of the wrist, left arm; RWRA = dorsal midpoint of the wrist, right arm; LFIN = third metacarpophalangeal joint, dorsal left hand; RFIN = third metacarpophalangeal joint, dorsal right hand; LPIP3 = third proximal interphalangeal joint, dorsal left hand; RPIP3 = third interphalangeal joint, dorsal right hand

The overall reach volume for each arm was determined while the children performed arm movements following standardized instructions. Each arm of the TD children was tested three times. The first two sessions, performed with the original marker placement, were used to evaluate intra-session reliability. The third session, performed after removal of all markers, a short break and subsequent marker replacement, was used to evaluate inter-session reliability, in comparison to the second trial. For comparison of the left and right arms, data from the first session with each participant were used. Participants with CP performed only the two first sessions without marker removal and replacement.

A coordinate system around the trunk was created, with the origin defined as the point between the dorsal marker on the spinous process of the seventh cervical vertebrae and the ventral marker between the medial ends of the clavicles. Distal markers were placed on the elbow, wrist, third metacarpophalangeal joint and third proximal interphalangeal joint as above.

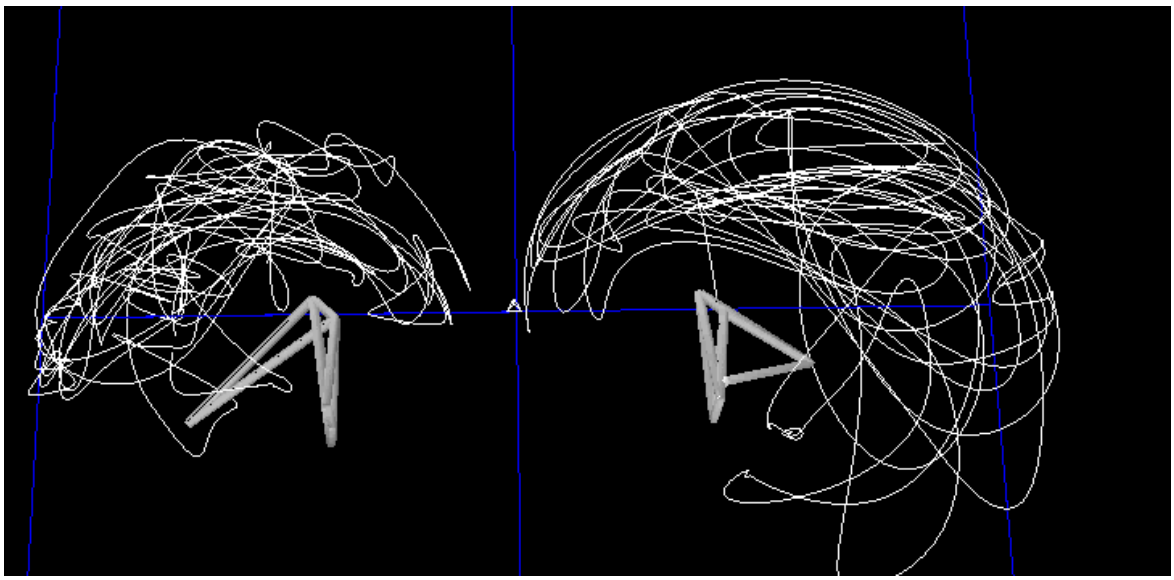

Figure 2.
Superior view of the virtual shape, unilateral (left) CP

The 3D reach volume for each of these markers with respect to the trunk coordinate system was computed as a virtual shape generated by its trajectory and the volume of that shape computed using a Convex Hull algorithm [180] that computes the smallest volume which includes all positions of the marker along the trajectory. To enable inter-subject comparisons this volume was divided by arm length cubed. The standard deviations (SD) of the volumes were determined from the 3 trials.

In order to determine the distribution of this reach volume relative to the subject's body, the overall volume was divided into to two hemispheres, each of which was subsequently divided into four quadrants.

3.2.8 Accelerometry

The physical activity of the subject in his/her own home and school environments was monitored by four uniaxial accelerometers attached with soft elastic bands to both wrists, the waist and around the right ankle [136] with the sum of acceleration recorded by each monitor being converted into "counts" [181, 182]. Since walking and running require reciprocal movement by both legs, one accelerometer on the ankle in combination with one on the waist is sufficient to monitor ambulatory activity. With accelerometers on both wrists, the dominant and the non-dominant sides can be compared. Total body activity was evaluated from the information provided by the accelerometer around the waist [133, 134, 183].

Physical activity was assessed during waking hours for four consecutive days, including two weekdays and an entire weekend [184] both prior to the injections as well as three weeks and three months later. The data were obtained separately from the four accelerometers [135] were synchronized with respect to time and processed employing a customized program developed in MatLab® (MathWorks, Natick, MA, US). For analysis, active wear time was calculated by subtracting non-wear time from the total recording time (raw data).

The data for each day were analyzed independently dividing active time into time spent performing 'voluntary manual activity' or 'ambulatory activity' (walking, running, jumping). Voluntary activity was defined as an acceleration of the waist <25 counts/second

(cps) [183] together with acceleration of one or both arms. Ambulatory activity was defined as acceleration of the waist >25 cps together with acceleration of the leg [183]. Bimanual activity was defined as both arms demonstrating an acceleration >0 cps and the waist an acceleration <25 cps, and expressed as a percentage of the time spent performing voluntary activity. The time spent in physical activity is expressed relative to the total active wear time and its intensity as mean acceleration (cps)

Activity	Definition
Ambulatory activity (W>25 cps + L>0 cps)	t_a/active time
mean acceleration of the leg	sum(L)/t_a
mean acceleration of the dominant arm	sum(dom)/t_a
mean acceleration of the non-dominant arm	sum(ndom)/t_a
mean acceleration of the waist	sum(W)/t_a
Voluntary activity of arms (W<25 cps + arm_{dom}>0 cps and/or W<25 cps + arm_{ndom}>0 cps)	t_v/active time
time spent using the dominant arm (%)	t_{dom}/t_v
time spent using the non-dominant arm (%)	t_{ndom}/t_v
mean acceleration of the dominant arm	sum($_{dom}$)/t_v
mean acceleration of the non-dominant arm	sum($_{ndom}$)/t_v
time spent using the arms simultaneously	t_{sim}/t_v
mean acceleration of the dominant arm during simultaneous use of both arms	sum($_{dom}$)/t_v
mean acceleration of the non-dominant arm during simultaneous use of both arms	sum($_{ndom}$)/t_v

Table 1. Definition of activities

active time= total recording time subtracted with non-wear time, cps=counts/second, t=total time spent performing an activity, t_a=time spent in ambulatory activity, sum=the sum of acceleration (counts), t_{dom}=total time when acceleration of the dominant arm was >0, t_{ndom}=total time when acceleration of the non-dominant arm was >0, t_v=time spent by the arms performing voluntary activity, t_{sim}=time when acceleration of both arms was >0, W=waist worn accelerometer, L=leg worn accelerometer

3.2.9 Switching the brand of BoNT-A

Following a process of procurement by the Stockholm County Council, Botox® was replaced by Dysport®. We carried out a prospective population-based observational study on all children with CP who received BoNT-A treatment to reduce muscle tone elevated due to spasticity or dystonia. Baseline data on the duration and effect of the previous injection were collected for four months prior to the switch, so that each child contributed with evaluation data of 1-2 treatments with Botox® and 1-3 treatments with Dysport® during the study period.

Parents were asked to evaluate the duration and magnitude of the effect (on an ordinal scale of 0 = "no effect" to 10 "best possible effect") of the previous injection, as well as side-effects. The duration of the effect of each treatment was documented by the attending physician using a standardized protocol, in which he/she also recorded the goal of the treatment (functional goals, pain reduction and/or facilitating activities of daily living) together with the weight of the child, the muscles injected and dose and dilution of the product injected. The cost of the of BoNT-A in 2014 (Botox®) and 2015 (Dysport®) to the

Dept. of Pediatric Orthopedic Surgery at Astrid Lindgren Children's Hospital was acquired from the Stockholm County Council and then divided by the number of treatments per year to obtain a cost/treatment.

3.3 STATISTICAL ANALYSES

3.3.1 Paper I

Differences between the flexors and extensors of the wrist were analyzed as related values with the Wilcoxon signed-ranks test. Differences between the muscles of patients with CP and controls were examined with the Mann-Whitney test. Spearman's rank correlation coefficient was used to test for correlation between the percentage of each isoform of MyHC and passive wrist extension or the GMFCS, MACS, and House classifications. We used the PASW1 Statistics 18.0 (IBM, Armonk, NY, US) for all analyses. Data were reported as means ±SD and the level of significance set at $p<0.05$.

3.3.2 Paper II

Differences between levels of mRNA, protein or collagen content were assessed with the Mann-Whitney-Wilcoxon test. Statistical analyses were performed with the Prism 7 Software (GraphPad Software, Inc, CA, US) and the level of significance set at $p<0.05$.

3.3.3 Paper III

The distribution of the data was shown to be approximately normal and parametric statistical analyses were therefore applied.

The Interclass Correlation Coefficients (ICC) (SPSS 24, IBM, NY, US) for the total volume within reach of the four markers (elbow, wrist, MCP3 and PIP3) and for the volume encompassed by these markers in each sector were calculated. Intra-session reliability was computed from the volumes measured in trials 1 and 2 and inter-session reliability from the volumes measured in trials 2 and 3, in both cases using a two-way random model of absolute agreement. The ICC value range from 0 to 1 with a value of 1 reflecting a perfect reliability. The reliability findings were interpreted according to Fleiss [185] with an ICC greater that 0.75 considered excellent.

To assess the disagreement between measurements, the standard error of measurement (SEM) was calculated. In addition, the smallest detectable change (SDC) was calculated to evaluate the new procedure. To analyze for differences in the volumes measured with the dominant and the non-dominant arms, a general linear model for analysis of variance (ANOVA) for repeated measures was employed. The level of significance was set as $p<0.05$.

3.3.4 Paper IV

To account for repeated measures and within-subject variance and correlated data, a linear mixed model was used to analyze outcomes with introduction of an interaction term to evaluate heterogeneity. The statistical analyses were performed with the IBM SPSS Statistics 24 software (IBM, NY, US) and the level of significance set at $p<0.05$.

3.3.5 Paper V

The characteristics of the patients and their disease were described, employing median and range or relative frequencies for continuous and categorical data, respectively. Doses of BoNT-A are presented as means ±SD.

3.4 ETHICAL CONSIDERATIONS

Prior to the collection of material and data, all studies were approved by the regional ethical committee of Karolinska Institutet. Both parents, and, whenever possible, the children themselves (depending on age and mental capacity) received written and oral information about the planned study and provided informed consent before entering.

4 RESULTS AND DISCUSSION

4.1 PAPER I

The most important findings of *Paper 1* were as follows: 1) The wrist flexors of children with CP contain a higher proportion of the fast and fatigable MyHC IIx than TD children. 2) Wrist flexors contain a higher proportion of MyHC IIx than wrist extensors. 3) The wrist flexors of children with CP contain a reduced proportion of the fast MyHC IIa isoform. 4) Wrist flexors contain a lower proportion of MyHC IIa than wrist extensors. 5) The level of MyHC I in children with CP was lowered in their wrist extensors only. 6) No correlations between the levels of MyHC isoforms and GMFCS, MACS, House classifications or passive extension of the wrist were observed.

Children with CP often move one or both arms involuntarily as a result of co-contraction or because of mirror movements (i.e., involuntary simultaneous and similar movement of the opposite hand while performing a task with one hand [186]. Usually, mirror movements are more pronounced in the less affected hand while using the more affected hand is being used [187]. In cases of major brain injury resulting in structural reorganization of the corticospinal tract, the main motor pathway, an ipsilateral, rather than contralateral control of the hand most affected is seen more often [188]. Through such reorganization, the manual ability can be maintained, but at the expense of more frequent mirror movements [186].

The reduced arm movement in children with CP during both manual activities and walking (arm swing) may explain for the upregulation of MyHC IIx observed which is in line with previous reports [189]. This expression is higher when the disability is more severe and spasticity worse [176], since voluntary activation is thought to down-regulate MyHC IIx to a greater extent than either involuntary contractions or mirror movements [60, 67].

We found no correlation between the levels of the different isoforms of MyHC and the functional classification (GMFCS, MACS or House) or severity of contracture. Either our study population was too small to allow any such correlation to be detected or other factors exert a stronger influence on the expression of MyHC. The wide variation in frequency and intensity with which the arms are utilized in daily life, alterations in brain signaling that result in different degrees of muscle spasticity and stiffness, or perhaps other trophic factors secreted by motor nerves might be involved. Our findings on changes in contractile proteins in children with CP motivate further research on: motor control, weakness and contracture development in this context.

4.2 PAPER II

The most important observations in *Paper 2* were as follows: 1) Ribosome biogenesis in skeletal muscle is impaired in children with CP. 2) The expression of genes encoding pro-inflammatory cytokines is elevated in the skeletal muscle of children with CP. 3) The number of satellite cells in their muscles is decreased, and 4) The amount of collagen surrounding bundles of muscle fibers and the levels the products of genes involved in collagen production are higher in these children.

The growth of muscles in children with CP is impaired compared to that in TD children, as shown in several cross-sectional studies [42, 48, 190]. Skeletal muscle associated with CP is not only thinner and weaker, but also stiffer [44], all three important contributors to the reduced range of motion observed [56]. The anabolic response of skeletal muscle and their

development of hypertrophy is largely dependent on the number of ribosomes they contain [84, 85], which essentially determines the rate of protein synthesis [81].

Mechanical loading of skeletal muscle cells enhances their production of ribosomes [82, 83]. Moreover, in newborn mice, inadequate nutrition results in fewer ribosomes and slower synthesis of skeletal muscle protein, resulting in stunting [191]. Indeed, inadequate nutrition and subsequently inhibited growth are well-recognized problems in children with CP as well [192].

The skeletal muscle of children with CP/ABI was found to contain significantly reduced levels of two transcription factors essential for ribosome biogenesis, i.e., Transcription Induction Factor-1A (TIF-1A) and Upstream Binding Factor (UBF). Consistent with these findings, the level of the 45S pre-ribosomal ribonucleic acid (pre-rRNA) transcript in these muscles was also reduced, as was the level of mature 28S ribosomal ribonucleic acid (rRNA). Therefore, we propose that the slower growth of children with CP may, at least in part, result from lowered translational capacity of skeletal muscle due to altered neurological signaling and/or inadequate nutrition [193].

The health and function of skeletal muscle also depend on the satellite cells they contain and we found 40% fewer satellite cells in children with CP/ABI than in TD children, in agreement with previous reports on the legs of the former [91, 194]. Recently, the necessity of satellite cells for hypertrophy of skeletal muscle has been questioned. Genetically modified mice, whose satellite cells have been ablated during adulthood respond normally to a two-week hypertrophic stimulus. However, the situation in the young and growing animal differs: When satellite cells were deleted from these same mice at a younger age, satellite cell-mediated addition of myonuclei was required for muscle hypertrophy, indicating that these cells are indeed needed for skeletal muscle hypertrophy in the growing individual [92].

Further, studies on mice indicate that in addition to their roles in the growth and repair of skeletal muscle, satellite cells are also involved in regulating production of the ECM [93]. The observation of an expansion of perimysial extracellular matrix in association with a 31% increase in intramuscular collagen content in connection with CP/ABI agrees with the higher levels of the transcripts of genes involved in collagen production that we observed (CTGF, TGFB1, TGFBR2, LTPBP1, LOX). We also observed a twofold increase in the level of COL1A mRNA. The dual role in muscle protein synthesis and ECM production, shown so far only in mice, motivates further studies on the potential involvement of satellite cells in contracture development in humans.

Skeletal muscle disorders such as myositis and muscular dystrophies are characterized by impaired growth and an increase in the amount of ECM at the expense of functional muscle tissue, driven in part by cytokines [107, 195]. The present investigation revealed that in muscles of children with CP/ABI the levels of cytokines that can modulate the expression of genes involved in both hypertrophy and collagen production were elevated. Moreover, the level of messenger RNA (mRNA) encoding transforming growth factor-beta (TGF-beta), which up-regulates expression of myostatin (MSTN), a potent negative regulator of muscle mass [196] was enhanced in these same children. Indeed, their level of MSTN mRNA was more than doubled.

MSTN signaling results in muscle atrophy by suppressing protein synthesis and promoting protein degradation and impairing cell proliferation [197, 198]. Furthermore, MSTN is involved in regulating skeletal muscle fibrosis [199] and has negative effects *in vitro* on the

proliferation of satellite cells from both mice and humans [107, 200]. In addition, MSTN regulates fibroblast proliferation and secretion of components [201]. Although the expression of pro-inflammatory genes in the muscle of children with CP was elevated, there was no inflammatory cell infiltration, as found in Duchenne muscular dystrophy or myositis [202]. Therefore, we propose that contracture development and expansion of the ECM in connection with CP represent a distinct pathophysiology.

4.3 PAPER III

The major findings in *Paper III* were as follows: 1) The 3D Reach Test for the arms exhibits excellent inter- and intra-session reliability in TD children as well as 2) excellent feasibility and reliability in a pilot study on five young adults with CP.

The aim here was to develop a simple, but robust 3D Reach Test for one aspect of arm functionality. The test was designed to measure the overall reach and relate the location of this volume to the positions of the trunk and head. In this particular case, we evaluated the reliability of the test in TD children, and performed a pilot study on its feasibility and applicability in young adults with CP.

Both the intra- and inter-session reliability are excellent, with ICCs for total volume of 0.82-0.90 (elbow, wrist, MCP3, PIP3) and the inter-session ICCs 0.80-0.93 (elbow, wrist, MCP3, PIP3), respectively. There was no difference between the right and left arm with respect to any of the four total volumes of reach (elbow, wrist, MCP3, PIP). The ipsilateral anterior superior and inferior volumes together account for approximately 50% of the total volume. For the different sectors, the ICC for intra-session reliability was 0.23-0.96 (mean 0.74) and for inter-session reliability 0.43-0.97 (mean 0.74).

The test demonstrated excellent reliability in young adults with CP, both with respect to overall and sector volumes. For this group, ICCs for the intra-session reliability were between 0.98-0.99 for total volume (elbow, wrist, MCP3, PIP3), and between 0.67-0.99 (mean 0.93) for the 8 different sectors. The volumes of reach were significantly lower for the non-dominant than the dominant arm. The ipsilateral sectors represented a larger proportion of the total volume and there was a larger difference between the volumes of the ipsilateral and contralateral sectors in the case of the non-dominant than dominant arm. For the wrist, MCP3 and PIP3 markers, the total volume of the dominant arm was twice as great. The even higher ICC for this group could be attributed to their age (these subjects were older and could followed instructions more closely, but also to the arm movements typical for people with CP. Whether reliability is correlated with age remains to be determined. In all cases, even for the dominant arm, the total volumes for all markers (elbow, wrist, MCP3 and PIP3) were larger in TD children than in those with CP.

The anatomic landmarks utilized here for marker placement are well-defined and easy to palpate. Indeed, the high inter-session reliability confirms that this placement is reproducible, strengthening the test's applicability as a routine tool for research or clinical use. Measurements, marker placement, practice and data capture for each arm took an average of 10 minutes overall to perform and no one reported any discomfort during the test. The 3D Reach Test is highly standardized, minimizing the risk for bias originating from the evaluation or situation. Division of the obtained 3D reach volume obtained into sectors makes it possible to see the change in the location of this volume as well, information that could be of importance when evaluating arm surgery.

In light of its excellent reliability, we believe that the 3D Reach Test could be a valuable tool for evaluating the effect of treatment effect in patients with limited arm movement,

e.g., those with CP. This test can be used over a wide range of ages, having shown good reliability in children as young as five. Moreover, we also believe that the 3D Reach Test can be applied in an elderly population, since it is performed mainly in a seated position, with only about 15 seconds of standing.

4.4 PAPER IV

The most important observations of *Paper IV* were these: 1) Contrary to the intended clinical outcome, the ambulatory activity of children with CP declines following BoNT-A injection into the legs. 2) Contrary to clinical goals, accelerometer-based follow-up reveals that arm swing does not improve and the arms do not become more similar with respect to quantity of motion following BoNT-A treatment of the arm most affected. 3) These effects were not discovered in connection with routine clinical follow-up.

Prior to the BoNT-A injections, the mean percentage of time the entire group spent performing ambulatory activity was 5.6%. Contrary to the treatment goal three weeks after injections into the legs, this value had declined to 4.7%, and was 3.9% after three months a 30% reduction in a value that was already quite low. One possible explanation is that the weakness experienced was unfamiliar, since a feeling of overly weak muscles is one of the typical undesirable side-effects of BoNT-A reported [158, 159]. Naturally, a further decline in ambulation by these patients, who are already sedentary, is undesirable and probably enhances the risk of chronic detrimental effects on health.

Treatment of the legs with BoNT-A is designed to improve gait by reducing spasticity, as well as an unopposed strength of the muscles injected. Equinus is diminished by injecting the gastrocnemius and soleus muscles and knee extension improved by injecting the hamstrings muscles. Contrary to our own hypothesis, injection of leg muscles did not alter the acceleration of the waist during ambulatory activity nor did we see any decline in the asymmetry of arm swing. In children whose more affected non-dominant arm was injected, this arm exhibited significantly lower acceleration than the dominant arm during ambulatory activity, whereas there was no such difference among those receiving treatment in the legs only. Although BoNT-A was injected into the more affected, non-dominant arm primarily to reduce arm flexion during walking and thus make arm swing more symmetrical, unfortunately, these injections did not improve the symmetry of arm swing.

BoNT-A was injected into the non-dominant, more severely affected arm to make it a better helping hand and improve movement. After such injection, the dominant arm was used 21% more for voluntary activity than the non-dominant arm, versus 2.5% for the corresponding value without injection. Three weeks and three months after injection into the non-dominant arm, the time the dominant and non-dominant arms were engaged increased and decreased slightly respectively, although not significantly. Acceleration of the dominant and non-dominant arm was similar prior to injection and had not changed significantly at the time of follow-up. Thus, these children demonstrated a larger difference between the arms with respect to time spent performing voluntary activity than those who had indications for BoNT-A injection into the legs only.

This finding confirms the indications for treatment, but in contrast to our hypothesis, BoNT-A injection into the arm more affected did not enhance its use. This absence of any BoNT-A treatment effect on arm usage for voluntary activity, could be due to muscle weakness and/or pain, another common side-effect following BoNT-A injections [158, 159]. On the other hand, BoNT-A is also used to treat pain and in this context is believed to act both by directly reducing spasticity and through pharmacological pathways [161].

An alternative explanation could involve an effect of BoNT-A on the central nervous system, which has been suggested to occur via modulation of intrafusal fibers in the muscles injected. This would, in turn, influence sensory afference to the spinal cord, the brainstem and cortical areas. With altered spindle activity, feedback would likely alter the spinal and cortical circuitry, thereby also affecting muscles other than the ones initially injected [162]. In addition to such reorganization of the central nervous system, BoNT-A appears to exert direct actions on the spinal cord that probably involve axonal trafficking of active neurotoxin, since there is no evidence that therapeutic doses of BoNT-A injected intramuscularly can cross the blood-brain barrier [162]. Mazzocchio and co-workers [163] have proposed that BoNT-A may change the excitability of spinal pathways or cause central chemo-denervation via retrograde axonal transport to the spinal cord.

For children with indications for injection of BoNT-A into the arm, the time spent performing bimanual activity (as a percentage of the total time spent in voluntary activity) was longer both before and after injection than for children receiving BoNT-A in the legs only. For children injected into the non-dominant arm, the time spent performing bimanual activity did not change. However, for those treated in the legs only, the proportion of voluntary bimanual activity had risen three weeks later. We also know that these children walked less at this same time-point, so one possible interpretation is that when the effect of the BoNT-A is maximal, the children prefer to play sitting down than on foot.

Three weeks after being injected in one or both legs and one arm, the difference in the acceleration of the arms during bimanual activity by children with CP had increased whereas after treatment of the legs only, there was no such difference. One possible explanation for this surprising finding, the opposite of what was expected, is the occurrence of mirror movements described in *Paper 1* above.

A review of the medical records revealed that for those children who received BoNT-A injections in the legs only, the arms were not evaluated specifically. Moreover, for the children injected in the non-dominant arm, the non-injected dominant arm was not evaluated.

In summary, we demonstrate here that monitoring children with CP in their own home and school environment with accelerometers following BoNT-A injections provides novel and valuable information not obtained through routine clinical follow-up. Three weeks after such injection into the legs the children walked less and their capacity for walking had not recovered by the three-month follow-up. Neither the observed difference between the arms with respect to time spent in voluntary activity nor arm swing symmetry during ambulation were altered by injections into the most paretic arm. We recommend future characterization of both remote and generalized effects of BoNT-A injections.

4.5 PAPER V

The main findings of *Paper V* were the following: 1) Parents reported comparable effects of similar duration with few, similar and transient side-effects when Botox® was replaced by Dysport® (with a conversion factor of 1:2). 2) The procured cost per treatment was 4029 SEK for Botox® and 41% lower (2380 SEK) for Dysport®.

A total of 170 children, 159 with CP, received treatments in connection with 341 visits to the hospital during the study period, with reports 176 treatments with Botox® and 111 treatments with Dysport®. Of the 16 arm muscles injected, the biceps brachii, adductor pollicis and flexor carpi ulnaris were treated most often. In the case of the legs, the three muscles most injected out of 14, were the gastrocnemius, hamstrings and gracilis.

The treatment goals were functional improvement in 190 cases, facilitation of activities of daily living in 35 and pain reduction in 29. In 87 cases, two or more goals were combined with pain reduction being one of these goals in 85% of these children. Botox® was injected into 89 patients at an average dose of 9.4 (SD, 3.22) U/kg body weight. Dysport® was injected into 252 patients at an average dose of 18.4 (SD, 6,55) U/kg body weight, i.e., the conversion factor of 1:2 was achieved. Parents reported comparable effects of similar duration for these two BoNT-A products. Side-effects were reported after 9/176 injections of Botox® and 5/111 injections of Dysport® with weakness and transient pain being most common. No less than 99.3% of the information targeted was actually collected.

The annual cost for BoNT-A was 1,120,021 SEK in 2014 and 716,267 SEK in 2015. The cost per treatment was 4029 SEK for Botox® and 2380 SEK for Dysport®. Botox® and Dysport® were equal in reported effect and duration in children with CP when Dysport® was diluted to a concentration of 100 U/ml and injected at a dose twice as high as with Botox®. This conversion factor of 1:2, lower than in many previous studies, resulted in a cost reduction of 41%. A similar cost reduction of 37% was found when adults with cervical dystonia were treated with Dysport® instead of Botox® (conversion factor 2:1) [203]. The expense could have been reduced even more if Dysport® could be obtained in vials containing less than 300 U. Botox® was available in vials containing smaller amounts, resulting in less waste.

After dilution to 100 U/ml the volume of Dysport® injected was the same as for 50 U/ml Botox®. The effect of different dilutions of BoNT-A is under debate. Both animal and human studies indicate that more extensive dilution enhances the efficacy of each U of toxin by increasing the volume administrated and thereby the extent of diffusion [204-206]. However, other studies, including two on children with spastic CP [207, 208], have concluded that the efficacy is independent of dilution [207, 209, 210]. Injection of larger volumes can be more painful and has been reported to give more side-effects due to the spread of toxin to nearby muscles [207, 210].

We conclude that replacing Botox® with Dysport® reduces cost by 41% with no loss in the magnitude or duration of effect, maintenance of safety and without any increase in associated costs. Our study population was relatively large, including all children with CP among a well-defined one-fourth of the entire Swedish population who received BoNT-A. Even though parents were asked about the effect of previous treatments, a standard aspect of care, the study design was prospective.

5 LIMITATIONS

Even though CP is the most common cause of physical disability in children, it is difficult to collect large numbers of skeletal muscle biopsies, since the children often need to be anaesthetized or sedated in this connection. The best opportunity is in conjunction with surgery or injection of botulinum toxin. However, with an incidence of 2-3/1000 children in combination with the criteria for anesthesia or sedation, few children with CP are eligible. The large variations in disease severity and muscle composition also require a larger number of subjects than we had to obtain sufficient statistical power. Control biopsies from TD children are also difficult to obtain for the same reasons. Collecting biopsies from muscles exposed during open reduction of dislocated fractures is complicated, since these operations are often performed outside office hours.

Cross-sectional studies on muscle samples from children with CP and contracture formation of differing severity indicate progression of this pathology, although the inter-individual variability is extensive. Moreover, to follow actual morphological changes, muscle samples should be collected from the same muscle of the same child repeatedly and few children undergo repeated surgical procedures that provide access to the same muscle.

The muscles of the body differ in many respects, and biopsies from different muscles must be compared with caution. Sometimes a group of muscles with similar function can be combined, but comparing corresponding muscles from children with CP and TD children is preferable. The muscle composition also changes as the child grows and information on development in association with puberty can provide valuable insights.

Specific limitations of the present studies:

In *Paper I*, the control children were slightly younger (mean age 10, range 7-13 years) than the those with CP (mean age 14, interval 8-17 years). At the department where this research was conducted, trauma in children younger than 15 is treated and, therefore, no TD children older than over 14 years of age could be included. At this same department, children with CP are treated until the age of 18. Tendon transfers in the forearm are usually performed on older teenagers with CP.

Biopsies were taken only from muscles directly accessible during surgery (open fracture reduction or tendon transfer), so that these biopsies derived from slightly different muscles. All muscles in the human body demonstrate their specific phenotypes, but the FCU and FCR, as well as ECRB and ECU have similar functions and architecture and could therefore be grouped.

With the SDS-PAGE procedure employed, small amounts of developmental myosin are difficult to distinguish from other forms of MyHCs. Earlier immunohistochemical investigations techniques have revealed that children with CP have more very small fibers expressing developmental myosin in their wrist and elbow flexors than control [45, 47]. These small cells were interpreted as an indication of ongoing regeneration, rather than immaturity, since all larger cells expressed only mature MyHC. However, the net amount of developmental myosin in spastic flexors was very small.

In *Paper II*, we included both patients with CP and acquired brain injury (ABI). We did so because the two patients with ABI suffered their injuries at a young age (7 and 8 years), had

similar fixed contractures and were considered candidates for a similar surgical procedure. Control biopsies were taken at autopsy within 24 hours of death.

Paper IV is limited by the small number of patients, the heterogeneity of the subtype of CP, and the variation in age and muscles injected, reflecting the variety of patients treated at our department. In addition, some data were lost due to technical problems with the accelerometers and the failure of some children to participate in the entire follow-up.

In *Paper V*, the effect of treatment was reported by the parents, i.e., not in an objectively measurable manner. The five experienced pediatric orthopedic surgeons who participated had slightly different ways of describing the goals that the parents, children and treating physician, physiotherapists and occupational therapists agreed upon. Analyses related specifically to goal fulfillment were therefore difficult to perform.

Paper V could not have a blinded design, since we were required to inform the parents and children about the change in products. In addition, the entire multi-professional team was aware of the change in brand, since all underwent training about dosing and diluting the new drug to ensure patient safety. Neither the parents, children nor staff involved expressed any expectation or hope of anything more than a neutral effect of the product change, which could result in expectation bias.

6 SUMMARY AND CONCLUSIONS

The major findings described here are as follows:

6.1 PAPER I

- The wrist flexors of children with CP contain a higher proportion of the fast and fatigable MyHC IIx isoform than TD children.
- Wrist flexors contain a higher proportion of MyHC IIx than wrist extensors.
- The wrist flexors of children with CP contain a lowered proportion of the fast MyHC IIa isofrom.
- Wrist flexors contain a lower proportion of MyHC IIa than wrist extensors.
- The wrist extensors of children with CP contain a lower proportiopn of the slow MyHC I isoform.

Both their brain injury and the various requirements imposed on the flexors and extensors of the wrist affect the expression of MyHC in children with CP. Reduced voluntary usage of the arms and a partial alteration in neuronal signaling from the injured brain can both contribute to the elevated levels of MyHC IIx seen in these children.

6.2 PAPER II

- The biogenesis of ribosomes is impared in the skeletal muscle of children with CP.
- The expression of genes encoding pro-inflammatory cytokines is elevated in this same tissue.
- The number of satellite cells in the skeletal muscle of children with CP is reduced.
- More intramuscular collagen surrounds bundles of muscle fibers and the levels of transcripts of genes involved in collagen production are higher in this same tissue.

The underlying cause of contracture development in children with CP is their brain injury and the consequent alteration in neuronal signaling. Pro-inflammatory cytokines may mediate both reduced growth and undesirable production of extracellular matrix in connection with such development of muscle contractures and decreased production of ribosomes may also be a contributing factor. The finding of fewer satellite cells and an elevated amount of intramuscular collagen in the biceps brachii muscle of children with CP are consistent with previous findings on other muscles of these patients.

6.3 PAPER III

- The 3D Reach Test for the arms demonstrates excellent inter- and intra-session reliability in TD children.
- This 3D reach Test shows excellent feasibility and reliability in a pilot study on five young adults with CP.
- The results are independent of which arm is dominant in the case of TD children, but indicates a clear side difference in individuals with unilateral CP.

The 3D Reach Test for the arms demonstrates excellent inter- and intra-session reliability, excellent feasibility and takes only 10 minutes to perform with no discomfort to the subject. We recommend the 3D Reach Test for use in clinical practice, as well as for investigating e.g., the efficacy of an intervention.

6.4 PAPER IV

- Accelerometer-based follow-up reveals that, contrary to the intended clinical outcome, ambulatory activity declines following BoNT-A injection into the legs.
- Contrary to clinical goals, such follow-up shows that arm swing does not improve and the arms do not become more similar with respect to quantity of motion following BoNT-A treatment of the arm most affected.
- These effects were not discovered through routine clinical follow-up.

Monitoring children with CP in their own home with accelerometers following BoNT-A treatment reveals previously unknown effects on physical activity that are not detected by routine clinical follow-up.

6.5 PAPER V

- The parents of children with CP report effect of similar magnitude and duration, with few, similar and transient side-effects following the replacement of Botox® with Dysport® for treatment of spasticity and pain.
- The cost per treatment was 4,029 SEK for Botox® and 2,380 SEK for Dysport®.

When Botox® was replaced by a dose of Dysport® twice as high, the parents of children with CP perceived the treatment to be equally effective, while the procured cost was lowered 41%.

The results of *Paper I* regarding MyHC expression in the muscles of children with CP are proposed to reflect altered neuronal signaling, differences in muscular requirements and reduced activity due to established contractures. Whether the enhanced level of MyHC IIx in these muscles is caused by the reduced voluntary usage of the arms remains to be proven. Increased usage might favor the expression of the MyHC I and MyHC IIa isoform which could potentially promote endurance.

The several potential causes of skeletal muscle contractures found in *Paper II* include reduced biogenesis of ribosomes, enhanced expression of genes encoding pro-inflammatory cytokines, reduced numbers of satellite cells, higher levels of transcripts of genes involved in collagen production and the larger amount of collagen surrounding bundles of muscle fibers.

Previous evaluations of functional gains by children with CP from BoNT-A treatment have shown varying results, although reduction of spasticity is usually observed. *Paper III and IV* describe novel and promising approaches to evaluating such treatment. *Paper IV* reveals that treatment with botulinum toxin can give rise to unexpected and sometimes undesirable effects which should be taken into account when planning follow-up. Particularly if the treatment goals are functional gain, assessment of muscles and/or limbs not injected should be included in the evaluation.

Furthermore, BoNT-A treatment is expensive in terms of both the cost of the drug itself and the cost for the staff involved in the injection, follow-up and training post-treatment. In *Paper V*, we have shown that the cost of the drug can be reduced by more than 40% with preserved effect and duration simply by switching to another product, thereby freeing up more resources for training and follow-up.

7 FUTURE PERSPECTIVES

In order for us to understand contracture development in children with CP more has to be done. I believe that repeated biopsies from the same child during childhood and adolescence could give more valuable insights into the mechanisms underlying contracture formation. Today, we do not know whether the biochemical and histological changes that we observe in CP muscle are the cause or the consequence of the contracture.

Spasticity peaks at about four years of age and then gradually levels out at the age of 12 [53]. Contracture formation however, starts early and continues progressively during growth, with more rapid development during puberty [54, 55, 57]. The early biopsy most likely holds the key to understanding contracture formation. Muscle sampling at a young age would enable a comparison between muscle before and after the establishment of a fixed contracture that would give us information on the state of the muscle before the development of the contracture. Tentative findings to explore further in young CP muscle is for example the increased levels of TNF and IL-6 and the reduced biogenesis of ribosomes that we found in muscle with fixed contractures.

Detailed information on the effects of BoNT-A on muscle, especially repeated injections, in children with CP is also warranted. We know that the short-term effect of BoNT-A is reduced spasticity [137, 146-148] and that BoNT-A initially increases the ROM even if the obtained ROM cannot be preserved over time [155]. Rather than one leading to the other, spasticity and contracture development seem to be two parallel phenomena, as contracture development continuous despite substantial and permanent spasticity reduction through e.g. SDR [211]. Important questions to answer are whether the process of contracture development is just so strong that it overruns efforts to prevent the development of a contracture or if BoNT-A treatment, in worst case, could even accelerate the development. Contrary to what could be expected, studies on rats following BoNT-A injection have shown an increased gain of the stretch reflex by an increased excitability of spinal motor neurons actually counteracting the intended spasticity reduction [212]. An increased muscle stiffness has also been found [213]. Whether the same findings also happen in humans remain to be proven. Furthermore, it's also of great importance to elucidate if BoNT-A treatment affects skeletal muscle growth and/or ECM production, processes affected in skeletal muscle with contractures.

To answer some of the questions above we have collected a unique material with repeated biopsies from the biceps brachii and gastrocnemius muscles of children with cerebral palsy. The first biopsy was taken prior to the initiation of BoNT-A treatment from BoNT-A naïve skeletal muscle. Follow-up biopsies were taken 3-6 and approximately 12 and 24 months after the first BoNT-A injection in conjunction with the next injection. As spasticity is often high at young age and contractures typically develop somewhat later in life, the first biopsy was often collected before a fixed contracture was established. To be able to answer mechanistic questions on the BoNT-A effect, ideally, repeated biopsies from children with CP but without BoNT-A injections should also be collected. However, as surgery on i.e. gastrocnemius or biceps brachii muscles are performed after the establishment of a contracture consecutive biopsies from early age without any relation to treatment is difficult to obtain. Animal studies could be an alternative, but also poses problems as there still is no ideal animal model for CP.

As satellite cells are important for skeletal muscle growth [89, 90] but also play a role in the regulation of ECM production [93, 94], these cells are of great interest for the study of contracture development in children with CP. Recently, Domenighetti and co-workers [214] showed a loss of myogenic potential for satellite cells isolated from CP muscle. The

authors speculate that this was due to altered expression of genetic programs associated with muscle stem cell differentiation and muscle fiber formation, plausibly conditioned by an alternative DNA methylation pattern. Further, Fry and collaborators [94] demonstrated that satellite cells communicate with interstitial fibro-genic cells to ensure a proper ECM production in skeletal muscle during hypertrophic growth through the secretion of exosomes. Whether these two processes are involved in contracture formation in skeletal muscle of children with CP are still unexplored. In the future, research efforts should be focused on comparisons between muscle of children with CP and TD children as well as comparisons of muscle with and without a fixed contracture with respect to e.g. epigenetic mechanisms. Several layers of regulatory mechanisms control gene expression including DNA methylation as studied by Domenighetti et al, but also other potential mechanisms such as histone modification through acetylation and/or methylation and RNA interference via miRNAs. With more knowledge on the epigenetic modifications of satellite cells and the communication between satellite cells and fibro-genic cells there might be a potential way of inhibiting contracture development in the future.

The 3D Reach Test shows promising results on reliability and feasibility in both TD children and young adults with CP, which is an inspiration for further development of the test. Further reliability tests may be warranted in younger children, both TD and with CP, to establish reliability across a wider age span. The 3D Reach Test could also be of interest in in an elderly population, warranting evaluation in this group also. Given the excellent reliability we have found in our test group, good or excellent reliability in other groups can be expected. Once reliability is confirmed for an age-group, evaluation on treatment might follow in different diagnosis with restricted arm movement.

Further, we need to perform a study for the clinical use and interpretability of the test in children with CP. We plan to compare our new 3D Reach Test to already available clinical tests in order to examine whether an existing test, or part of an existing test, could be replaced or whether the 3D Reach Test adds information to that gained from other tests. Melbourne Assessment 2 (MA2) provides the possibility to analyze the elements “Range of movement” and “Accuracy of reach” separately [112] in children 2.5-15 years of age, making the MA2 a good candidate for a bench-mark test for the proposed comparison.

8 POPULÄRVETENSKAPLIG SAMMANFATTNING

Cerebral pares (CP) är den vanligaste orsaken till fysiskt rörelsehinder i världen idag. Termen CP används idag som ett paraplybegrepp för bestående avvikelser avseende rörelse och hållning samt problem med kommunikation, kognition, tolkning av sinnesintryck och varseblivning till följd av en skada som drabbat den omogna hjärnan. Hjärnskadan kan uppstå under fosterstadiet, i samband med förlossning eller under de första levnadsåren. Trots att hjärnskadan i sig är icke-progressiv, yttrar den sig på olika sätt i rörelseapparaten i olika åldrar.

Minskad ledrörlighet och ökat muskelmotstånd vid passiv rörelse är vanligt. Spasticitet, en komponent i det ökade rörelsemotståndet, är som störst runt fyra års ålder. Minskad ledrörlighet, också kallat kontraktur, utvecklas under hela barndomen och tonåren. Orsaken till minskad ledrörlighet vid CP är inte känd. För att i framtiden bättre kunna behandla, och kanske i framtiden förebygga minskad ledrörlighet, behöver vi få mer kunskap om vad som händer i muskeln när rörligheten försämras. I denna avhandling ingår två arbeten som beskriver muskler hos barn med CP.

Många olika insatser görs för att hjälpa barn med CP till bättre rörelse och självständighet. Korrigering av skelett och mjukdelar med ortopedisk kirurgi är en, att minska spasticitet i muskler med botulinumtoxin en annan och träning ytterligare en insats. Oavsett behandling är det viktigt att följa upp resultatet för att kunna planera den fortsatta behandlingen och utveckla behandlingen av barn med CP. I denna avhandling har vi vidareutvecklat och testat upprepbarheten i ett utvärderingsinstrument som mäter tredimensionell räckvidd i armarna på barn utan rörelsehinder. Vi har även testat metoden på unga vuxna med CP. Vidare har vi utvärderat botulinumtoxin-behandling av barn med CP på ett nytt sätt genom att med fyra stycken rörelsemätare före och efter behandling följa dem under fyra dagar i deras hem- och skol-miljö.

Botulinumtoxin-behandling av barn med CP är en dyrt och det är tidskrävande för både barn, föräldrar och vårdpersonal. För att använda våra resurser så effektivt som möjligt, inte minst för att göra behandlingen tillgänglig för alla som behöver den, har vi ett ansvar att göra det så billigt och kostnadseffektivt som möjligt med bibehållen kvalitet. Priset på botulinumtoxin är en aspekt att ta hänsyn till. I denna avhandling har vi genomfört ett kontrollerat byte från ett dyrare till ett billigare botulinumtoxin samtidigt som vi följt upp behandlingseffekten, hur länge medicinen verkar och om den nya behandlingen har fler eller andra biverkningar än den tidigare.

Det viktigaste jag har kommit fram till i min avhandling är följande:

- Signalsubstanser som normalt verkar vid inflammation (proinflammatoriska cytokiner) skulle kunna ha en roll i utvecklingen av minskad ledrörlighet hos barn med CP genom att både hämma muskeltillväxt och att göra muskeln styvare genom att stimulera bildningen av bindväv i muskeln.
- Minskad muskeltillväxt hos barn med CP skulle kunna orsakas av en försämrad produktion av protein till följd av försämrad funktion i muskelcellens ”proteinfabrik” (ribosomen).
- Bicepsmuskeln i överarmen hos barn med CP innehåller ett minskat antal muskelstamceller (satellitceller) och en ökad mängd bindväv mellan muskelfiberbuntarna.
- Det nya testet för att mäta tredimensionell räckvidd uppvisar en hög upprepbarhet hos barn utan rörelsehinder och en mycket hög upprepbarhet för unga vuxna med CP samt

en mycket hög genomförbarhet i båda grupperna. Det nya testet är snabbt och enkelt att genomföra.

- En alternativ uppföljning av barn med CP som fått behandling med botulinumtoxin med fyra stycken rörelsemätare (accelerometarar) fångar förändringar som traditionell uppföljning på sjukhus missar. Till exempel så minskar aktiviteten till fots tre veckor efter behandling och denna nedgång består fortfarande efter tre månader.
- Vi har visat att vi genom bytet från ett botulinumtoxin (Botox®) till ett annat (Dysport®) kunde reducera läkemedelskostnaden med 41% med bevarad effekt, bevarad tid för effekten och utan att biverkningsprofilen ändrades.

I vårt fortsatta arbete hoppas vi kunna bringa ytterligare klarhet i varför ledrörligheten minskar hos barn med CP genom att ta ett muskelprov från samma barn vid flera tillfällen och helst med ett första muskelprov taget innan ledrörligheten påverkats. Detta skulle ge oss möjlighet att bättre avgöra vad av våra, och andras, tidigare fynd som är orsak till den minskade ledrörligheten och vad som är en konsekvens av den samma.

Vidare hoppas vi i framtiden kunna svara på frågan om huruvida injektioner med botulinumtoxin, särskilt upprepade sådana, påverkar antalet muskelstamceller, muskelns tillväxt eller produktionen av bindväv i muskeln. Muskelstamcellernas roll behöver även utredas vidare såtillvida att vi behöver lära oss mer om hur tidpunkten för hjärnskadan vid CP påverkar muskelstamcellernas funktion och antal och om barn med CP har färre muskelstamceller från början eller om de förlorar stamceller successivt under uppväxten. Vi hoppas också kunna utreda om de signalsubstanser som normalt verkar vid inflammation påverkar tillväxten i såväl muskel som i skelettets tillväxtzoner.

Det tredimensionella testet för räckvidd behöver testas vidare för yngre barn utan rörelsehinder och för barn och ungdomar med CP för att bekräfta användbarheten i dessa grupper. En undersökning behöver också göras för att bekräfta att det nya testet kan ersätta andra mer tidskrävande tester för att senare kunna komma i klinisk användning.

9 ACKNOWLEDGMENTS

I wish to express my sincere gratitude to all of you who have supported me and contributed to this thesis in different ways, and especially to the following:

All the children and young adults who participated in my studies, your contribution was decisive!

Eva Pontén, pediatric orthopedic surgeon and my principal supervisor. Thank you for introducing me to science, sharing your extensive knowledge about cerebral palsy and helping me reach this goal. I never thought I could do this!

Lanie Gutierrez-Farewik, professor in biomechanics and my co-supervisor. You are my true inspiration, thinking so fast and making things look so easy and at the same time being such a warm, caring and supportive friend, standing by me throughout the entire journey, from the admission seminar until the finish line. Thank you so much!

Gustavo Nader, associate professor of kinesiology and also my co-supervisor. Thank you for allowing me to familiarize myself with the work in your laboratory at CMM. I learned a lot! Being part of your group for a while gave me insight into a new world. Thank you very much!

Hans Forssberg, professor in pediatric neurology and for many years one of my co-supervisors. Thank you for keeping a watchful eye on me and my progress.

Ferdinand von Walden, doctor, co-supervisor and true friend. Formally my co-supervisor for only somewhat less than a year, but in reality, my supervisor, support and coach for many years. Working together with you is so enjoyable! You are smart, fast, hard-working and so helpful and generous with your time and knowledge, in addition to having three jobs, four kids and a soccer team to coach. I am impressed! Without you, I would never have had a chance to reach this goal and I can´t thank you enough!

Henrik Smeds, doctor, external mentor and dear friend. Thank you for keep me on track. Every time I went astray on this journey, you helped me find the way back. Every time we speak, I gain new self-confidence and trust in the project. Thank you, from the bottom of my heart!

Yvette Hedström, Chang Liu, Hanna Borgström, Lars Björk, Ola Gremark, Per Stål, Huina Mao, Sandra Vikerfors, Josefin Jansson Edqvist, Maria Hagströmer, Kristina Tedroff, Gustaf Befrits and Carl Johan Tedroff, friends and co-authors. Without you there would be no papers. And no thesis! A special thanks to **Kristina** for the moral support when I lost faith in myself and my project and for the opportunity to plan and execute *Paper V* together with you.

Åsa B, Josefin EN, Elin L, Cecilia L, Kicki L, Marie E, Åsa H, Elena, Dan J, Anna-Clara E, Lena KS, Anki E, Johan G, Mimmi Ö, Johan vH, Marie A, Eva BM, Linda N, Eli G, Catarina E, Britt-Marie Z, Linda H, Patrick B, Birgitta H, Pähr E, Jacques R, Mominul I, Jenny HG, Per Å, Nikos K, fellow researchers, former and present, at Karolinska Institutet. For the joy, inspiration, support and your help with anything and everything. A special thanks to technician **Mikael Reimeringer**, always so kind, helpful and professional. Your knowledge about computers is invaluable!

Fellow students of the research school for clinicians in molecular medicine. Twenty weeks of education, fun and friendship!

None-Marie Kemp, Anna Sandberg and Agneta Nordenskjöld, staff and director of studies at the Department of Women´s and Children´s Health. Thank you for help and support with every question that came up - and there were a lot!

Eva Broström, associate professor and my employer at Karolinska (Institutet and University Hospital!). In addition to being a brilliant scientist, you are a very supportive and encouraging boss! I simply cannot understand how you find the hours you need. Thank you for your unwavering support. You are the best boss I have ever worked for!

Stephanie Böhm, pediatric orthopedic surgeon and fellow Patient Flow Captain (sic!). A highly skilled surgeon and a warm and supportive friend and colleague. If you hadn´t covered for me for almost a year, I wouldn't have had a chance to complete my thesis. Thank you very much!

Colleagues at Astrid Lindgren Children´s Hospital, in particular **pediatric orthopedic surgeons and the management group in our patient area.** Karolinska provides a fantastic working environment with respect to both co-workers, knowledge and experience with every aspect of pediatrics and nursing. It has been extremely inspiring to work with you all!

Two of you who have been especially important to me, **Mikael Rolfs and Anja Berglund**, unfortunately left the hospital on the same day, which was not a good day for me! Working at the children's hospital is not the same without you!

The Department of Orthopedic Surgery in Västerås, and especially my supervisor there **Ola Vikerfors.** Thank you for teaching me the basics, and so much more, about orthopedic surgery. The best beginning of a career as an orthopedic surgeon that one could wish for!

Sjukvårdens ledarskapsakademi. The best network you can imagine! Thanks for the joy, discussions, friendship and support, as well as the inspiration to always keep developing as a leader.

Friends, neighbors and former classmates, the “real” life outside work. Thanks for friendship, dinners, everyday meetings and helpfulness, which I appreciate so much!

IBF Offensiv Lidingö, and especially **Christoffer Wallin** and the **P01 Team**; the floorball club, my fellow coach and our fabulous boys. Coaching floorball games is probably the best way to forget everything else for a while. Thanks for all the joy, drama and distraction!

Magnus Areskog and Barbro Wijma, godfather and godmother, associate professor and professor. Thank you for the inspiration! Attending your dissertations and your dissertation parties in the early 1980´s lit something inside me.

Staffan, Kerstin and Kyllan; uncle, aunts and godmother. Thank you for being around since my childhood and following me, my career and my family with such keen interest. I am so impressed that you always remember my children´s birthdays. Thank you!

Johan & Sophie, Josephine & Sarah, Eva & Anders, Hanna, Andrea & Fred, brothers- and sisters-in-law and your children. Thank you for your interest in me and my work, for family dinners and discussions on all subjects imaginable. Sharing summer life with you in Falsterbo has been a privilege.

Brita Ekwall, my mother-in-law. Thank you for welcoming me into the Ekwall family and for showing your trust in me. Preparing Falsterbo for the next generation the way you and Åke did is true love. I wish Åke could have been here today.

Tomas Gantelius, my brother, with family **Matilda, Tage, Selma, Olov and Maja**. The best brother I can imagine, always close, supportive and interested and always an excellent conversational partner. Watching the cousins get together when we meet is true joy and happiness!

Lena and Lars-Åke Johansson, mamma och pappa. Your support throughout my life means everything to me. Always believing in me, supporting my decisions, listening to whatever I feel I need to tell you. Always curious about my life and my family. Thank you! For everything!

Ebba, my dearest friend and the love of my life! Without your support, patience and encouragement, this thesis would never have been finished. You are so much: a wonderful wife, a fantastic mother, my dearest friend and an incredibly competent professional, all in one. I admire you and I am truly impressed the way you handle life! With you, our everyday life is exciting. Travelling and experiencing new things with you is even better. Watching our wonderful children grow is the greatest gift of all. I love you!

Edvin, Liv och Tom. Ni är de mest värdefulla personerna i mitt liv! Att få följa er i vardagen, att hjälpa er att växa och mogna är mitt livs viktigaste uppgift. Ni är mitt livs största glädje. Jag älskar er!

Funding for this thesis was provided by the following organizations: Norrbacka-Eugeniastiftelsen, Stiftelsen Promobilia, Jerringfonden, Sällskapet Barnavård and Karolinska Institutet KID-funding as well as through the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and Karolinska Institutet.

10 REFERENCES

1. Graham, H.K., et al., *Cerebral palsy.* Nat Rev Dis Primers, 2016. **2**: p. 15082.
2. Colver, A., C. Fairhurst, and P.O. Pharoah, *Cerebral palsy.* Lancet, 2014. **383**(9924): p. 1240-9.
3. *Prevalence and characteristics of children with cerebral palsy in Europe.* Dev Med Child Neurol, 2002. **44**(9): p. 633-40.
4. Westbom, L., G. Hagglund, and E. Nordmark, *Cerebral palsy in a total population of 4-11 year olds in southern Sweden. Prevalence and distribution according to different CP classification systems.* BMC Pediatr, 2007. **7**: p. 41.
5. Chounti, A., et al., *Sex differences in cerebral palsy incidence and functional ability: a total population study.* Acta Paediatr, 2013. **102**(7): p. 712-7.
6. Paneth, N., T. Hong, and S. Korzeniewski, *The descriptive epidemiology of cerebral palsy.* Clin Perinatol, 2006. **33**(2): p. 251-67.
7. Himpens, E., et al., *Prevalence, type, distribution, and severity of cerebral palsy in relation to gestational age: a meta-analytic review.* Dev Med Child Neurol, 2008. **50**(5): p. 334-40.
8. Little, W.J., *On the incidence of abnormal parturition, difficult labour, premature birth and asphyxia neonatorum on the mental and physical condition of the child, especially in relation to deformities.* Trans. Obstet. Soc., 1862. **3**: p. 293–344.
9. Osler, W., *The Cerebral Palsies of Children.* 1889.
10. Freud, S., *Zur Kenntnis der cerebralen Diplegien des Kindesalters (im Anschluss an die Little's Krankheit).* 1893.
11. Mutch, L., et al., *Cerebral palsy epidemiology: where are we now and where are we going?* Dev Med Child Neurol, 1992. **34**(6): p. 547-51.
12. Rosenbaum, P., et al., *A report: the definition and classification of cerebral palsy April 2006.* Dev Med Child Neurol Suppl, 2007. **109**: p. 8-14.
13. Edwards, A.D. and S. Tan, *Perinatal infections, prematurity and brain injury.* Curr Opin Pediatr, 2006. **18**(2): p. 119-24.
14. Barkovich, A.J., et al., *A developmental and genetic classification for malformations of cortical development: update 2012.* Brain, 2012. **135**(Pt 5): p. 1348-69.
15. Volpe, J.J., *Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances.* Lancet Neurol, 2009. **8**(1): p. 110-24.
16. Oskoui, M., et al., *Clinically relevant copy number variations detected in cerebral palsy.* Nat Commun, 2015. **6**: p. 7949.
17. Bax, M., et al., *Proposed definition and classification of cerebral palsy, April 2005.* Dev Med Child Neurol, 2005. **47**(8): p. 571-6.
18. Blair, E., N. Badawi, and L. Watson, *Definition and classification of the cerebral palsies: the Australian view.* Dev Med Child Neurol Suppl, 2007. **109**: p. 33-4.

19. Bax, M., C. Tydeman, and O. Flodmark, *Clinical and MRI correlates of cerebral palsy: the European Cerebral Palsy Study.* Jama, 2006. **296**(13): p. 1602-8.

20. Himmelmann, K., et al., *Risk factors for cerebral palsy in children born at term.* Acta Obstet Gynecol Scand, 2011. **90**(10): p. 1070-81.

21. Dahlseng, M.O., et al., *Risk of cerebral palsy in term-born singletons according to growth status at birth.* Dev Med Child Neurol, 2014. **56**(1): p. 53-8.

22. Villamor, E., K. Tedroff, and S. Cnattingius, *Maternal Obesity and Cerebral Palsy in Offspring-Reply*. Jama, 2017. **317**(24): p. 2550.

23. Apgar, V., *A Proposal for a New Method of Evaluation of the Newborn Infant. Originally published in July 1953, volume 32, pages 250-259.* Anesth Analg, 2015. **120**(5): p. 1056-9.

24. Persson, M., et al., *Five and 10 minute Apgar scores and risks of cerebral palsy and epilepsy: population based cohort study in Sweden.* Bmj, 2018. **360**: p. k207.

25. Back, S.A., et al., *Late oligodendrocyte progenitors coincide with the developmental window of vulnerability for human perinatal white matter injury*. J Neurosci, 2001. **21**(4): p. 1302-12.

26. Himmelmann, K., et al., *Dyskinetic cerebral palsy: a population-based study of children born between 1991 and 1998.* Dev Med Child Neurol, 2007. **49**(4): p. 246-51.

27. *Surveillance of cerebral palsy in Europe: a collaboration of cerebral palsy surveys and registers. Surveillance of Cerebral Palsy in Europe (SCPE).* Dev Med Child Neurol, 2000. **42**(12): p. 816-24.

28. Himmelmann, K. and P. Uvebrant, *The panorama of cerebral palsy in Sweden part XII shows that patterns changed in the birth years 2007-2010.* Acta Paediatr, 2018. **107**(3): p. 462-468.

29. Himmelmann, K. and P. Uvebrant, *The panorama of cerebral palsy in Sweden. XI. Changing patterns in the birth-year period 2003-2006.* Acta Paediatr, 2014. **103**(6): p. 618-24.

30. Arner, M., et al., *Hand function in cerebral palsy. Report of 367 children in a population-based longitudinal health care program.* J Hand Surg Am, 2008. **33**(8): p. 1337-47.

31. Palisano, R., et al., *Development and reliability of a system to classify gross motor function in children with cerebral palsy.* Dev Med Child Neurol, 1997. **39**(4): p. 214-23.

32. Eliasson, A.C., et al., *The Manual Ability Classification System (MACS) for children with cerebral palsy: scale development and evidence of validity and reliability.* Dev Med Child Neurol, 2006. **48**(7): p. 549-54.

33. Eliasson, A.C., et al., *Mini-MACS: development of the Manual Ability Classification System for children younger than 4 years of age with signs of cerebral palsy.* Dev Med Child Neurol, 2017. **59**(1): p. 72-78.

34. House, J.H., F.W. Gwathmey, and M.O. Fidler, *A dynamic approach to the thumb-in palm deformity in cerebral palsy.* J Bone Joint Surg Am, 1981. **63**(2): p. 216-25.

35. Koman, L.A., et al., *Quantification of upper extremity function and range of motion in children with cerebral palsy.* Dev Med Child Neurol, 2008. **50**(12): p. 910-7.

36. Waters, P.M., et al., *Interobserver and intraobserver reliability of therapist-assisted videotaped evaluations of upper-limb hemiplegia.* J Hand Surg Am, 2004. **29**(2): p. 328-34.

37. Forssberg, H., *Cerebral Palsy: Science and Clinical Practice.* 2014: Chapter 14: Neural basis of motor control, p. 199-224.

38. Bjornson, K.F., et al., *Ambulatory physical activity performance in youth with cerebral palsy and youth who are developing typically.* Phys Ther, 2007. **87**(3): p. 248-57.

39. Capio, C.M., et al., *Fundamental movement skills and physical activity among children with and without cerebral palsy.* Res Dev Disabil, 2012. **33**(4): p. 1235-41.

40. Peterson, M.D., et al., *Chronic Conditions in Adults With Cerebral Palsy.* Jama, 2015. **314**(21): p. 2303-5.

41. Peterson, M.D., P.M. Gordon, and E.A. Hurvitz, *Chronic disease risk among adults with cerebral palsy: the role of premature sarcopoenia, obesity and sedentary behaviour.* Obes Rev, 2013. **14**(2): p. 171-82.

42. Barber, L., et al., *Medial gastrocnemius muscle volume and fascicle length in children aged 2 to 5 years with cerebral palsy.* Dev Med Child Neurol, 2011. **53**(6): p. 543-8.

43. Barrett, R.S. and G.A. Lichtwark, *Gross muscle morphology and structure in spastic cerebral palsy: a systematic review.* Dev Med Child Neurol, 2010. **52**(9): p. 794-804.

44. von Walden, F., et al., *Forearm Flexor Muscles in Children with Cerebral Palsy Are Weak, Thin and Stiff.* Front Comput Neurosci, 2017. **11**: p. 30.

45. Ponten, E., et al., *Spastic wrist flexors are more severely affected than wrist extensors in children with cerebral palsy.* Dev Med Child Neurol, 2005. **47**(6): p. 384-9.

46. Rose, J., et al., *Muscle pathology and clinical measures of disability in children with cerebral palsy.* J Orthop Res, 1994. **12**(6): p. 758-68.

47. Ponten, E.M. and P.S. Stal, *Decreased capillarization and a shift to fast myosin heavy chain IIx in the biceps brachii muscle from young adults with spastic paresis.* J Neurol Sci, 2007. **253**(1-2): p. 25-33.

48. Noble, J.J., et al., *Lower limb muscle volumes in bilateral spastic cerebral palsy.* Brain Dev, 2014. **36**(4): p. 294-300.

49. Pitcher, C.A., et al., *Muscle morphology of the lower leg in ambulant children with spastic cerebral palsy.* Muscle Nerve, 2018.

50. Gracies, J.M., *Pathophysiology of spastic paresis. I: Paresis and soft tissue changes.* Muscle Nerve, 2005. **31**(5): p. 535-51.

51. Lance, J.W., *Symposium synopsis. In: Feldman RG , Koella WP , Young RR , editors. Spasticity: disordered motor control.* Chicago: Year Book Medical Publishers, 1980: p. p. 485–494.

52. Sanger, T.D., et al., *Classification and definition of disorders causing hypertonia in childhood.* Pediatrics, 2003. **111**(1): p. e89-97.

53. Hagglund, G. and P. Wagner, *Development of spasticity with age in a total population of children with cerebral palsy.* BMC Musculoskelet Disord, 2008. **9**: p. 150.

54. Nordmark, E., et al., *Development of lower limb range of motion from early childhood to adolescence in cerebral palsy: a population-based study.* BMC Med, 2009. **7**: p. 65.

55. Hagglund, G. and P. Wagner, *Spasticity of the gastrosoleus muscle is related to the development of reduced passive dorsiflexion of the ankle in children with cerebral palsy: a registry analysis of 2,796 examinations in 355 children.* Acta Orthop, 2011. **82**(6): p. 744-8.

56. Herskind, A., et al., *Muscle growth is reduced in 15-month-old children with cerebral palsy.* Dev Med Child Neurol, 2016. **58**(5): p. 485-91.

57. Hedberg-Graff, J., et al., *Upper-limb contracture development in children with cerebral palsy: a population-based study.* Dev Med Child Neurol, 2018.

58. Sinkjaer, T., et al., *Muscle stiffness in human ankle dorsiflexors: intrinsic and reflex components.* J Neurophysiol, 1988. **60**(3): p. 1110-21.

59. Sinkjaer, T., et al., *Non-reflex and reflex mediated ankle joint stiffness in multiple sclerosis patients with spasticity.* Muscle Nerve, 1993. **16**(1): p. 69-76.

60. Sinkjaer, T. and I. Magnussen, *Passive, intrinsic and reflex-mediated stiffness in the ankle extensors of hemiparetic patients.* Brain, 1994. **117 (Pt 2)**: p. 355-63.

61. Lieber, R.L., *Skeletal Muscle Structure, Function and Plasticity: The physiological basis of rehabilitation.* 2010(Third Edition): p. 15-16.

62. Ponten, E., S. Gantelius, and R.L. Lieber, *Intraoperative muscle measurements reveal a relationship between contracture formation and muscle remodeling.* Muscle Nerve, 2007. **36**(1): p. 47-54.

63. Lieber, R.L. and J. Friden, *Spasticity causes a fundamental rearrangement of muscle-joint interaction.* Muscle Nerve, 2002. **25**(2): p. 265-70.

64. Lieber, R.L., et al., *Sarcomere length changes after flexor carpi ulnaris to extensor digitorum communis tendon transfer.* J Hand Surg Am, 1996. **21**(4): p. 612-8.

65. Lieber, R.L. and J. Friden, *Intraoperative measurement and biomechanical modeling of the flexor carpi ulnaris-to-extensor carpi radialis longus tendon transfer.* J Biomech Eng, 1997. **119**(4): p. 386-91.

66. Lieber, R.L. and J. Friden, *Musculoskeletal balance of the human wrist elucidated using intraoperative laser diffraction.* J Electromyogr Kinesiol, 1998. **8**(2): p. 93-100.

67. Pette, D. and R.S. Staron, *Myosin isoforms, muscle fiber types, and transitions.* Microsc Res Tech, 2000. **50**(6): p. 500-9.

68. Baldwin, K.M. and F. Haddad, *Skeletal muscle plasticity: cellular and molecular responses to altered physical activity paradigms.* Am J Phys Med Rehabil, 2002. **81**(11 Suppl): p. S40-51.

69. Andersen, J.L., et al., *Myosin heavy chain isoform transformation in single fibres from m. vastus lateralis in spinal cord injured individuals: effects of long-term functional electrical stimulation (FES).* Pflugers Arch, 1996. **431**(4): p. 513-8.

70. Castle, M.E., T.A. Reyman, and M. Schneider, *Pathology of spastic muscle in cerebral palsy.* Clin Orthop Relat Res, 1979(142): p. 223-32.

71. Lieber, R.L., et al., *Long-term effects of spinal cord transection on fast and slow rat skeletal muscle. II. Morphometric properties.* Exp Neurol, 1986. **91**(3): p. 435-48.

72. Purslow, P.P., *Strain-induced reorientation of an intramuscular connective tissue network: implications for passive muscle elasticity.* J Biomech, 1989. **22**(1): p. 21-31.

73. Lieber, R.L., et al., *Inferior mechanical properties of spastic muscle bundles due to hypertrophic but compromised extracellular matrix material.* Muscle Nerve, 2003. **28**(4): p. 464-71.

74. Booth, C.M., M.J. Cortina-Borja, and T.N. Theologis, *Collagen accumulation in muscles of children with cerebral palsy and correlation with severity of spasticity.* Dev Med Child Neurol, 2001. **43**(5): p. 314-20.

75. de Bruin, M., et al., *Intramuscular connective tissue differences in spastic and control muscle: a mechanical and histological study.* PLoS One, 2014. **9**(6): p. e101038.

76. Pitcher, C.A., et al., *Ultrasound characterization of medial gastrocnemius tissue composition in children with spastic cerebral palsy.* Muscle Nerve, 2015. **52**(3): p. 397-403.

77. Smith, L.R., et al., *Hamstring Contractures in Children With Spastic Cerebral Palsy Result from a Stiffer ECM and Increased In Vivo Sarcomere Length.* J Physiol, 2011.

78. Smith, L.R., et al., *Novel transcriptional profile in wrist muscles from cerebral palsy patients.* BMC Med Genomics, 2009. **2**: p. 44.

79. White, R.B., et al., *Dynamics of muscle fibre growth during postnatal mouse development.* BMC Dev Biol, 2010. **10**: p. 21.

80. Benard, M.R., et al., *Effects of growth on geometry of gastrocnemius muscle in children: a three-dimensional ultrasound analysis.* J Anat, 2011. **219**(3): p. 388-402.

81. Millward, D.J., et al., *Relationship between protein synthesis and RNA content in skeletal muscle.* Nature, 1973. **241**(5386): p. 204-5.

82. von Walden, F., et al., *Mechanical loading induces the expression of a Pol I regulon at the onset of skeletal muscle hypertrophy.* Am J Physiol Cell Physiol, 2012. **302**(10): p. C1523-30.

83. Nader, G.A., et al., *Resistance exercise training modulates acute gene expression during human skeletal muscle hypertrophy.* J Appl Physiol (1985), 2014. **116**(6): p. 693-702.

84. Stec, M.J., et al., *Ribosome biogenesis may augment resistance training-induced myofiber hypertrophy and is required for myotube growth in vitro.* Am J Physiol Endocrinol Metab, 2016. **310**(8): p. E652-e661.

85. von Walden, F., et al., *mTOR signaling regulates myotube hypertrophy by modulating protein synthesis, rDNA transcription, and chromatin remodeling.* Am J Physiol Cell Physiol, 2016. **311**(4): p. C663-c672.

86. Grummt, I., *Regulation of mammalian ribosomal gene transcription by RNA polymerase I.* Prog Nucleic Acid Res Mol Biol, 1999. **62**: p. 109-54.

87. Moss, T., et al., *A housekeeper with power of attorney: the rRNA genes in ribosome biogenesis.* Cell Mol Life Sci, 2007. **64**(1): p. 29-49.

88. Russell, J. and J.C. Zomerdijk, *RNA-polymerase-I-directed rDNA transcription, life and works.* Trends Biochem Sci, 2005. **30**(2): p. 87-96.

89. Hawke, T.J. and D.J. Garry, *Myogenic satellite cells: physiology to molecular biology.* J Appl Physiol, 2001. **91**(2): p. 534-51.

90. Gros, J., et al., *A common somitic origin for embryonic muscle progenitors and satellite cells.* Nature, 2005. **435**(7044): p. 954-8.

91. Smith, L.R., H.G. Chambers, and R.L. Lieber, *Reduced satellite cell population may lead to contractures in children with cerebral palsy.* Dev Med Child Neurol, 2013. **55**(3): p. 264-70.

92. Murach, K.A., et al., *Differential requirement for satellite cells during overload-induced muscle hypertrophy in growing versus mature mice.* Skelet Muscle, 2017. **7**(1): p. 14.

93. Kinney, M.C., et al., *Reduced skeletal muscle satellite cell number alters muscle morphology after chronic stretch but allows limited serial sarcomere addition.* Muscle Nerve, 2017. **55**(3): p. 384-392.

94. Fry, C.S., et al., *Myogenic Progenitor Cells Control Extracellular Matrix Production by Fibroblasts during Skeletal Muscle Hypertrophy.* Cell Stem Cell, 2017. **20**(1): p. 56-69.

95. Van Heest, A.E., J.H. House, and C. Cariello, *Upper extremity surgical treatment of cerebral palsy.* J Hand Surg Am, 1999. **24**(2): p. 323-30.

96. Park, E.S., E.G. Sim, and D.W. Rha, *Effect of upper limb deformities on gross motor and upper limb functions in children with spastic cerebral palsy.* Res Dev Disabil, 2011. **32**(6): p. 2389-97.

97. Chow, J.W., et al., *Muscle activation during the tennis volley.* Med Sci Sports Exerc, 1999. **31**(6): p. 846-54.

98. Lieber, R.L., B.M. Fazeli, and M.J. Botte, *Architecture of selected wrist flexor and extensor muscles.* J Hand Surg Am, 1990. **15**(2): p. 244-50.

99. Hagglund, G., H. Lauge-Pedersen, and P. Wagner, *Characteristics of children with hip displacement in cerebral palsy.* BMC Musculoskelet Disord, 2007. **8**: p. 101.

100. Pingel, J., E.M. Bartels, and J.B. Nielsen, *New perspectives on the development of muscle contractures following central motor lesions.* J Physiol, 2017. **595**(4): p. 1027-1038.

101. Farmer, S.E. and M. James, *Contractures in orthopaedic and neurological conditions: a review of causes and treatment.* Disabil Rehabil, 2001. **23**(13): p. 549-58.

102. Fergusson, D., B. Hutton, and A. Drodge, *The epidemiology of major joint contractures: a systematic review of the literature.* Clin Orthop Relat Res, 2007. **456**: p. 22-9.

103. Tedroff, K., et al., *Does loss of spasticity matter? A 10-year follow-up after selective dorsal rhizotomy in cerebral palsy.* Dev Med Child Neurol, 2011. **53**(8): p. 724-9.

104. Tedroff, K., et al., *Long-term effects of botulinum toxin A in children with cerebral palsy.* Dev Med Child Neurol, 2009. **51**(2): p. 120-7.

105. Gough, M. and A.P. Shortland, *Could muscle deformity in children with spastic cerebral palsy be related to an impairment of muscle growth and altered adaptation?* Dev Med Child Neurol, 2012. **54**(6): p. 495-9.

106. Lundberg, I.E. and G.A. Nader, *Molecular effects of exercise in patients with inflammatory rheumatic disease.* Nat Clin Pract Rheumatol, 2008. **4**(11): p. 597-604.

107. Burks, T.N. and R.D. Cohn, *Role of TGF-beta signaling in inherited and acquired myopathies.* Skelet Muscle, 2011. **1**(1): p. 19.

108. Smith, L.R., et al., *Transcriptional abnormalities of hamstring muscle contractures in children with cerebral palsy.* PLoS One, 2012. **7**(8): p. e40686.

109. Roubenoff, R., *Molecular basis of inflammation: relationships between catabolic cytokines, hormones, energy balance, and muscle.* JPEN J Parenter Enteral Nutr, 2008. **32**(6): p. 630-2.

110. de Bruin, M., M.J. Smeulders, and M. Kreulen, *Why is joint range of motion limited in patients with cerebral palsy?* J Hand Surg Eur Vol, 2013. **38**(1): p. 8-13.

111. Randall, M., et al., *Reliability of the Melbourne assessment of unilateral upper limb function.* Dev Med Child Neurol, 2001. **43**(11): p. 761-7.

112. Randall, M., et al., *Rasch analysis of The Melbourne Assessment of Unilateral Upper Limb Function.* Dev Med Child Neurol, 2014. **56**(7): p. 665-72.

113. Krumlinde-Sundholm, L., et al., *The Assisting Hand Assessment: current evidence of validity, reliability, and responsiveness to change.* Dev Med Child Neurol, 2007. **49**(4): p. 259-64.

114. Louwers, A., et al., *Development of the Assisting Hand Assessment for adolescents (Ad-AHA) and validation of the AHA from 18 months to 18 years.* Dev Med Child Neurol, 2016. **58**(12): p. 1303-1309.

115. Greaves, S., et al., *Development of the Mini-Assisting Hand Assessment: evidence for content and internal scale validity.* Dev Med Child Neurol, 2013. **55**(11): p. 1030-7.

116. Davids, J.R., et al., *Validation of the Shriners Hospital for Children Upper Extremity Evaluation (SHUEE) for children with hemiplegic cerebral palsy.* J Bone Joint Surg Am, 2006. **88**(2): p. 326-33.

117. Bohannon, R.W. and M.B. Smith, *Interrater reliability of a modified Ashworth scale of muscle spasticity.* Phys Ther, 1987. **67**(2): p. 206-7.

118. Mutlu, A., A. Livanelioglu, and M.K. Gunel, *Reliability of Ashworth and Modified Ashworth scales in children with spastic cerebral palsy.* BMC Musculoskelet Disord, 2008. **9**: p. 44.

119. Ansari, N.N., et al., *Ashworth Scales are unreliable for the assessment of muscle spasticity.* Physiother Theory Pract, 2006. **22**(3): p. 119-25.

120. Wagner, L.V. and J.R. Davids, *Assessment tools and classification systems used for the upper extremity in children with cerebral palsy.* Clin Orthop Relat Res, 2012. **470**(5): p. 1257-71.

121. Klingels, K., et al., *A systematic review of arm activity measures for children with hemiplegic cerebral palsy.* Clin Rehabil, 2010. **24**(10): p. 887-900.

122. Thorley, M., et al., *Reliability of the quality of upper extremity skills test for children with cerebral palsy aged 2 to 12 years.* Phys Occup Ther Pediatr, 2012. **32**(1): p. 4-21.

123. Gilmore, R., L. Sakzewski, and R. Boyd, *Upper limb activity measures for 5- to 16-year-old children with congenital hemiplegia: a systematic review.* Dev Med Child Neurol, 2010. **52**(1): p. 14-21.

124. Kreulen, M., et al., *Three-dimensional video analysis of forearm rotation before and after combined pronator teres rerouting and flexor carpi ulnaris tendon transfer surgery in patients with cerebral palsy.* J Hand Surg Br, 2004. **29**(1): p. 55-60.

125. Sanger, T.D., *Arm trajectories in dyskinetic cerebral palsy have increased random variability.* J Child Neurol, 2006. **21**(7): p. 551-7.

126. Malfait, N. and T.D. Sanger, *Does dystonia always include co-contraction? A study of unconstrained reaching in children with primary and secondary dystonia.* Exp Brain Res, 2007. **176**(2): p. 206-16.

127. Mailleux, L., et al., *Clinical assessment and three-dimensional movement analysis: An integrated approach for upper limb evaluation in children with unilateral cerebral palsy.* PLoS One, 2017. **12**(7): p. e0180196.

128. Gage, J.R., *Gait analysis. An essential tool in the treatment of cerebral palsy.* Clin Orthop Relat Res, 1993(288): p. 126-34.

129. Gage, J.R., *The role of gait analysis in the treatment of cerebral palsy.* J Pediatr Orthop, 1994. **14**(6): p. 701-2.

130. Chen, K.Y. and D.R. Bassett, Jr., *The technology of accelerometry-based activity monitors: current and future.* Med Sci Sports Exerc, 2005. **37**(11 Suppl): p. S490-500.

131. Troiano, R.P., et al., *Evolution of accelerometer methods for physical activity research.* Br J Sports Med, 2014. **48**(13): p. 1019-23.

132. Matthew, C.E., *Calibration of accelerometer output for adults.* Med Sci Sports Exerc, 2005. **37**(11 Suppl): p. S512-22.

133. Capio, C.M., C.H. Sit, and B. Abernethy, *Physical activity measurement using MTI (actigraph) among children with cerebral palsy.* Arch Phys Med Rehabil, 2010. **91**(8): p. 1283-90.

134. Clanchy, K.M., et al., *Validity of accelerometry in ambulatory children and adolescents with cerebral palsy.* Eur J Appl Physiol, 2011. **111**(12): p. 2951-9.

135. Michielsen, M.E., et al., *Quantifying nonuse in chronic stroke patients: a study into paretic, nonparetic, and bimanual upper-limb use in daily life.* Arch Phys Med Rehabil, 2012. **93**(11): p. 1975-81.

136. Uswatte, G., et al., *Ambulatory monitoring of arm movement using accelerometry: an objective measure of upper-extremity rehabilitation in persons with chronic stroke.* Arch Phys Med Rehabil, 2005. **86**(7): p. 1498-501.

137. Novak, I., et al., *A systematic review of interventions for children with cerebral palsy: state of the evidence.* Dev Med Child Neurol, 2013. **55**(10): p. 885-910.

138. Flett, P.J., *Rehabilitation of spasticity and related problems in childhood cerebral palsy.* J Paediatr Child Health, 2003. **39**(1): p. 6-14.

139. Ward, A.B., *A summary of spasticity management--a treatment algorithm.* Eur J Neurol, 2002. **9 Suppl 1**: p. 48-52; dicussion 53-61.

140. Smyth, M.D. and W.J. Peacock, *The surgical treatment of spasticity.* Muscle Nerve, 2000. **23**(2): p. 153-63.

141. Tardieu, C., et al., *For how long must the soleus muscle be stretched each day to prevent contracture?* Dev Med Child Neurol, 1988. **30**(1): p. 3-10.

142. Green, W.T., *Tendon transplantation of the flexor carpi ulnaris for pronation-flexion deformity of the wrist.* Surg Gynecol Obstet, 1942. **(75)**: p. 337-342.

143. Alexander, R.D., et al., *Wrist arthrodesis in children with cerebral palsy.* J Pediatr Orthop, 2000. **20**(4): p. 490-5.

144. Lieber, R.L. and S.C. Bodine-Fowler, *Skeletal muscle mechanics: implications for rehabilitation.* Phys Ther, 1993. **73**(12): p. 844-56.

145. Fattal-Valevski, A., L. Sagi, and D. Domenievitz, *Botulinum Toxin A Injections to the Upper Limbs in Children With Cerebral Palsy: Duration of Effect.* J Child Neurol, 2010.

146. Hoare, B.J., et al., *Botulinum toxin A as an adjunct to treatment in the management of the upper limb in children with spastic cerebral palsy (UPDATE).* Cochrane Database Syst Rev, 2010(1): p. CD003469.

147. Ade-Hall, R.A. and A.P. Moore, *Botulinum toxin type A in the treatment of lower limb spasticity in cerebral palsy.* Cochrane Database Syst Rev, 2000(2): p. CD001408.

148. Corry, I.S., et al., *Botulinum toxin A in the hemiplegic upper limb: a double-blind trial.* Dev Med Child Neurol, 1997. **39**(3): p. 185-93.

149. Elkamil, A.I., et al., *Botulinum neurotoxin treatment in children with cerebral palsy: a population-based study in Norway.* Eur J Paediatr Neurol, 2012. **16**(5): p. 522-7.

150. Franzen, M., G. Hagglund, and A. Alriksson-Schmidt, *Treatment with Botulinum toxin A in a total population of children with cerebral palsy - a retrospective cohort registry study.* BMC Musculoskelet Disord, 2017. **18**(1): p. 520.

151. Heinen, F., et al., *The updated European Consensus 2009 on the use of Botulinum toxin for children with cerebral palsy.* Eur J Paediatr Neurol, 2010. **14**(1): p. 45-66.

152. Comella, C.L., J. Jankovic, and M.F. Brin, *Use of botulinum toxin type A in the treatment of cervical dystonia.* Neurology, 2000. **55**(12 Suppl 5): p. S15-21.

153. Lowe, N.J., et al., *Botulinum toxin type A in the treatment of primary axillary hyperhidrosis: a 52-week multicenter double-blind, randomized, placebo-controlled study of efficacy and safety.* J Am Acad Dermatol, 2007. **56**(4): p. 604-11.

154. Holds, J.B., et al., *Motor nerve sprouting in human orbicularis muscle after botulinum A injection.* Invest Ophthalmol Vis Sci, 1990. **31**(5): p. 964-7.

155. Tedroff, K., et al., *Botulinum toxin A treatment in toddlers with cerebral palsy.* Acta Paediatr, 2010. **99**(8): p. 1156-62.

156. Love, S.C., et al., *Botulinum toxin assessment, intervention and after-care for lower limb spasticity in children with cerebral palsy: international consensus statement.* Eur J Neurol, 2010. **17 Suppl 2**: p. 9-37.

157. Fehlings, D., et al., *Botulinum toxin assessment, intervention and follow-up for paediatric upper limb hypertonicity: international consensus statement.* Eur J Neurol, 2010. **17 Suppl 2**: p. 38-56.

158. Lukban, M.B., R.L. Rosales, and D. Dressler, *Effectiveness of botulinum toxin A for upper and lower limb spasticity in children with cerebral palsy: a summary of evidence.* J Neural Transm (Vienna), 2009. **116**(3): p. 319-31.

159. Swinney, C.M., et al., *Severity of cerebral palsy and likelihood of adverse events after botulinum toxin A injections.* Dev Med Child Neurol, 2018. **60**(5): p. 498-504.

160. Meyns, P., et al., *Arm swing during walking at different speeds in children with Cerebral Palsy and typically developing children.* Res Dev Disabil, 2011. **32**(5): p. 1957-64.

161. Sandahl Michelsen, J., G. Normann, and C. Wong, *Analgesic Effects of Botulinum Toxin in Children with CP.* Toxins (Basel), 2018. **10**(4).

162. Palomar, F.J. and P. Mir, *Neurophysiological changes after intramuscular injection of botulinum toxin.* Clin Neurophysiol, 2012. **123**(1): p. 54-60.

163. Mazzocchio, R. and M. Caleo, *More than at the neuromuscular synapse: actions of botulinum neurotoxin A in the central nervous system.* Neuroscientist, 2015. **21**(1): p. 44-61.

164. Lowing, K., et al., *Effects of Botulinum Toxin-A and Goal-Directed Physiotherapy in Children with Cerebral Palsy GMFCS Levels I & II.* Phys Occup Ther Pediatr, 2017. **37**(3): p. 268-282.

165. Heinen, F., et al., *European consensus table 2006 on botulinum toxin for children with cerebral palsy.* Eur J Paediatr Neurol, 2006. **10**(5-6): p. 215-25.

166. CDC, *Data & statistics for cerebral palsy.* https://www.cdc.gov/ncbddd/cp/data.html., 2016.

167. Novak, I., et al., *Clinical prognostic messages from a systematic review on cerebral palsy.* Pediatrics, 2012. **130**(5): p. e1285-312.

168. Burbaud, P., et al., *Botulinum toxin treatment in neurological practice: how much does it really cost? A prospective cost-effectiveness study.* J Neurol, 2011. **258**(9): p. 1670-5.

169. Bihari, K., *Safety, effectiveness, and duration of effect of BOTOX after switching from Dysport for blepharospasm, cervical dystonia, and hemifacial spasm dystonia, and hemifacial spasm.* Curr Med Res Opin, 2005. **21**(3): p. 433-8.

170. Rystedt, A., D. Nyholm, and H. Naver, *Clinical experience of dose conversion ratios between 2 botulinum toxin products in the treatment of cervical dystonia.* Clin Neuropharmacol, 2012. **35**(6): p. 278-82.

171. Wohlfarth, K., et al., *Dose equivalence of two commercial preparations of botulinum neurotoxin type A: time for a reassessment?* Curr Med Res Opin, 2009. **25**(7): p. 1573-84.

172. Brin, M.F. and A. Blitzer, *Botulinum toxin: dangerous terminology errors.* J R Soc Med, 1993. **86**(8): p. 493-4.

173. Marchetti, A., et al., *Retrospective evaluation of the dose of Dysport and BOTOX in the management of cervical dystonia and blepharospasm: the REAL DOSE study.* Mov Disord, 2005. **20**(8): p. 937-44.

174. Russman, B.S., A. Tilton, and M.E. Gormley, Jr., *Cerebral palsy: a rational approach to a treatment protocol, and the role of botulinum toxin in treatment.* Muscle Nerve Suppl, 1997. **6**: p. S181-93.

175. Larsson, L. and R.L. Moss, *Maximum velocity of shortening in relation to myosin isoform composition in single fibres from human skeletal muscles.* J Physiol, 1993. **472**: p. 595-614.

176. Ponten, E., M. Lindstrom, and F. Kadi, *Higher amount of MyHC IIX in a wrist flexor in tetraplegic compared to hemiplegic cerebral palsy.* J Neurol Sci, 2008. **266**(1-2): p. 51-6.

177. Lindstrom, M. and L.E. Thornell, *New multiple labelling method for improved satellite cell identification in human muscle: application to a cohort of power-lifters and sedentary men.* Histochem Cell Biol, 2009. **132**(2): p. 141-57.

178. Junqueira, L.C., G. Bignolas, and R.R. Brentani, *Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections.* Histochem J, 1979. **11**(4): p. 447-55.

179. Munretto, J., E. Gutierrez-Farewik, *Methods Using Motion Analysis for Examining the Upper Limb in children with Hemiplegic Cerebral Palsy.* 2005.

180. Barber, C.B., D.P. Dopkin, and H.T. Huhdanpaa, *The Quickhull Algorithm for Convex Hulls.* ACM Transactions on Mathematical Software, 1996(22): p. 469-483.

181. Freedson, P.S., E. Melanson, and J. Sirard, *Calibration of the Computer Science and Applications, Inc. accelerometer.* Med Sci Sports Exerc, 1998. **30**(5): p. 777-81.

182. Hagstromer, M., et al., *Participation in organized weekly physical exercise in obese adolescents reduced daily physical activity.* Acta Paediatr, 2009. **98**(2): p. 352-4.

183. Evenson, K.R., et al., *Calibration of two objective measures of physical activity for children.* J Sports Sci, 2008. **26**(14): p. 1557-65.

184. Trost, S.G., et al., *Using objective physical activity measures with youth: how many days of monitoring are needed?* Med Sci Sports Exerc, 2000. **32**(2): p. 426-31.

185. Fleiss, J.L., *The design and analysis of clinical experiments. New York: John Wiley & Sons.* 1986.

186. Klingels, K., et al., *Do mirror movements relate to hand function and timing of the brain lesion in children with unilateral cerebral palsy?* Dev Med Child Neurol, 2016. **58**(7): p. 735-42.

187. Kuhtz-Buschbeck, J.P., et al., *Quantitative assessment of mirror movements in children and adolescents with hemiplegic cerebral palsy.* Dev Med Child Neurol, 2000. **42**(11): p. 728-36.

188. Carr, L.J., *Development and reorganization of descending motor pathways in children with hemiplegic cerebral palsy.* Acta Paediatr Suppl, 1996. **416**: p. 53-7.

189. Pette, D., *The adaptive potential of skeletal muscle fibers.* Can J Appl Physiol, 2002. **27**(4): p. 423-48.

190. Barber, L.A., et al., *Medial gastrocnemius muscle volume in ambulant children with unilateral and bilateral cerebral palsy aged 2 to 9 years.* Dev Med Child Neurol, 2016. **58**(11): p. 1146-1152.

191. Fiorotto, M.L., et al., *Ribosome abundance regulates the recovery of skeletal muscle protein mass upon recuperation from postnatal undernutrition in mice.* J Physiol, 2014. **592**(23): p. 5269-86.

192. Andrew, M.J. and P.B. Sullivan, *Growth in cerebral palsy.* Nutr Clin Pract, 2010. **25**(4): p. 357-61.

193. Day, S.M., et al., *Growth patterns in a population of children and adolescents with cerebral palsy.* Dev Med Child Neurol, 2007. **49**(3): p. 167-71.

194. Dayanidhi, S., et al., *Reduced satellite cell number in situ in muscular contractures from children with cerebral palsy.* J Orthop Res, 2015.

195. Nader, G.A., et al., *A longitudinal, integrated, clinical, histological and mRNA profiling study of resistance exercise in myositis.* Mol Med, 2010. **16**(11-12): p. 455-64.

196. McPherron, A.C., A.M. Lawler, and S.J. Lee, *Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member.* Nature, 1997. **387**(6628): p. 83-90.

197. McFarlane, C., et al., *Human myostatin negatively regulates human myoblast growth and differentiation.* Am J Physiol Cell Physiol, 2011. **301**(1): p. C195-203.

198. McFarlane, C., et al., *Myostatin induces cachexia by activating the ubiquitin proteolytic system through an NF-kappaB-independent, FoxO1-dependent mechanism.* J Cell Physiol, 2006. **209**(2): p. 501-14.

199. Li, Z.B., H.D. Kollias, and K.R. Wagner, *Myostatin directly regulates skeletal muscle fibrosis.* J Biol Chem, 2008. **283**(28): p. 19371-8.

200. Allen, R.E. and L.K. Boxhorn, *Inhibition of skeletal muscle satellite cell differentiation by transforming growth factor-beta.* J Cell Physiol, 1987. **133**(3): p. 567-72.

201. Petrov, V.V., R.H. Fagard, and P.J. Lijnen, *Stimulation of collagen production by transforming growth factor-beta1 during differentiation of cardiac fibroblasts to myofibroblasts.* Hypertension, 2002. **39**(2): p. 258-63.

202. Mann, C.J., et al., *Aberrant repair and fibrosis development in skeletal muscle.* Skelet Muscle, 2011. **1**(1): p. 21.

203. Trosch, R.M., et al., *A Retrospective, Single-Center Comparative Cost Analysis of OnabotulinumtoxinA and AbobotulinumtoxinA for Cervical Dystonia Treatment.* J Manag Care Spec Pharm, 2015. **21**(10): p. 854-60.

204. Rystedt, A., C. Swartling, and H. Naver, *Anhidrotic effect of intradermal injections of botulinum toxin: a comparison of different products and concentrations.* Acta Derm Venereol, 2008. **88**(3): p. 229-33.

205. Kim, H.S., et al., *Effect of muscle activity and botulinum toxin dilution volume on muscle paralysis.* Dev Med Child Neurol, 2003. **45**(3): p. 200-6.

206. Carruthers, A., et al., *A randomized, evaluator-blinded, two-center study of the safety and effect of volume on the diffusion and efficacy of botulinum toxin type A in the treatment of lateral orbital rhytides.* Dermatol Surg, 2007. **33**(5): p. 567-71.

207. Lee, J.H., et al., *Effects of different dilutions of botulinum toxin type A treatment for children with cerebral palsy with spastic ankle plantarflexor: a randomized controlled trial.* J Rehabil Med, 2009. **41**(9): p. 740-5.

208. Lee, L.R., et al., *Botulinum toxin for lower limb spasticity in children with cerebral palsy: a single-blinded trial comparing dilution techniques.* Am J Phys Med Rehabil, 2004. **83**(10): p. 766-73.

209. Francisco, G.E., C. Boake, and A. Vaughn, *Botulinum toxin in upper limb spasticity after acquired brain injury: a randomized trial comparing dilution techniques.* Am J Phys Med Rehabil, 2002. **81**(5): p. 355-63.

210. Boyle, M.H., et al., *High versus low concentration botulinum toxin A for benign essential blepharospasm: does dilution make a difference?* Ophthalmic Plast Reconstr Surg, 2009. **25**(2): p. 81-4.

211. Tedroff, K., K. Lowing, and E. Astrom, *A prospective cohort study investigating gross motor function, pain, and health-related quality of life 17 years after selective dorsal rhizotomy in cerebral palsy.* Dev Med Child Neurol, 2015. **57**(5): p. 484-90.

212. Pingel, J., et al., *Muscle disuse caused by botulinum toxin injection leads to increased central gain of the stretch reflex in the rat.* J Neurophysiol, 2017. **118**(4): p. 1962-1969.

213. Pingel, J., et al., *Botulinum toxin injection causes hyper-reflexia and increased muscle stiffness of the triceps surae muscle in the rat.* J Neurophysiol, 2016. **116**(6): p. 2615-2623.

214. Domenighetti, A.A., et al., *Loss of myogenic potential and fusion capacity of muscle stem cells isolated from contractured muscle in children with cerebral palsy.* Am J Physiol Cell Physiol, 2018. **315**(2): p. C247-c257.