JYU DISSERTATIONS 751

Pedro Frederico Valadão

The EXECP Project

A Neuromechanical Examination of Hyper-Resistance Within an Exercise Intervention for Children and Young Adults with Cerebral Palsy

UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF SPORT AND
HEALTH SCIENCES

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Esitetään Jyväskylän yliopiston liikuntatieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Liikunta-rakennuksen salissa L304
maaliskuun 8. päivänä 2024 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Sport and Health Sciences of the University of Jyväskylä,
in building Liikunta, auditorium L304, on March 8, 2024 at 12 o'clock noon.

JYVÄSKYLÄN YLIOPISTO
UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2024

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ISBN 978-951-39-9943-8 (PDF)
URN:ISBN:978-951-39-9943-8
ISSN 2489-9003

Permanent link to this publication: http://urn.fi/URN:ISBN:978-951-39-9943-8

ABSTRACT

Valadão, Pedro
The EXECP Project: a neuromechanical examination of hyper-resistance within an exercise intervention for children and young adults with cerebral palsy.
Jyväskylä: University of Jyväskylä, 2024, 108 p.
(JYU Dissertations
ISSN 2489-9003; 751)
ISBN 978-951-39-9943-8

Spastic cerebral palsy (CP) is characterized by muscle weakness, limited joint flexibility, motor incoordination and hyper-resistance (i.e., increased resistance to passive muscle stretch). All these symptoms hinder motor function, resulting in reduced physical activity levels, which increases many cardiometabolic risk factors. The purpose of this thesis was to develop a combined strength, flexibility and gait training intervention (EXECP intervention) to address the debilitating symptoms above-mentioned. The EXECP intervention was successful in increasing motor function measured by a gait performance test (six minutes walking test) and the gross motor function measure. Furthermore, muscle strength and joint flexibility improved with the EXECP intervention for most muscles, except the ankle plantarflexors and dorsiflexors. Overall, the results showed that the EXECP intervention was safe and efficient in improving motor function, without any adverse effects. Importantly, three months after the EXECP intervention ceased and the CP participants were re-evaluated, most of the improvements induced by the intervention regressed back to pre-intervention levels. The deadaptation after the intervention suggests that training should be a life-long choice for people with CP. The present study also proposed a correction to a hyperreflexia (i.e., exacerbated stretch reflex responses) assessment method called the stretch reflex threshold (SRT). The proposed correction had a significant effect on the evaluation of the SRT and the new approach is recommended for future studies. Furthermore, this study performed a thorough comparison between individuals with CP and typically developed (TD) controls regarding hyper-resistance, hyperreflexia and joint neuromechanical variables. The comparison between groups showed several significant differences in most studied variables. Ankle joint neuromechanical variables and neurophysiological variables of hyperreflexia were not useful in explaining the neuromechanical variables of hyperreflexia or peak torque during stretch. The SRT of both muscles had a good positive correlation with peak torque at high stretch velocities and was significantly different between CP and TD groups, suggesting it is a useful diagnostic variable.

Keywords: Cerebral Palsy, training, neuromechanics.

TIIVISTELMÄ (ABSTRACT IN FINNISH)

Valadão, Pedro
EXECP-projekti: Lihasjäykkyyden neuromekaaninen tarkastelu liikunnallisessa kuntoutuksessa lapsilla ja nuorilla, joilla on CP-vamma
Jyväskylä: University of Jyväskylä, 2024, 108 s.
(JYU Dissertations
ISSN 2489-9003; 751)
ISBN 978-951-39-9943-8

CP-oireyhtymä johtuu sikiöaikana tai pian syntymän jälkeen syntyneestä aivojen vauriosta. Spastiselle CP-oireyhtymälle on ominaista liiallinen lihasjäykkyys, rajoitettu nivelten liikelaajuus, lihasheikkous, ongelmat motorisessa koordinaatiossa ja mm. eriytyneiden liikkeiden suorittamisessa. Kaikki nämä oireet haittaavat motorisia toimintoja, mikä osaltaan johtaa fyysisen aktiivisuuden vähenemiseen, täten lisäten monia kardiometabolisia riskitekijöitä. Tämän väitöskirjan tarkoituksena oli kehittää yhdistetty voima-, liikkuvuus- ja kävelyharjoittelua sisältävä interventio (EXECP-interventio), jolla puututaan edellä mainittuihin liikuntakykyä heikentäviin oireisiin. Väitöskirjassa käytettiin monipuolisia neurofysiologisia ja biomekaanisia tutkimusmenetelmiä. Vallalla olevaan venytysrefleksivasteiden arviointimenetelmään, jota kutsutaan venytysrefleksi-kynnykseksi (SRT, stretch reflex threshold), ehdotettiin muutosta. Ehdotetulla muutoksella oli merkittävä vaikutus SRT:n mielekkääseen tulkintaan, ja uutta lähestymistapaa suositellaan tuleviin tutkimuksiin. Tutkittaessa liiallisen lihasjäykkyyden mahdollisia taustatekijöitä, CP-ryhmän ja kontrollihenkilöiden välinen vertailu osoitti merkittäviä eroja lihasjäykkyydessä, nivelmekaniikassa, venytysrefleksivasteissa ja muissa selkäydintason hermostollisissa prosesseissa. Tutkitut muuttujat eivät korreloineet yliherkistyneen venytysrefleksin kanssa. Molempien lihasten venytysrefleksikynnyksellä oli positiivinen korrelaatio mitatun liiallisen lihasjäykkyyden kanssa suurilla venytysnopeuksilla. Tärkeimpänä löydöksenä EXECP-interventio onnistui parantamaan liikuntakykyä (kuuden minuutin kävelytestin tulosta) ja karkeamotorista toimintakykyä. Lisäksi alaraajojen lihasvoima ja nivelten liikelaajuus paranivat EXECP-intervention ansiosta lukuun ottamatta nilkan ojentaja- ja koukistajalihaksia. Kaiken kaikkiaan tulokset osoittivat, että EXECP-interventio oli turvallinen ja tehokas parantamaan liikuntakykyä ilman haittavaikutuksia. Huomattavaa oli, että kolme kuukautta sen jälkeen, kun EXECP-interventio oli päättynyt, suurin osa intervention aiheuttamista parannuksista palautui takaisin interventiota edeltävälle tasolle. Intervention jälkeinen palautuminen viittaa siihen, että harjoittelun tulisi olla elinikäinen valinta henkilöillä, joilla on CP-vamma.

Asiasanat: CP-vamma, liikuntaharjoittelu, neuromekaniikka

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ACKNOWLEDGEMENTS

I start by thanking my parents for all their unconditional support and enthusiasm with all my endeavors. My wife Samira, who has my deepest gratitude for always being there to support me through all these years. I want to acknowledge my first supervisor professor Mauro Heleno Chagas, who with his knowledge and professional conduct set me off on the research path.

The idea of working with cerebral palsy came from an exchange of emails between me, Dr. Jens Bo Nielsen and my main supervisor Dr. Taija Finni. I had never worked with clinical populations, and Dr. Nielsen suggested that my background in strength training and my neuromechanical research interest would be a nice combination if applied to create an intervention for people with cerebral palsy. I am grateful for Dr. Nielsen's advice, it made me think outside my reference frame, and I have thoroughly enjoyed the experience in clinical practice. I am extremely grateful to Dr. Taija Finni, who was the central pillar of the EXECP project, always providing me with guidance and support. Furthermore, Dr. Finni was immensely helpful in project logistics, recruiting dozens of students to participate and finding funding for the expenses, just to name a few. Finally, Dr. Finni was always supportive and understanding with the project's pace, which was slow due to its size and complexity.

I also want to express my gratitude to my other two supervisors. Dr. Janne Avela was always available to discuss and give insightful comments and suggestions. Dr. Avela assisted me in creating the electrical stimulation protocol and was the project's first pilot subject. Dr. Harri Piitulainen taught me a lot about magnetoencephalography, research topic that I would not be able to pursue in this project without his assistance. Dr. Piitulainen helped immensely in the manuscript writing process, his comments have developed my writing skills considerably.

I was lucky to have many intelligent collaborators who taught me a lot, to them my sincere gratitude. Dr. Eero Haapala provided valuable information about cardiometabolic topic in cerebral palsy. Dr. Francesco Cenni helped immensely in the data analysis by creating roughly half of all analysis code used in the present work. Dr. Tiina Parviainen was very helpful in establishing the magnetoencephalography testing protocol and analyses. Dr. Lynn Bar-On, as the only physiotherapist in the project with clinical experience, provided invaluable discussions for the analyses and manuscript writing of articles II and III.

I am very grateful to the University of Jyväskylä for providing excellent infrastructure, funding and qualified personnel, which were essential to the completion of this thesis. At the Neuromuscular Research Center, Mrs. Sirpa Roivas, Mr. Jouni Tukiainen and Mr. Markku Ruuskanen provided excellent assistance, which undoubtedly contributed to the quality of the data acquisition. At the Centre for Interdisciplinary Brain Research Dr. Viki-Veikko Elomaa and Dr. Simo Monto provided excellent assistance in the magnetoencephalography measurements, which without both, would not have succeeded. To the more than twenty bachelor's and master's degree students that participated in the EXECP

project, my sincere gratitude, you were the everyday driving force that made such ambitious project possible.

Funding from multiple sources was critical in enabling the EXECP project. Thank you very much Olvi Foundation, Finnish Cultural Foundation, Research Foundation of Cerebral Palsy Alliance, Academy of Finland, Jane and Aatos Erkko Foundation and Ministry of Education and Culture.

Lastly, I would like to express my immense gratitude to the participants and families that dedicated so much time to make this project possible. I became friends with the families and participants with cerebral palsy, after all we spent nine months in constant contact! I learned a lot from the families and participants, things that you do not learn in research articles or clinical textbooks, and for that I am very thankful. Hopefully I was able to help them understand cerebral palsy and physical exercise a little bit better. My biggest desire is that EXECP intervention made a good impression on the positive life-changing effects of exercising, and that it may transform into a constant habit throughout their lives.

Jyväskylä 04.09.2023

Pedro Frederico Valadão

ORIGINAL PUBLICATIONS AND AUTHOR CONTRIBUTION

This thesis is based on the following original research articles, which will be referred to in the text by their Roman numerals:

I Valadão, P., Piitulainen, H., Haapala, E., Parviainen, T., Avela, J & Finni, T. (2021). Exercise intervention protocol in children and young adults with cerebral palsy: the effects of strength, flexibility and gait training on physical performance, neuromuscular mechanisms and cardiometabolic risk factors (EXECP). BMC Sports Science, Medicine and Rehabilitation 13 (17), 1–19.

II Valadão, P., Bar-On, L., Cenni, F., Piitulainen, H., Avela, J & Finni, T. (2022). Revising the stretch reflex threshold method to measure stretch reflex hyperreflexia in cerebral palsy. Frontiers in Bioengineering and Biotechnology 10, 897852.

III. Valadão, P., Cenni, F., Bar-On, L., Piitulainen, H., Avela, J & Finni, T. Neuromechanical analysis of hyper-resistance and hyperreflexia in children and young adults with cerebral palsy. Submitted for publication.

IV Valadão, P., Cenni, F., Piitulainen, H., Avela, J & Finni, T. (2024). Effects of EXECP intervention on muscle strength, motor function and joint flexibility in people with cerebral palsy. Medicine & Science in Sports & Exercise 56 (1), 1-12.

The thesis author was involved in all phases of this research project: creating the experimental design and intervention, executing the EXECP intervention with six CP participants and instructing ten trainers responsible for the other interventions, calibrating all equipment used in the testing sessions, performing data acquisition and analyses and performing the statistical analyses. The thesis author drafted the manuscripts, edited based on co-author's comments and served as corresponding author for all the manuscripts. Lastly, the author was also responsible for the logistics of scheduling tests and travel arrangements for the participants regarding intervention execution and testing.

ABBREVIATIONS

6MWT	Six minutes walking test
95% CI	95% confidence intervals
AP	Action potential
CP	Cerebral palsy
EMG	Electromyography
GLMM	Generalized linear mixed models
GMFCS	Gross motor function classification system
GMFM	Gross motor function measure
H/Hmax	H-reflex normalized by the maximum H-reflex
H/M	H-reflex normalized by the preceding M-wave
Hmax	Maximum Hoffman reflex
HR	Hyper-resistance
H-reflex	Hoffman reflex
IQR	Interquartile range
M/Mmax	M-wave normalized by the maximum M-wave
MG	Medial gastrocnemius
Mmax	Maximum M-wave
MO	Maturity offset
MTU	Muscle-tendon unit
PAD	Post-activation depression
PKE	Passive knee extension
R^2	Coefficient of determination
RCT	Randomized controlled trial
RMS	Root mean square
ROM	Range of motion
SD	Standard deviation
SE	Standard error
Sol	Soleus
SR	Stretch reflex
SRT	Stretch reflex threshold
TD	Typically developing
TSRT	Tonic stretch reflex threshold
αMN	Alpha motoneuron
B	Parameter estimates

CONTENTS

1 INTRODUCTION

Cerebral Palsy (CP) is a neurodevelopmental condition caused by a lesion in the developing fetal or infant brain. During development, the lesion leads to secondary symptoms such as muscle weakness, incoordination and reduced joint flexibility (Hanssen et al. 2021; Kalkman et al. 2020; Lieber and Fridén 2019; Moreau et al. 2012; Wiley and Damiano 1998), which hinder motor function for everyday activities such as walking and climbing stairs (García et al. 2016; Hanna et al. 2009; Smits et al. 2013). Furthermore, these secondary symptoms induce lower physical activity levels (Carlon et al. 2013; Nieuwenhuijsen et al. 2009) and cardiometabolic performance (García et al. 2016), increasing the risk of chronic diseases such as cardiovascular problems (Maltais et al. 2014; Verschuren et al. 2016). Thus, there is an urgent need for therapeutic interventions capable of breaking this downward spiral of inactivity and loss of function.

Strength training is among the most studied interventions in CP, and it is effective if established training guidelines (Faigenbaum et al. 2009; Garber et al. 2011; Verschuren et al. 2016) are followed (Gillett et al. 2018; Hanssen et al. 2022; Kirk et al. 2016; Liang et al. 2020; MacPhail and Kramer 1995; Merino-Andrés et al. 2022; Moreau et al. 2013; Nikolaos et al. 2019; Novak et al. 2020; Park and Kim 2014; Scholtes et al. 2010, 2012; Taylor et al. 2013; van Vulpen et al. 2017). While successful in increasing muscle strength, some interventions failed (Hanssen et al. 2022; Kirk et al. 2016; Moreau et al. 2013; Scholtes et al. 2010, 2012; Taylor et al. 2013) while others succeeded to improve motor function (Gillett et al. 2018; MacPhail and Kramer 1995; Moreau et al. 2013; Nikolaos et al. 2019; van Vulpen et al. 2017).

The diverging results in motor function gains induced by exercise interventions seems to arise from four main reasons. Firstly, the neural and morphological adaptations are highly specific to the strength training methods (Folland and Williams 2007; Kasper 2019; Moreau et al. 2013), which varied in these studies. Secondly, functional tasks involve a complex interaction between muscle strength, joint flexibility (i.e., maximum joint range of motion) and motor coordination. Thirdly, functional tasks involve activation of several muscle groups spanning across multiple joints. Thus, an adequate intervention should

train all relevant muscles with naturalistic patterns of neural activation. Fourthly, adherence to the training principles of individualization, overload, progression, and periodization are vital for an adequate strength training program (Kasper 2019). The training load must be constantly adjusted to overload the neuromuscular system to induce adaptations, considering the individual capabilities and peculiarities of each person. Furthermore, the training program must also consider the person's recovery, otherwise the progressive overload may lead to overtraining.

Stretching is another broadly used intervention to prevent or treat loss of joint flexibility, although its efficacy is still unclear (Harvey et al. 2017; Kalkman et al. 2020; Pin et al. 2006; Walhain et al. 2021; Wiart et al. 2008). Longitudinal studies applying manual passive stretching therapies for 6–9 weeks have generally reported an increase in joint range of motion (ROM) with no mechanical changes (e.g., resting fascicle length), suggesting that increased stretch tolerance played a major role in the ROM increase (Hösl et al. 2018; Kalkman et al. 2019; Theis et al. 2015; Weppler and Magnusson 2010). Little is known about the effects of more prolonged stretching interventions (Weppler and Magnusson 2010), and it is possible that structural changes (e.g., sarcomerogenesis) require more time. Kalkman et al. (2019) demonstrated that combined plantarflexors strength training and passive static stretching for children with CP was more effective in increasing resting fascicle length than solely stretching. The mechanistic explanation proposed by the authors is that strength training increased tendon stiffness, which decreased the muscle relative stiffness, and thus increased the stretch stimulus to the muscle.

Gait training has been shown to be safe, feasible and able to improve walking ability in children and young adults with CP (Booth et al. 2018). Especially, the inclined treadmill setup seems particularly effective, as it requires a greater ankle dorsiflexion in the swing phase and serves as a stretch for the ankle plantarflexors in the stance phase (Han et al. 2009; Leroux et al. 1999). Daily gait training with an inclined treadmill has been demonstrated to increase walking speed, dorsiflexion strength and active ROM, and reduce ankle stiffness in only 4–6 weeks (Lorentzen et al. 2017; Willerslev-Olsen et al. 2014).

Functional tasks such as walking and climbing stairs require muscle strength, joint flexibility and motor coordination; thus, it is reasonable to propose that an intervention should address all these aspects. Thus, the EXECP (i.e., EXErcise CP) intervention was developed for children and young adults with CP, which combines strength training for the lower limbs and trunk muscles, flexibility training for the lower limbs and gait training on an inclined treadmill.

While the main goal is to induce adaptations to increase motor function and allow people with CP to live a physically active life, the first fundamental step is to understand the differences between CP and typically developing (TD) controls. Physical capacities such as muscle strength and joint flexibility are straightforward to measure, however a common symptom of the upper motor neuron syndrome called hyper-resistance (HR), is a very complex phenomenon. HR is defined as an abnormal increase in passive resistance to muscle stretch, and

it can be problematic for several reasons: firstly, performing everyday motor tasks such as squatting and walking requires ample excursion on several joints, which may be hindered by the increased energy expenditure to overcome the stretch resistance and/or a suboptimal joint range of motion (ROM; Fox et al. 2018; Hicks et al. 2008); secondly, since there is a high resistance to achieve a joint position where the muscle is lengthened, the muscle will tend to stay in a short position to avoid unnecessary energy expenditure. Keeping a muscle in a shortened position for a prolonged period may trigger morphological changes in the muscle-tendon unit (Farmer and James 2001; Gracies 2005a; Lieber and Fridén 2019), which can result in permanent loss of joint range of motion (i.e., muscle contractures). Furthermore by hindering usual motor tasks, physical activity may also decrease, and inactive muscles tend to further weaken and maladapt (Farmer and James 2001; Gracies 2005a). Thus, due to its highly detrimental effects, HR must be monitored continuously and treated adequately.

The etiology of HR is a complex interaction between altered tissue properties, velocity-dependent hyperreflexia (i.e., early and/or exacerbated stretch reflex responses) and other involuntary background muscle activation (van den Noort et al. 2017). Identifying the contribution of each underlying component causing HR is of vital importance for designing treatments. Although HR requires only joint torque to be measured during a stretch, differentiating the contribution of its three components is far from trivial: electromyography (EMG), angular position and torque must be recorded concomitantly during slow and fast stretches. Furthermore, hyperreflexia can be quantified either by finding the joint angle at the stretch reflex (SR) onset (i.e., stretch reflex threshold, SRT) seen in EMG or by quantifying the SR EMG-RMS to the stretch. The SRT has been used since the 90 s (Levin and Feldman 1994), but unfortunately researchers did not take into account the SR latency (i.e., time between the SR onset at the muscle and its appearance on the EMG signal), thus measurements of hyperreflexia with this method carry a systematic error that has not been considered. Since HR can be caused by different contributions of its components, the ability to differentiate between components is crucial for choosing individualized interventions and for keeping track of possible side effects. Furthermore, demonstrating that training interventions do not negatively affect HR and its three components is crucial for broader adoption to be ensued, as many concerns about potential side effects still exist.

Therefore, the main purposes of this thesis were to (1) develop, execute, and test the effects of a multicomponent exercise intervention for children and young adults with spastic CP (articles I, IV), (2) study the effects of the proposed latency correction on the stretch reflex threshold and tonic stretch threshold methods to assess hyperreflexia (article II) and (3) perform a cross-sectional comparison of the neuromechanical responses to passive stretch and neurophysiological variables of hyperreflexia between CP and TD, and examine the correlation between the studied variables (article III).

2 LITERATURE REVIEW

2.1 Cerebral Palsy

2.1.1 Definition and Epidemiology

Originally reported as "cerebral paresis" by Little in 1861 (Little 1861), the CP definition has evolved during the 19th century along with the progressive understanding of its etiology and features. The current clinically accepted definition of CP was proposed by (Rosenbaum et al. 2007):

"Cerebral palsy describes a group of permanent disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, perception, cognition, communication, and behavior; by epilepsy, and by secondary musculoskeletal problems".

Rosembaum et al., (2006) offer important clarifications about this definition, some particularly pertinent to the current thesis. Firstly, CP is a heterogeneous condition both in terms of etiology as well as in type and severity of impairments. Secondly, the original brain insult that caused CP is non-progressive and permanent, but secondary symptoms such as muscle weakness, incoordination and limited joint flexibility tend to worsen over time. Thirdly, abnormal fine and gross motor function is a key feature in CP, but it is often accompanied by several other symptoms, again attesting to the heterogeneity of symptoms and impairments. Three important remarks about this definition are necessary: 1) by "non-progressive disturbance" the authors are probably contrasting the brain lesion to other conditions such as multiple sclerosis in which the brain maladapts with time. However, it is important to acknowledge that the brain is highly plastic and many changes do occur after the lesion; 2) the involvement of abnormal spinal cord development is unmentioned, and the focus remains on the

brain despite the severity or even presence of physical impairments being poorly predicted by brain image (Brandenburg et al. 2019). Furthermore, several fundamental spinal mechanisms, such as post-activation depression, presynaptic inhibition, reciprocal IA inhibition and recurrent inhibition have been reported to be abnormal in people with hyper-resistance, a common feature in the most prevalent type of CP (Nielsen et al. 2005); 3) the secondary symptoms (i.e., muscle weakness, incoordination and limited joint flexibility) induce lower levels of physical activity, which in turn causes worsening of the secondary symptoms, in a risky downward spiral. Furthermore, lower physical activity is associated with increased risk of chronic diseases such as cardiovascular problems (Maltais et al. 2014; Verschuren et al. 2016).

Cerebral palsy is the most common motor disability of childhood (Graham et al. 2016). Population-based registries of CP in US and Europe have found a CP prevalence ranging from 1.7 to 3.6 per 1000 live births (Kirby et al. 2011; SCPE 2002; Winter et al. 2002; Yeargin-Allsopp et al. 2008). The primary symptoms of CP include hyper-resistance, dyskinesias, ataxia, hyperreflexia and in rare cases hypotonia (Brandenburg et al. 2019). HR is an increased velocity-dependent resistance to muscle stretch (van den Noort et al. 2017). Dyskinesias such as dystonia, athetosis and chorea, are involuntary, often sustained movements, commonly due to an injury or pathology of deep brain structure (Sanger et al. 2010). Ataxia involves incoordination of movement, typically due cerebellar injury or maldevelopment (Krägeloh-Mann and Horber 2007). Hyperreflexia is an abnormal stretch reflex (SR) response to muscle stretch (van den Noort et al. 2017). Hypotonia (i.e., decreased muscle tone) is most commonly associated with muscle or peripheral nerve disorder (Lisi and Cohn 2011), although it can occur in central nervous system, genetic and metabolic disorders. Its underlying mechanisms are poorly understood (Lisi and Cohn 2011).

Usually, CP is broadly divided into four motor types: spastic, ataxic, dyskinetic and mixed, although various combinations of the above-mentioned symptoms are present in people with CP (Sanger et al. 2010). Spastic CP is the most prevalent type of CP with over 80% of all diagnosed cases (Himmelmann et al. 2005) and is roughly classified topographically as unilateral (hemiplegia) or bilateral (diplegia or quadriplegia). The main feature of spastic CP is hyper-resistance, which will be discussed in detail in the next section. Regarding the etiology of spastic CP, Brandenburg et al., (2019) cites the following known risk factors: "asphyxia, hemorrhagic or ischemic stroke (prenatal or neonatal), infection (prenatal or neonatal), brain malformation, trauma (inflicted or accidental), non-progressive genetic disorders/differences, and dysmaturation". Furthermore, Brandenburg et al. (2019) points out that while considerable effort to understand how the above-mentioned risk factors cause or contribute to the neurodevelopmental injury in the brain, there is scant information about the impact of these risk factors on spinal cord development. Considering that hyper-resistance is thought to be due to loss of descending inhibition to the motoneurons (Graham et al. 2016; Trompetto et al. 2014), and that several spinal circuits have been found abnormal in spastic CP (Nielsen et al. 2005), more

research into spinal cord development in spastic CP seems crucial for the pathophysiological mechanisms to be fully understood.

2.1.2 Motor function

Motor function is broadly divided into gross and fine motor function. Gross motor function is the ability of the neuromuscular system to activate and coordinate several muscles to perform motor tasks such as walking, climbing stairs and squatting. Fine motor function involves the activation of smaller muscle groups in more precise motor tasks, such as drawing and object manipulation. Cerebral palsy can affect both (Himmelmann et al. 2005), but its effect on lower limb gross motor function is particularly important as it limits physical activity, locomotion, and participation in everyday activities. The gross motor function measure (GMFM) has been shown to be a valid, reliable and responsive observational instrument to assess gross motor function in CP (Russell et al. 1989, 2000, 2013). This instrument has five dimensions: a) lying and rolling, b) sitting, c) crawling and kneeling, d) standing and e) walking, running, and jumping. Each dimension has a set of tests, with well-defined scoring criteria, so that the examiner can observe the person's motor action and score accordingly. Figure 1 shows an example of dimension D.

Item		D: STANDING	SCORE				NT
*	52.	ON THE FLOOR: PULLS TO STD AT LARGE BENCH	0☐	1☐	2☐	3☐	52.
*	53.	STD: MAINTAINS, ARMS FREE, 3 SECONDS	0☐	1☐	2☐	3☐	53.
*	54.	STD: HOLDING ON TO LARGE BENCH WITH ONE HAND, LIFTS R FOOT, 3 SECONDS	0☐	1☐	2☐	3☐	54.
*	55.	STD: HOLDING ON TO LARGE BENCH WITH ONE HAND, LIFTS L FOOT, 3 SECONDS	0☐	1☐	2☐	3☐	55.
*	56.	STD: MAINTAINS, ARMS FREE, 20 SECONDS	0☐	1☐	2☐	3☐	56.
*	57.	STD: LIFTS L FOOT, ARMS FREE, 10 SECONDS	0☐	1☐	2☐	3☐	57.
*	58.	STD: LIFTS R FOOT, ARMS FREE, 10 SECONDS	0☐	1☐	2☐	3☐	58.
*	59.	SIT ON SMALL BENCH: ATTAINS STD WITHOUT USING ARMS	0☐	1☐	2☐	3☐	59.
*	60.	HIGH KN: ATTAINS STD THROUGH HALF KN ON R KNEE, WITHOUT USING ARMS	0☐	1☐	2☐	3☐	60.
*	61.	HIGH KN: ATTAINS STD THROUGH HALF KN ON L KNEE, WITHOUT USING ARMS	0☐	1☐	2☐	3☐	61.
*	62.	STD: LOWERS TO SIT ON FLOOR WITH CONTROL, ARMS FREE	0☐	1☐	2☐	3☐	62.
*	63.	STD: ATTAINS SQUAT, ARMS FREE	0☐	1☐	2☐	3☐	63.
*	64.	STD: PICKS UP OBJECT FROM FLOOR, ARMS FREE, RETURNS TO STAND	0☐	1☐	2☐	3☐	64.

FIGURE 1 Test items on dimension D. Scores for each item can vary from 0 (does not initiate), 1 (initiates), 2 (partially completes) and 3 (completes). All specific parameters about time, distance, type of support, repetitions, etc. are clearly provided for each item. STD: standing; L: left; R: right; High KN: high knee position; NT: not tested. Source: Russell et al., (2013). Reproduced with permission from Gross Motor Function Measure (GMFM-66 & GMFM-88) User's Manual 3rd Edition (Dianne J Russell, Marilyn Wright, Peter L Rosenbaum, Lisa M Avery), published by Mac Keith Press (www.mackeith.co.uk) in its Clinics in Developmental Medicine Series, 2021, 9781911612490).

Since the GMFM is very time-consuming, a fast and easy to apply descriptive classification system called gross motor function classification system (GMFCS) was developed to stratify gross motor function in CP (Palisano et al. 1997, 2008; Russell et al. 2013). The GMFCS has 5 levels and four age bands, with levels

representing meaningful differences in gross motor function and age bands accounting for age-related differences in gross motor function. For example, a child classified in GMFCS level 1, with an age of 6–12 years old can walk indoors and outdoors and climb stairs without limitations. A child classified in GMFCS level 2 with the same age can also do the same, however the child has limitations walking on uneven surfaces and inclines, and needs to hold a rail to climb stairs. Research on the GMFCS supports interrater reliability and stability, as well as content, construct, discriminative, and predictive validity (Palisano et al. 1997, 2000, 2006; Rosenbaum et al. 2002; Wood and Rosenbaum 2000). Palisano et al., (2000) reported a significative negative correlation between GMFCS and GMFM ($r = -0.91$, $p < 0.001$), showing that the broad classification of gross motor function level was closely related to the detailed quantification of gross motor function measured by the GMFM. A few studies have followed hundreds of participants with CP (more than 75% spastic CP), measuring GMFM throughout time to track gross motor function development (Hanna et al. 2009; Rosenbaum et al. 2002; Smits et al. 2013). Figure 2 depicts the gross motor development curves measured with the GMFM in time, stratified by the five GMFCS. Hanna et al., (2008) found a stable plateau of motor function for GMFCS levels 1–2, while the other three levels experienced a peak around 7–8 years, and then a gradual decline in function over the subsequent years. Oppositely, Smits et al., (2013) reported that all five levels had a stable plateau of function over the ages of 1 to 24 years. Physical interventions capable of increasing GMFM or at least avoiding its deterioration over time are fundamental. Furthermore, the loss of gross motor function with further aging has been well documented in the CP population (Bottos et al. 2001; Jahnsen et al. 2004).

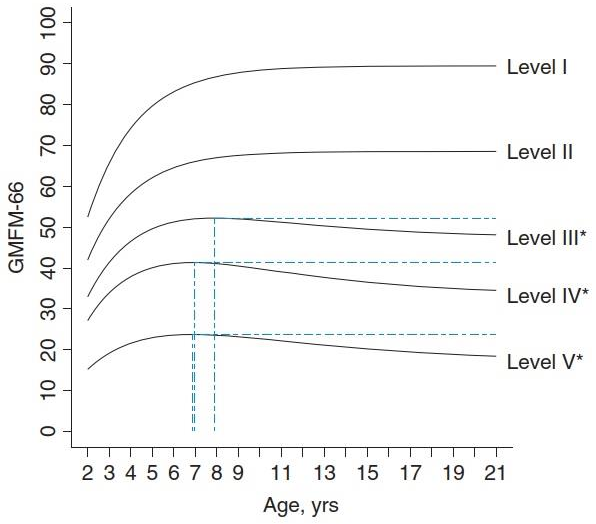

FIGURE 2 Motor development curves for children and young adults with cerebral palsy. Source: Hanna et al., (2009). Dashed lines illustrate age and score at peak GMFM-66. Reproduced with kind permission by the publisher John Wiley and Sons. License: 5617640627482.

The six minutes walking test (6MWT) is a widely used, easy to apply, and reliable test to assess walking ability in CP (Maher et al. 2008). The distance covered in the 6MWT is reported to reflect functional capacity and has been recommended as a submaximal exercise test for children with CP of GMFCS 1 to 3 (Verschuren et al. 2011; Verschuren and Balemans 2015). Fitzgerald et al., (2016) has provided references values for each of the three GMFCS levels, reporting statistically significant differences between GMFCS levels.

Gross motor function involves the coordinated activation of several muscle groups to generate movement throughout a range of motion. Thus, a successful gross motor action has three important requirements: muscle strength, neuromuscular coordination, and joint flexibility. Adequate muscle strength in the agonist muscles is necessary to generate movement, while activation of synergistic muscles is required for body stability. Movement throughout a range of motion inherently requires that enough range of motion is available, otherwise a physical blockage to the movement will hinder the motor action. Lastly, having enough flexibility and strength to execute a motor action is not sufficient, motor coordination to activate all agonist and synergist muscles in the correct timing, while inhibiting the antagonists is crucial. Therefore, an intervention to increase gross motor function should be able to tackle all three requirements to successfully increase gross motor function in people with spastic CP.

2.2 Hyper-resistance in cerebral palsy

HR refers to a abnormally high resistance to stretch given by the muscle-tendon unit (MTU), which can be quantified subjectively with perception scales such as the Ashworth scale (Bohannon and Smith 1987), or objectively using equipment capable of measuring torque (e.g., Bar-On et al. 2014). High HR can be problematic for several reasons: firstly, performing everyday motor tasks such as squatting and walking requires ample excursion on several joints, which may be hindered by the increased energy expenditure to overcome the stretch resistance and/or suboptimal joint ROM (Fox et al. 2018; Hicks et al. 2008); secondly, since there is a high resistance to achieve a joint position where the muscle is lengthened, the muscle will tend to stay in a short position to avoid unnecessary energy expenditure. Keeping an inactive joint in a shortened position for a prolonged period may trigger morphological changes in the MTU (Farmer and James 2001; Gracies 2005a; Lieber and Fridén 2019), which can result in permanent loss of joint range of motion (i.e., muscle contractures); and lastly, by hindering usual motor tasks, physical activity will decrease, and inactive muscles tend to further weaken and maladapt (Farmer and James 2001; Gracies 2005a). Due to its highly detrimental effects, hyper-resistance must be monitored continuously and treated adequately.

During clinical examination in CP, it is common for the clinician to passively move the person's joint throughout the entire range of motion. The clinician evaluates joint flexibility (i.e., maximum joint ROM) and the amount of

resistance that the neuromuscular system is imposing to the stretch. Although hyper-resistance is easily recognizable on clinical tests, its etiology is a complex interaction between tissue properties (i.e., morphological changes in the MTU), hyperreflexia (i.e., early and/or exacerbated stretch reflex response) and increased involuntary background muscle activation (Lorentzen et al. 2017; van den Noort et al. 2017; Willerslev-Olsen et al. 2013). Figure 3 depicts the conceptual framework for hyper-resistance and its assessment requirements.

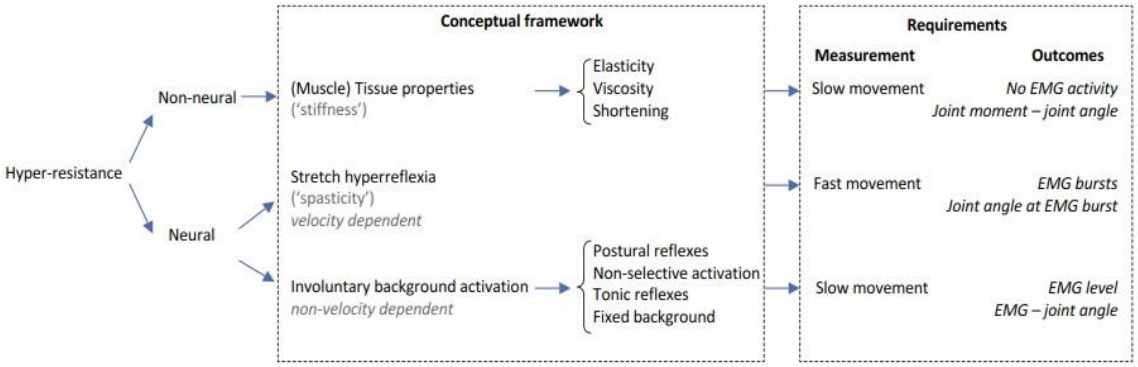

FIGURE 3 Hyper-resistance conceptual framework. Source: adapted from van den Noort et al., (2017). Reproduced with kind permission by the publisher John Wiley and Sons. License: 5617671250619.

Lorentzen et al., (2010) reported that trained neurologists were only able to determine increased reflex activity (i.e., hyperreflexia) in adults with lesions in the descending motor pathways (e.g., multiple sclerosis, stroke) in about 60% of the cases. This result suggests that clinical evaluation alone is unreliable, and an objective biomechanical and electrophysiological evaluation is necessary to distinguish between hyper-resistance components. Treatments to reduce hyperreflexia such as Botulinum toxin A and selective dorsal rhizotomy may be useful for a person with hyper-resistance mediated by the neural components (i.e., hyperreflexia and increased background muscle activation), while completely ineffective if the main component is altered tissue properties (Willerslev-Olsen et al. 2013). Since hyper-resistance is a main topic on this thesis, each component will be discussed separately in the following sections.

2.2.1 Tissue Properties

The non-neurological, strictly mechanical component of muscle HR consists of morphological alterations that are thought to occur as a result of neural dysregulation (i.e., maladaptation in the neuromuscular signaling between supraspinal and spinal neurons, and the muscle). In spastic CP, muscle morphological changes such as atrophy, loss of sarcomeres, accumulation of intramuscular connective tissue, increased fat content and extracellular matrix hypertrophy have been reported (Graham et al. 2016; Hanssen et al. 2021; Mathewson and Lieber 2015). Although spastic CP muscles are shorter, smaller

and with reduced diameter fibers, they are generally found to have higher stiffness than TD muscles (i.e., higher torque-angle derivative; Lieber and Fridén 2019; Willerslev-Olsen et al. 2018). Since muscle volume has a high positive correlation with joint stiffness (Chleboun et al. 1997), this inversion attests to the significance of the morphological maladaptation in spastic CP muscle. These morphological alterations happen very early in spastic CP, Willerslev-Olsen et al., (2018) reported lower medial gastrocnemius muscle volume compared to TD controls already at one year of age. Furthermore, passive stiffness was statistically higher in CP children compared to TD at 27 months of age, and at around 30 months of age, children with CP already deviated from the upper 95% confidence interval of TD children stiffness. These results indicate that reduced muscle growth may be involved in the pathophysiology of contractures.

Joint contracture is characterized by a permanent loss of movement amplitude, and it is the most severe clinical case of increased passive muscle resistance. In muscles with contractures, there are less sarcomeres (i.e., muscle's contractile unit), that are up to two times the normal length, even though the muscle fiber is shorter than in TD (Graham et al. 2016; Lieber and Fridén 2019). Furthermore, the MTU with a contracture is under extremely high passive mechanical tension, contributing immensely to HR (Graham et al. 2016; Lieber and Fridén 2019). The muscle's altered viscoelastic properties may also be a mechanical, velocity-dependent contributing factor to the measured HR (Taylor et al. 1990; Wu et al. 2010).

2.2.2 Hyperreflexia

2.2.2.1 Muscle spindles and the stretch reflex

Before discussing hyperreflexia, a simplified description of the muscle spindle mechanoreceptor and the SR pathway is appropriate. Muscle spindles are small, encapsulated sensory receptors that have a fusiform shape and are arranged in parallel with the extrafusal muscle fibers that make up the main body of the muscle. Due to its placement, the intrafusal fibers change in length concomitantly with the muscle's extrafusal fibers. Muscle spindles monitor muscle length and rate of length change, providing the central nervous system with proprioceptive information (e.g., position of limbs in space; Winter et al. 2005). Furthermore, muscle spindles are also responsible for triggering the stretch reflex, causing muscle activation that is important to protect the muscle from excessive deformation (Kandel et al. 2000). In addition to the protective role, the stretch reflex can enhance muscle function through the stretch-shorten cycle (Komi et al. 2003).

The stretch reflex starts with the mechanical deformation of the intrafusal muscle fibers, which generates actions potentials by activating mechanically gated ion channels in the afferent axons coiled around the muscle spindle. The action potentials travel through the IA afferent nerve towards the sensory neuron's soma located near the spinal cord, in a dorsal root ganglion. The sensory neuron forms a monosynaptic excitatory connection with the alpha motoneurons

(αMN) in the ventral horn of the spinal cord that innervate the same muscle (Kandel et al. 2000). Additionally, afferent input via inhibitory connections is sent to the αMNs of the antagonist muscles preventing active stretching, and upstream to the supraspinal neurons. Henneman (1985) demonstrated that a single IA afferent from a muscle spindle can have synapses with more than 80% of the homonymous αMN pool. If the monosynaptic excitatory afferent input from the IA axons can depolarize the αMN, an efferent volley from the later will be sent towards the muscle, causing muscle activation. Figure 4 depicts the stretch reflex pathway.

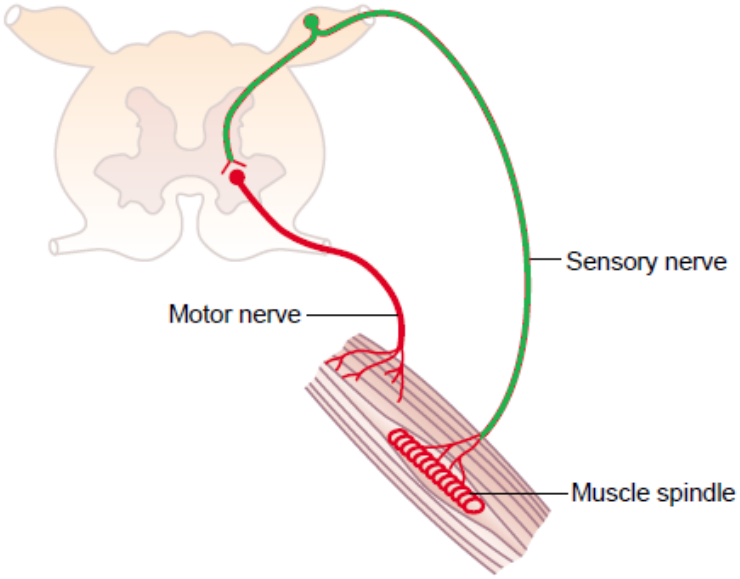

FIGURE 4 Stretch reflex pathway. The muscle spindle is shown coiled around the extrafusal muscle fibers, and it sends its signal through the sensory nerve (i.e., IA afferents, green colored). The signal passes through the sensory neuron and depolarizes the alpha motoneuron, which sends its efferent signal through the motor nerve to cause muscle activation (red colored). Note: although depicted separately, both sensory and motor nerves are bundled together. Source: adapted from Wikimedia commons

(https://commons.wikimedia.org/wiki/File:Aferentn%C3%AD_a_eferentn%C3%AD_dr%C3%A1ha.png)

2.2.2.2 Neuromechanical assessment of hyperreflexia

Hyperreflexia is the neural, velocity-dependent, involuntary muscle activation component of hyper-resistance. Stretch hyperreflexia is defined as an exacerbated stretch reflex response to a muscle stretch, characterized by a decreased threshold and an increased response magnitude (Gracies 2005b). Two main methods have been developed to quantify hyperreflexia. The first method is using surface EMG to quantify the stretch reflex response (e.g., Bar-On et al. 2014), usually using the root mean square (RMS) of the EMG signal in a given time window (e.g., 50 ms) immediately after the SR. The SR response is positively correlated with stretch velocity; hence hyperreflexia is deemed velocity-

dependent (Bar-On et al. 2014, 2018). Physiologically this dependency is expected, since higher stretch velocities will trigger more muscle spindles, which will send more afferent signal and hence depolarize more αMNs and causing greater muscle activation.

The second method to assess hyperreflexia is to determine the joint angle at the SR EMG onset, that is, the joint angle corresponding to the appearance of the SR EMG signal, also termed the SRT. The SRT can be measured at different stretch velocities, and it is generally assumed that higher stretch velocities will result in earlier onset joint angles (Levin and Feldman 1994). The "decreased threshold" for the stretch reflex stated in the hyperreflexia definition is related to the SRT in two ways: 1) at slow stretch velocities (e.g., $50°\cdot s^{-1}$), muscles with hyperreflexia respond with SRs whereas TD muscles remain silent; 2) within the same stretch velocity, the SRT in muscles with hyperreflexia occur earlier in the stretch as compared to TD muscles. To the best of my knowledge, none has been clearly demonstrated with an experimental study, although the first point is tacitly clear from clinical examination. The current thesis will test both ways to assess SRT, as both are important to discuss the usefulness of this variable to assess hyperreflexia.

The SRT has been used for almost three decades (Levin and Feldman 1994), in numerous studies (e.g., Blanchette et al. 2016; Calota et al. 2008; Frenkel-Toledo et al. 2021; Germanotta et al. 2017), however, researchers have unfortunately forgotten to consider the SR latency for the SRT calculation. SR latency is the duration between the SR being mechanically initiated at the muscle spindles (i.e., SR onset) to its appearance in the EMG signal (i.e., SR EMG onset; pathway depicted in Figure 4). For a given SR latency, the difference between the joint angle at the SR onset and at the SR EMG onset (i.e., SRT) will have a positive linear relationship with the stretch velocity. For example, if we consider a SR latency of 30 ms and two stretches performed at $50°\cdot s^{-1}$ and $300°\cdot s^{-1}$, the errors of using the angle at the SR EMG onset are 1.5° and 9° respectively, simply because the EMG onset is delayed by the ~30 ms monosynaptic SR latency. Thus, without the SR latency correction, the SRT is progressively overestimated to later angles as velocity increases. While the study by Levin & Feldman (1994) acknowledged the SR latency problem in calculating the SRT and proposed subtracting 30 ms as a mean latency for the SR, later studies implementing the method did not.

Another widely used method to assess hyperreflexia is called the Tonic Stretch Reflex Threshold (Calota and Levin 2009; TSRT; Levin and Feldman 1994). The TSRT estimates the joint angle in which involuntary muscle activity would hypothetically start in the absence of joint movement. The TSRT is the y-intercept of the linear regression line through the SRTs with stretch velocity, thus representing the joint angle at zero velocity. Figure 5 depicts the TSRT calculation using SRTs obtained at four different stretch velocities.

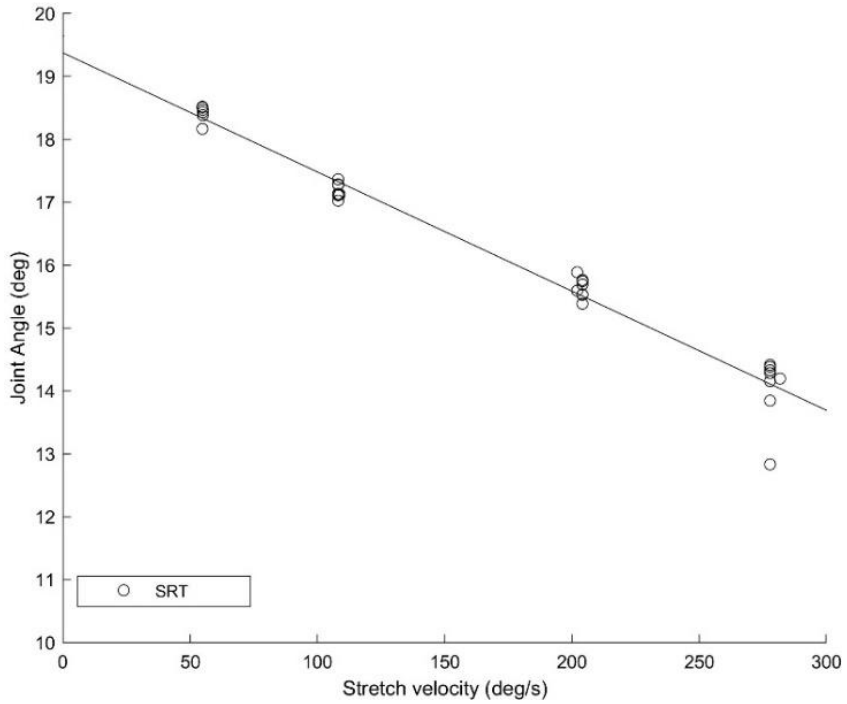

FIGURE 5 The tonic stretch reflex threshold is calculated as the y-intercept of the regression line between stretch velocity and the stretch reflex threshold (SRT). In this example, the TSRT equals 19.4°.

Since the TSRT method depends on the linear relationship between stretch velocity and the SRT, a high coefficient of determination (R^2) for the linear regression is required. If the linear regression has a low R^2, it would not make sense to extrapolate it to zero velocity (i.e., y-intercept), as by definition the regression would not explain the SRT-joint velocity relationship well. Several studies have reported a moderate to high R^2 for the linear regression between the SRTs and stretch velocity (Blanchette et al. 2016; Calota et al. 2008; Frenkel-Toledo et al. 2021; Germanotta et al. 2017). However, since these studies did not perform the SR latency correction, and the systematic error discussed above was inserted into the analysis, it remains to be verified if the R^2 would still be high after the correction is performed. Due to the systematic nature of the error (i.e., the higher the velocity, higher is the error), it is very likely that it increases the linearity of the joint velocity and SRT relationship, consequently inflating the R^2.

Previous studies have performed the linear regression between SRT and joint velocity with the opposite axes as shown in Figure 5 (e.g., Blanchette et al. 2016; Calota et al. 2008). Inverting the axes was chosen because linear functions ($y = mx + b$) declare x as the independent variable (i.e., joint velocity) and y as the dependent variable (i.e., SRT). Thus, the present linear model (Figure 5) predicts the SRT as a function of joint velocity values, while in previous studies the linear model predicted the joint velocity as a function of the SRT values, which is theoretically incorrect cause and effect relationship. Thus, the slope value (m in the linear function) that multiplies x is readily understandable in the axes' setup; showing how joint velocity affects SRT (i.e., gain), and it is not

comparable to the opposite axes' setup (i.e., predicting y as a function of x is not the same as predicting x as a function of y). Another practical reason is that the TSRT is available from the regression equation as the y-intercept (b in the linear function), while with the opposite axes' setup, the equation must be solved for x, adding an additional calculation step. Changes in axes setup between studies do not affect the R^2 value for the linear regression between joint velocity and SRT. However, the TSRT and slope values are different and cannot be compared between studies.

2.2.2.3 Hyperreflexia neural pathways

The Hoffman reflex (H-reflex) is an electrically induced spinal reflex that is used to probe the efficacy of the synaptic transmission in the IA reflex arc (Capaday 1997). Figure 6 and the following paraphrased description of events involved in eliciting the H-reflex are from Palmieri et al. (2004). A percutaneous electrical stimulation of a mixed peripheral nerve containing both motor and sensory axons will evoke preferentially an afferent sensory volley from the IA fibers to the αMNs (Figure 6A, event 2). The afferent volley will depolarize the αMNs, resulting in an efferent motor response (i.e., from αMNs to the muscle) recorded in EMG, called the H-reflex (Figure 6A, event 3). When the electrical stimulation intensity is increased, motor axons will also depolarize, generating a compound muscle action potential denominated M-wave (i.e., muscle response; Figure 6A, event 1). The higher intensity required to elicit an M-wave is explained by the motor axon's intrinsic properties and smaller diameter which results in a higher depolarization threshold (Pierrot-Deseilligny and Burke 2005). The M-wave has a shorter latency than the H-reflex since it is generated via direct stimulation of the motor nerve and hence there is no synaptic delay (Bischoff 2002).

At submaximal stimulation intensities, the H-reflex and M-wave do not activate the same αMNs: IA afferent volley will recruit MNs according to the size principle (Henneman 1957), while the electrical stimulation that elicits the M-wave activates initially larger diameter axons from fast motor units (Knikou 2008; Pierrot-Deseilligny and Burke 2005). The amplitudes of the H-reflex and M-wave will both increase fairly linearly with the stimulation intensity until the maximum H-reflex (Hmax), representing the fullest extent of reflex activation, and at higher stimulation intensities, the maximum M-wave (Mmax), representing the maximal muscle activation (Figure 6B; (Crone et al. 1999)). The action potentials (APs) generated in the motor axons not only travel orthodromically towards the muscle, but also antidromically towards the αMNs. Thus, after plateauing, the H-reflex will progressively decrease until completely disappears due to the collision between the orthodromic and antidromic volleys (Figure 6B).

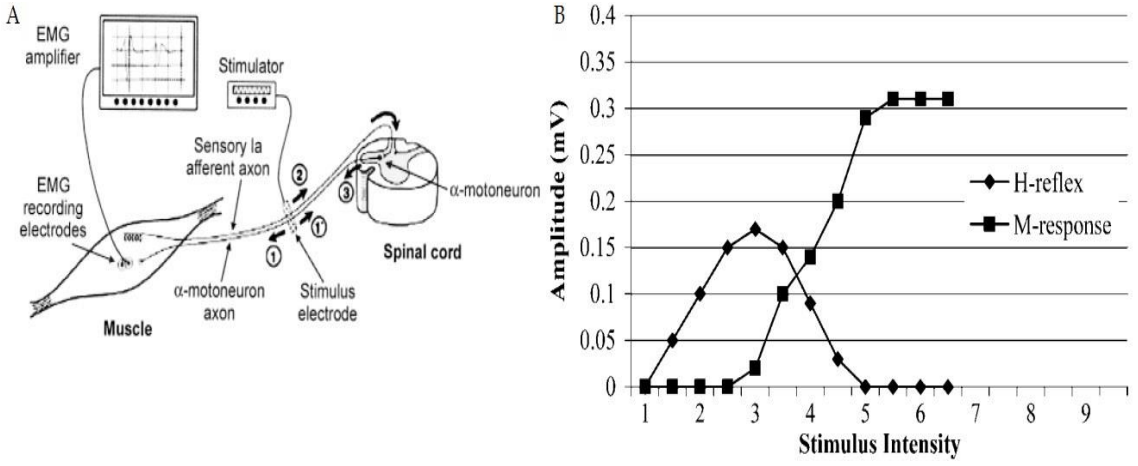

FIGURE 6 A: H-reflex and M-wave pathways. A low-intensity electric stimulus on the peripheral nerve elicits actions potentials (APs) selectively on the IA afferents due to their large axon diameter (event 2). These APs travel to the spinal cord, depolarizing the alpha motoneurons, which send APs to the muscle (event 3). Event 3 is recorded in the muscle as an H-reflex. Gradually increasing the stimulus intensity causes APs to occur in the thinner axons of the αMN (event 1), traveling in both directions: towards the muscle recorded as the M-wave, and towards the spinal cord colliding with APs from response 3, causing partial cancelation of the reflex response. At supramaximal stimulus intensities, orthodromic (towards the muscle) and antidromic (towards the spinal cord) APs occur in all alpha motoneuron axons; the former gives rise to a maximum M-wave, whereas the latter results in the complete cancellation of the H-reflex. B: H-reflex and M-wave recruitment curves. Source: text and figures adapted from Palmieri et al. (2004), figure A was adapted by Palmieri et al. (2004) from Aagaard et al. (2002).

The H-reflex has a similar pathway as the SR, with two main differences: 1) electrical stimulation generates APs at the IA afferent axons, bypassing the muscle spindle (Palmieri et al. 2004); 2) electrical stimulation generates a synchronous AP volley, whereas physiological muscle spindle activation generates an asynchronous AP volley. Taking these differences into consideration, the H-reflex can be used to probe the IA reflex arc sensitivity. Since the peak-to-peak amplitude or area of the H-reflex can vary widely among people due to several factors such as skin resistance, the M-max is used for normalization (Palmieri et al. 2004). Thus, the Hmax/Mmax ratio is used as an overall measure of IA reflex arc excitability.

Once afferent APs originating from diverse proprioceptors and exteroceptors are propagated into the central nervous system, the signal spreads to hundreds of thousands of neurons in a very complex manner, thus the ability to reduce a given afferent signal at source is very important. One such mechanism is called post-activation depression (PAD), a homosynaptic, activity-dependent mechanism that reduces presynaptic transmitter release, with a long-lasting depressive effect (i.e., 4–4 s) on the transmission of the IA reflex (Hultborn et al. 1996). Since hyperreflexia is a hyperactivity of the IA reflex arc, naturally

PAD is a primary candidate for modulating it. Indeed, PAD has been shown to be depressed in stroke patients (Lamy et al. 2009; Yang et al. 2015) and in CP (Achache et al. 2010). However, both studies with stroke patients did not normalize the H-reflex by the preceding M-wave, possibly creating errors in their data analysis and conclusions.

To assess PAD, H-reflexes are elicited at a fast (e.g., 1 Hz) and a slow (e.g., 0.1 Hz) stimulation frequencies. The slow frequency should be at least 0.1 Hz to ascertain that PAD effects are gone (Crone and Nielsen 1989; Pierrot-Deseilligny and Burke 2005) and serves a reference value (i.e., efficacy of the synaptic transmission in the IA arc without effects of PAD). The ratio between the mean values of fast and slow frequency H-reflex normalized amplitudes is the PAD measure (i.e., lower ratio means higher PAD). Two important methodological considerations are important for comparing the H-reflexes elicited at the two stimulation frequencies, and hence calculate PAD. Firstly, the stimulation intensity should be adjusted to elicit slow H-reflexes on the ascending limb of the recruitment curve (Figure 6B). The H-reflex at the ascending limb can be easily modulated (positively or negatively), while that is not the case for the descending limb (Crone et al. 1990; Knikou 2008). Secondly, the stimulation intensity should optimally evoke a clear M-wave because its ratio with the Mmax (i.e., M/Mmax) provides information about the effective stimulation intensity. To make sure that PAD was responsible for the H-reflex amplitude modulation on the fast stimulation frequency, it is important that the effective stimulation intensity remains constant between stimulation frequencies (Knikou 2008). Otherwise, the variation in the effective stimulation intensity will be a confounding variable. All three above mentioned PAD studies chose a stimulation intensity to elicit slow H-reflexes at 50% of the Hmax, which very likely did not elicit consistent M-waves and none of the three above-mentioned PAD studies mentioned the M/Mmax, thus the results must be interpreted with caution.

In theory, these artificial neurophysiological variables (i.e., Hmax/Mmax and PAD) should be directly related to hyperreflexia which should affect HR. Achache et al. (2010) reported a significant correlation between HR measured with the Ashworth scale (Bohannon and Smith 1987) and PAD ($r = 0.52$, $p = 0.01$), and no correlation between HR and Hmax/Mmax. However, to the best of my knowledge, the correlation between hyperreflexia neuromechanical variables and these neurophysiological variables have not been empirically tested.

2.2.3 Involuntary background activation

The non-velocity dependent neural component of hyper-resistance is called involuntary background activation; postural reflexes, non-selective activation, tonic reflexes, and fixed background tone are all part of this component (van den Noort et al. 2017). Furthermore, this component manifests at rest, in the absence of any phasic stretch, but is sensitive to tonic stretch intensity and duration (also referred to as spastic dystonia; Baude et al. 2019). Furthermore, various studies have demonstrated this length-dependent behavior in the involuntary background activation (Bar-On et al. 2014; Lebiedowska and Fisk 2009; Pandyan

et al. 2006). According to Baude et al. (2019), the increased background activation is likely related to increased involvement of brainstem descending pathways undergoing abnormal branching onto deafferented hyperexcitable motor neurons following higher lesions. Additionally, changes in membrane properties of alpha motoneurons could increase their sensitivity to IA afferent input, which in turn triggers persistent inward currents that lead to prolonged depolarization states (Gracies 2005b).

2.2.4 Hyper-resistance assessment

To assess the different components of HR the main strategy is to perform passive muscle stretches at different velocities. Very slow passive muscle stretches (e.g., $< 10°\cdot s^{-1}$) do not elicit stretch reflexes SR and are thus adequate to measure the passive mechanical properties of the MTU. The simplest and perhaps most used clinical measure obtained is the maximum ROM, which represents the MTU and its surrounding tissues' (e.g., articular capsule) maximum extensibility (i.e., joint flexibility). However, as discussed by Weppler & Magnusson (2010), other dimensions such as tension and time are invariably linked to this phenomenon, and passive biomechanical properties can be used to evaluate joint resistance (e.g., torque, stiffness, energy and hysteresis). Surface EMG is often used to ensure that the muscle is indeed relaxed during the passive stretch. As such, the passive ROM, calculated as the ROM in which the muscle remains completely relaxed, can be used to identify the presence of length-dependent involuntary muscle activation. Finally, passive peak torque describes the MTU's maximum passive resistance to the stretch, and it is influenced by the MTU's anatomical cross-sectional area and by its morphological properties (Chleboun et al. 1997; Lieber and Fridén 2019).

Fast stretches that elicit a SR evident in surface EMG, allows the detection of the SR onset joint angle (i.e., SRT) and quantification of the SR EMG response, both being key features of hyperreflexia (van den Noort et al. 2017). Furthermore, the comparison of the resistance to stretch in a slow and fast stretches allows the rough inference of which component is more prominent. For example, if the resistance to stretch is high at a slow stretch velocity, and does not increase substantially when stretch velocity is increased, it is reasonable to infer that the mechanical MTU properties were the most prominent factor influencing HR.

The effect of the increased background activity on HR is hard to assess due to the inherent difficulties of estimating force production from EMG activity (Farina et al. 2004). Furthermore, the increased background muscle activation may interact with the other two HR components by: 1) adding active torque to the slow stretch test, confounding the assessment of the passive mechanical component; 2) increasing muscle spindle sensitivity via alpha-gamma co-activation; 3) altering muscle-tendon dynamics as the increased active muscle stiffness will reduce muscle lengthening relative to the tendon during the stretch (Bar-On et al. 2018). Thus, monitoring EMG is important to verify if the muscle is relaxed during the slow stretch and to quantify the amount of background activity right before the initiation of the fast stretch. Due to differences in the

EMG signals between people (e.g., electrode placement in relation to motor units in the muscle, Vigotsky et al. 2017), background activity is usually normalized by the maximum isometric muscle action EMG of the same muscle. Thus, with the EMG normalization, EMG activation thresholds can be created to determine if muscle was under passive condition during the stretch (e.g., normalized EMG was lower than 3–5% of maximum isometric muscle action). An important caveat here is that surface EMG signal must be representative of the whole muscle being assessed, thus adequate electrode placement and interelectrode distance is crucial (Vigotsky et al. 2017).

Although hyperreflexia is deemed a neural component of hyper-resistance, the use of a passive muscle stretch to evaluate it merges neurophysiological (i.e., excitability of the IA pathway and muscle spindle) and mechanical (i.e., muscle-tendon dynamics) mechanisms. Muscle-tendon interaction during passive stretch depends on their relative stiffness, and thus the amount of stretch taken by tendon and muscle will affect the SR response (Avela et al. 2004; Bar-On et al. 2018; Rack et al. 1983). In spastic CP, muscle morphological changes (Graham et al. 2016) and altered muscle-tendon dynamics compared to TD controls have been described (Barber et al. 2012; Kalkman et al. 2018). Furthermore, several neural circuits capable of modulating the IA arc have been reported to be abnormal, such as PAD, presynaptic inhibition, reciprocal IA inhibition and recurrent inhibition (Nielsen et al. 2005). Thus, perhaps it is more adequate to classify hyperreflexia as a neuromuscular phenomenon, as both neural mechanisms and muscle-tendon mechanics will affect the SR. For example, it is conceivable that a person with a muscle contracture, having stretched muscle fibers and a MTU under stress, will have an early and strong SR in response to a stretch, because mechanically the muscle fiber and the intrafusal fibers will be stretched promptly (i.e., force is transmitted effectively due to high tissue stiffness). Thus, a complex mechanical-sensory-motor interaction within the neuromuscular system modulates hyperreflexia, and its understanding requires a thorough neuromechanical and neurophysiological examination.

2.3 Physical capacities and training

2.3.1 Training principles

With the widespread practice of sports and clinical rehabilitation, training methodologies to enhance muscle strength and joint flexibility have developed tremendously. Based on human physiology, a set of training principles applicable to all sports, rehabilitation and different populations have been put forth. The six core training principles are paraphrased below from the updated American College of Sports Medicine publication (Kasper 2019):

Overload. The neuromuscular, cardiorespiratory, and skeletal systems must be subjected to a higher degree of training stress than what is currently accustomed to induce compensatory improvements in those systems. However,

excessive overload and/or inadequate rest can result in overtraining, injury, and performance decrements.

Progression. The training stress must be gradually and systematically increased to maintain overload of the neuromuscular, cardiorespiratory, and skeletal systems, and thus continue to provoke training adaptations. The initial load parameters will cease to be effective once the person is more trained. As discussed in the overload principle, if the progression of training load is too fast, injuries and overtraining may occur.

Reversibility. The adaptations provoked by overloading a determined system will regress back to the pre-overload state after the training stimulus is removed. Bone density, muscle mass and aerobic capacity are all examples of adaptations that can be gained through overloading and lost due to the cessation of the stimulus. This principle implies that to keep certain wanted adaptations, for example higher muscle mass, training must be continuous to avoid the deadaptation process that will ensue once training stops.

Individualization. People vary substantially in physiology (e.g., age, current fitness, training history), psychology (e.g., motivation), environment (e.g., nutrition, lifestyle habits) and genetics. Thus, the training program generating progressive overload must consider the person's individuality.

Specificity. The adaptations induced by training are highly specific to the selected training load (e.g., volume, intensity, rest), training type (e.g., strength, flexibility, aerobic capacity) and execution patterns. Hence, the person's exact physiological demands must be understood, and the training program adjusted accordingly.

Periodization. Periodization is the systematic construction of the training program for a given time frame, considering load progression, recovery and aiming to induce specific adaptations. Different training methods can be used jointly or separately, but all must be organized in a logical progression, keeping in mind that adaptations that are not trained at all will be lost in time. Thus, the idea is generally to maintain certain adaptations with minimum training, while focusing on inducing other adaptations within a certain time frame. Sports periodization is often implemented using training cycles (i.e., microcycles, mesocycles and macrocycles), and aims to make the person achieve peak performance on a specific date (e.g., the most important tournament/competition in the year), while avoiding overtraining and injuries.

2.3.2 Muscle strength

Muscle strength can be defined as "the capacity of the neuromuscular system to produce force" (Schmidtbleicher 1993). The word "neuromuscular" is particularly important as it acknowledges the interaction between the nervous system and the skeletal muscles. From the first neuron at the motor cortex depolarizing to the cross-bridge mechanism activation to generate force, a complex electro-chemical-mechanical cascade of events takes place, which culminates in chemical energy being transformed into mechanical energy. Generally, the pulling force generated by the muscle acts across joints to rotate

body segments, with the tendons transmitting the force from muscle to bone. Due to the rotational nature of most joint movements, muscle force is multiplied by the moment arm (i.e., distance between the axis of rotation and the line of force acting on the joint) to yield torque. The external resistance is the product of the weight and the moment arm length measured from the axis of rotation to the resistance weight (Levangie et al. 2011). Specific terms for the interaction of muscle torque and external resistance have been created with the muscle actions classified as concentric, isometric and eccentric (Levangie et al. 2011). A concentric muscle action occurs when the muscle torque is greater than the external resistance (i.e., muscle shortens). An isometric muscle action happens when muscle torque and external resistance are equal (i.e., muscle remains at constant length). An eccentric muscle action occurs when muscle torque is lower than the external resistance (i.e., muscles lengthen). Considering important everyday gross motor tasks such as walking, sitting, and jumping, it is clear that all muscle action types are important. For example, squatting to open a low drawer requires initially an eccentric knee and hip extension and once the correct height is attained, an isometric muscle action for the same muscles to hold the position. Once the motor action of picking up something in the drawer is accomplished, a concentric muscle action for both knee and hip extensors is performed for the person to stand.

Schmidtbleicher proposed a very useful framework to think about muscle strength capacity. Figure 7 depicts the newest version of the strength framework. Unfortunately, the original work (Güllich and Schmidtbleicher 1999; Schmidtbleicher 1987) is published in German. To the best of my knowledge the only published reference in English was in the first edition of Komi's "Strength and Power in Sports" (Schmidtbleicher 1993). The newer strength model version includes important updates and will be presented here, however, Schmidtbleicher only presented it at conferences and symposiums, thus the original but inaccurate reference will be used here. Chagas and Lima (in Samulski et al. 2012) have published a book in Portuguese with the most recent model and it is possibly the best structured and discussed reference.

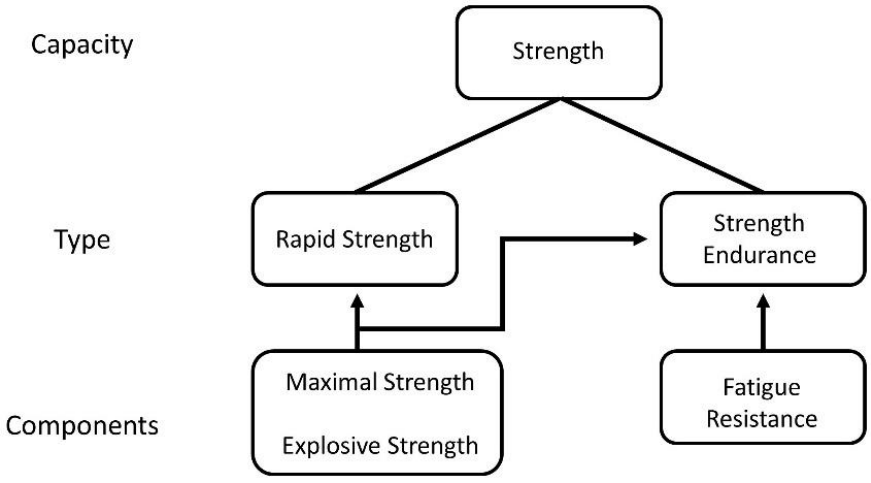

FIGURE 7 Muscle strength capacity framework (adapted from Schmidtbleicher, 1993).

According to the model, the muscle strength capacity can be divided into two types: rapid strength and strength endurance. Rapid strength is defined as "the neuromuscular system's capacity to produce the highest impulse (i.e., force-time integral) in the available time". Rapid strength depends on two main components: 1) explosive strength, which is the maximum rate of force development at the beginning of the muscle action; and 2) maximum strength, which is the highest torque value attained during a muscle action. Furthermore, another crucial parameter in the rapid strength concept is the duration of the applied force, which directly affects impulse. Furthermore, the available time to perform a given muscle action will dictate which component will have greater importance. On fast motor actions such as throwing a dart or jumping, explosive strength will be the main component influencing rapid strength, as not enough time will be available to achieve maximum strength. On the other hand, on slow motor actions, such as lifting a heavy weight from the floor, enough time will be available for maximum strength to be achieved, making it the preponderant component. As a reference, maximum strength usually takes at least 500–1500 ms to be achieved, sprinting and marathon running ground contact duration is 80–100 ms and 250 ms, respectively (Komi et al. 2003).

Strength endurance is defined as "the neuromuscular system's capacity to produce the greatest possible impulse summation under anaerobic and fatigue metabolic condition" (Frick 1993). Strength endurance is influenced by the impulse magnitude (maximum strength and explosive strength) and fatigue resistance (i.e., capacity to maintain impulse values as constant as possible during a certain amount of time). To differentiate strength endurance from aerobic endurance, Güllich & Schmidtbleicher (1999) proposed that a minimum of 30% of the maximum isometric strength is used on a muscle action. At this intensity level, muscle contraction lowers blood supply to the muscle (i.e., mechanically collapsing blood vessels) and therefore the muscle relies more on the anaerobic energy system. Both rapid strength and strength endurance can be manifested in the different muscle actions already described (i.e., concentric, isometric and eccentric) and also on a special type of muscle action called stretch-shorten cycle (Komi et al. 2003), in which an explosive concentric muscle action is preceded by a fast eccentric muscle action (e.g., countermovement jump).

Cross-sectional muscle strength comparisons between people with CP and TD controls have been extensively studied. Participants with CP have consistently lower maximum isometric, concentric and eccentric strength, and lower explosive strength than TD age and sex-matched counterparts (Barber et al. 2012; García et al. 2016; Geertsen et al. 2015; Hanssen et al. 2021; Moreau et al. 2012; Reid et al. 2010; Wiley and Damiano 1998). Muscle weakness in CP has both mechanical and neural contributions. Mechanical contribution includes muscle atrophy and overly stretched sarcomeres, which are at a disadvantageous length to produce force in its length-tension relationship (Hanssen et al. 2021; Lieber and Fridén 2019). The neural drive to generate joint torque is also hindered, both in its ability to selectively activate the agonists and synergist muscles, but also to inhibit the antagonist muscles (Gracies 2005b).

Interestingly, all above-mentioned studies comparing people with CP and TD controls tested rapid strength, and none tested strength endurance. Since both maximum strength and explosive strength were reduced in CP, it is reasonable according to Schmidtbleicher's strength model to assume that strength endurance in CP would also be lower than in TD controls. From a gross motor function perspective, it can be logically argued that gross motor function depends more on the explosive strength component than maximum strength as it is rarely necessary to reach the later during everyday motor activities (Tikkanen et al. 2013, 2016). Fatigue resistance is obviously an important component for gross motor function, since the repetition of movements at a lower strength level is necessary for playing, climbing stairs, etc. Even though maximum strength is not directly used on most everyday tasks, it positively affects strength endurance, and therefore it may be useful to be enhanced.

From a practical perspective, a strength training program for people with CP must consider some specific aspects, in addition to the training principles already discussed. Firstly, single joint and multi-joint exercises should be combined, as the former allow greater loading minimizing compensatory movements, whereas the latter offer a mixture of strength and motor control training that may be beneficial for motor function (Verschuren et al. 2011, 2016). To enhance autonomy and interaction within the training sessions, allowing the participants to choose the order of the exercises works well. However, the caveat is that the trainer must guide the choosing process complying with the general rule of avoiding fatigue build up, which could hinder execution quality and exercise intensity. Lastly, and perhaps most importantly, training sessions must be supervised, especially in the first months of training, for two main reasons. The first is that the muscle's ability to produce torque varies throughout the joint ROM due to the sarcomere force-length curve and the joint's lever arm (Lieber and Boakes 1988). Furthermore, as already discussed, CP joint ROM is often reduced (de Bruin et al. 2013) and muscle weakness is present (Maltais et al. 2014). Thus, allowing people with CP to perform isoinertial strength exercises (e.g., dumbbells, weight machines) by themselves leads to a movement with a smaller joint ROM compared to the total possible active ROM, consequently the non-optimum part of the torque-angle curve is not trained. Since strength gains are generally length-specific adaptations (Graves et al. 1989; Rhea et al. 2016), and increasing the torque production in the whole active curve seems very reasonable to allow functional benefits, an assisted training procedure should be adopted. The trainer needs to actively help the person on the concentric phase of the movement in the positions in which the person is not able to perform by himself. The exercise resistance can be selected based on the person's strength on the optimal angles, and the trainer needs to constantly assist on non-optimal angles. The eccentric phase can be performed unassisted, with the trainer providing constant feedback about movement velocity. Controlling eccentric velocity is an important aspect of motor control, since people with CP usually perform strength exercises with fast concentric bursts and have difficulty controlling slow eccentric muscle actions. The second reason for the importance of supervised sessions is to

monitor and control the training load progression, which is vital for a successful training program. Untrained people tend not to exert enough effort on strength training, and usually require someone to push them into the overload zone, and without it, the training program will most likely fail to induce significant adaptations.

2.3.3 Joint Flexibility

Joint flexibility is often defined as the maximum ROM of a given joint, representing the MTU's maximum extensibility. However, since torque must be applied to test MTU extensibility, other dimensions such as tension, cross-sectional area and time are invariably linked to this phenomenon. From these added dimensions, the biomechanical properties of stiffness (i.e., torque-angle derivative), energy (i.e., torque-angle integral), hysteresis (i.e., delta between loading and unloading energies), viscoelastic stress relaxation and creep can be derived (Weppler and Magnusson 2010). The skeletal muscle fibers are embedded into an extracellular matrix composed of a mesh of collagenous elements and macromolecules. The extracellular matrix is connected to the intramuscular connective tissues (i.e., endomysium, perimysium and epimysium), and plays an important role in lateral force transmission (Csapo et al. 2020). Due to the combination of both solid and fluid materials, muscles present a viscoelastic behavior. Elasticity implies that deformation (i.e., length changes) is directly proportional to the applied forces, and once tension ceases, the muscle returns to its initial length (provided that no plastic deformation occurred). Viscosity is characterized by time-dependency and rate change-dependency, where the rate of deformation is directly proportional to the applied forces (Taylor et al. 1990). Due to its viscoelastic nature, the skeletal muscle has two important acute responses to stretching exercises. The first is called stress relaxation and describes the drop in resistance torque when the muscle is stretched and held in a constant position. The behavior is both viscous (i.e., tension decreases over time) and elastic (i.e., some degree of tension is maintained over time). The second is called creep, and it is characterized by the continued increase in muscle length at a fixed tension load (Taylor et al. 1990).

Joint flexibility can be classified as passive and active (Roberts and Wilson 1999). Passive flexibility is the maximum ROM attained while both the stretched muscles and their antagonists are inactive). Active flexibility is the maximum ROM attained by the activation of the antagonists of the stretched muscles (e.g., knee extension ROM when knee extensors are actively rotating the joint). This distinction is important because passive flexibility describes the MTU's maximum extensibility, while active flexibility describes the strength capacity of the antagonist muscles to rotate the joint throughout the ROM. Considering gross motor function, both types of flexibility are important, for example, the lack of heel strike during gait may be due to reduced passive flexibility (i.e., triceps surae muscle extensibility is not enough to allow full dorsiflexion) and/or active flexibility (i.e., ankle dorsiflexors are not strong enough to sufficiently rotate the joint).

Stretching exercises can acutely increase joint flexibility and decrease joint passive resistance and stiffness (Avela et al. 1999; Morse et al. 2008; Weir et al. 2005). These changes are possible due to the viscoelastic behavior of the muscle (Taylor et al. 1990) and the alteration of the person's tolerance to stretch (i.e., how the nociceptive afferent inputs are handled by the nervous system; Magnusson et al. 1996). However, these acute effects are temporary and regress back to normal after 10–60 min (Avela et al. 1999; Palmer et al. 2022).

Due to the already discussed morphological alterations and muscle weakness, people with CP have lower active and passive joint flexibility as compared to TD controls. Despite the broad use of stretching to prevent or alleviate loss of joint flexibility in CP, its efficacy is unclear (Groppe et al. 2012; Kalkman et al. 2020; Pin et al. 2006; Walhain et al. 2021; Wiart et al. 2008). Longitudinal studies applying manual passive stretching therapies for 6–9 weeks have typically reported an increase in joint ROM with no mechanical changes (e.g., resting fascicle length), suggesting that increased stretch tolerance played a major role in the ROM increase (Hösl et al. 2018; Kalkman et al. 2019; Theis et al. 2015; Weppler and Magnusson 2010). Little is known about the effects of more prolonged stretching interventions (Weppler and Magnusson 2010), and it is possible that structural changes (e.g., sarcomerogenesis) require more time. The understanding of chronic adaptations to flexibility training is still incipient even in TD people (Chagas et al. 2016; Weppler and Magnusson 2010), and is further complicated in CP due to the developmental changes to motor drive and muscle physiology. Various studies have reported lower number of ribosomes and satellite cells in CP muscle (Gough and Shortland 2012; Lieber and Fridén 2019; Von Walden et al. 2018), and it has already been discussed that muscle growth is hindered as evidenced by the extremely stretched sarcomeres in muscles with contractures. Thus, there is an enormous gap of information regarding adaptations to flexibility training, and current understanding about CP muscle physiology suggests that it should have little capacity to adapt to the stretch stimulus.

One important hypothesis is that combining strength and flexibility training may be more effective. Kalkman et al. (2019) demonstrated that combined plantarflexors strength training and passive static stretching for children with CP was more effective in increasing resting fascicle length than solely stretching. The mechanistic explanation is that strength training increased tendon stiffness, which decreased the relative muscle stiffness, and thus increased the stretch stimulus to the muscle. Furthermore, it is reasonable to hypothesize that an active muscle that is constantly controlled by the central nervous system has a better chance to adapt than an inactive muscle receiving solely a stretch stimulus. Inactivity seems to be the main driver of disfunction in CP (Farmer and James 2001; Gracies 2005a), thus the paradigm of rehabilitation with the person laying down "receiving treatment", for example a passive muscle stretch, makes no sense with the current knowledge.

For functional motor tasks, evidently both active and passive flexibility are important, however there are practical limitations to be considered in CP. Due to

the usual increased joint stiffness and muscle weakness, active stretching exercises may be useful to increase muscle strength but will most likely be an inadequate stretch stimulus to the MTU. Inadequate because the active muscles are weak, and thus incapable of producing a high stretch intensity for an appropriate duration. Thus, in practice passive stretch is the easiest way to provoke adequate stretching stimulus in CP, although as discussed, its effectiveness to induce adaptations is currently under question. Another under-researched but promising modality is eccentric strength training, which combines both muscle activation and muscle stretch, theoretically being capable of adapting both physical capacities (i.e., muscle strength and joint flexibility). Eccentric muscle actions have a different motor control scheme when compared to concentric and isometric muscle actions (Duchateau and Baudry 2014; Duchateau and Enoka 2008, 2016; Valadão et al. 2018). Usually, eccentric muscle actions have less agonist muscle activation, which could lead to increased tension in the active muscle fibers, working as a more effective stretching stimulus. Finally, only mechanical adaptations to stretching have functional relevance for CP, and changes in the stretch tolerance offer no function benefit whatsoever. Thus, it is vital that future research establishes the effectiveness of the different stretching modalities and their combination with strength training to induce mechanical adaptations.

2.3.4 Gait Training

Booth et al. (2018) performed a systematic review and meta-analysis about the efficacy of functional gait training in CP. Joining 41 studies with a total sample of 619 children and young adults, only one study reported minor adverse effects relating to gait training: two children had minor leg discomfort which resolved without intervention, and one child had a blister on the foot. Improvements in walking speed and GMFM were widely reported, exceeding the clinically important threshold. Furthermore, the Danish research group led by Jens Bo Nielsen has reported increased walking speed, dorsiflexion strength and active ROM, and perhaps more importantly reduced ankle stiffness in only 4–6 weeks of training using an inclined treadmill (Lorentzen et al. 2017; Willerslev-Olsen et al. 2014). Thus, gait training for CP can be deemed safe and effective, with a level of evidence much greater than stretching and perhaps even than strength training.

Various gait training methods have been developed, which can roughly be divided into overground, treadmill, enhanced gait training (e.g., virtual reality, feedback, electrical stimulation, exoskeletons) and gait training with body weight support (Booth et al. 2018). Due to muscle weakness and limited joint flexibility, body weight support may be helpful, although assisted gait with exoskeletons seems much more promising as it is able to reinforce correct patterns of muscle activation. Since this technology is new, it is still unclear how the neuromuscular system will adapt to different stimuli reinforcing certain patterns and restraining others. From an evolutionary perspective, the neuromuscular system is always trying to use less energy. Thus, care must be taken to avoid the creation of new

patterns of muscle activation for gait, taking advantage of the external aid, instead of developing a functional activation pattern that can be used without it. Treadmill training is very convenient as it does not require a long pathway and can be performed at home. The inclined treadmill setup seems particularly effective, as it requires a greater ankle dorsiflexion in the swing phase and serves as a stretch for the ankle plantarflexors in the stance phase (Han et al. 2009; Leroux et al. 1999). Furthermore, a non-motorized treadmill can be theoretically beneficial, as the belt only moves if the person correctly performs hip extension torque. Oppositely, a motorized treadmill will continue to move regardless of the person pushing it correctly or not, which can be a potential downside.

Independently of the method used, perhaps the biggest problem in gait training is that it requires constant attention. Walking is by design an automated central nervous system function which requires very little attention to be performed. Verbal instructions to attain heel strike and to clear the foot during the swing phase quickly becomes tedious, and the person loses focus. As discussed above, the incline treadmill forces the person to dorsiflex during the swing phase, otherwise the person would hit his toes of the treadmill belt. However, what about the other possible important instructions that should be in the person's mind as he trains his gait? This is where virtual reality, electrical stimulation and different types of feedback can help, by providing constant feedback, visual goals, scores, and engaging the person in an immersive training environment. The challenge here is to use equipment that is affordable for clinics and even for individuals, as force plates and 3D motion capture systems are not affordable even to big hospitals. The combination of 3D goggles with a set of inertial measurement units could potentially provide an affordable alternative.

3 AIMS AND HYPOTHESES

The present thesis had two main aims. The first was to design (article I) and verify the effects of the multicomponent EXECP intervention on muscle strength, joint flexibility and motor function (article IV). The second aim was to perform a neuromechanical and neurophysiological comparison between participants with cerebral palsy and their typically developing age and sex-matched controls (articles II and III).

The specific aims and hypotheses were as follows:

1. To study the effects of the proposed latency correction on the stretch reflex threshold and tonic stretch threshold methods to assess hyperreflexia. (article II)

Hypothesis: A significant change in the stretch velocity-SRT regression slope would occur due to the latency correction, as it will necessarily shift higher velocity SRT to earlier joint angles. Although the change in the regression slope is predictable, it is impossible to predict how the regression line R^2 will change, and thus the adequacy of the TSRT method. Furthermore, it was hypothesized that the TSRT angle would not significantly change as the latency correction will have only a small effect on the lower velocity SRTs, and consequently will not change the y-intercept of the regression line considerably.

2. To compare neuromechanical responses to passive stretch and stretch reflex neurophysiological variables between CP and TD groups. Furthermore, correlations between the neuromechanical and neurophysiological variables were studied. (article III)

Hypothesis: Statistically significant differences between groups would be found for all studied variables. Furthermore, a good correlation between the neuromechanical variables of hyperreflexia and hyper-resistance, and a moderate correlation between ankle joint neuromechanical variables and

neurophysiological variables of hyperreflexia with the neuromechanical variables of hyperreflexia was expected.

3. To study the differences in muscle strength, joint flexibility and motor function between CP and TD groups. (article IV)

Hypothesis: CP participants would have significantly lower values for all studied variables compared to their TD controls.

4. To study the effects of the EXECP intervention on 1) lower limb and trunk concentric and isometric strength; 2) flexibility for the hip and knee joints and 3) motor function, measured by the six minutes walking test and the gross motor function measure. (article IV)

Hypothesis: The EXECP intervention would successfully increase performance in all studied variables.

5. To study the deadaptation process of the studied variables three months after the cessation of the EXECP intervention. (article IV)

Hypothesis: All variables would regress towards baseline values after the three-month retention period, not differing from control values.

4 MATERIALS AND METHODS

4.1 Participants

Recruitment was done throughout Finland by contacting hospitals, physiotherapy clinics, CP associations and online internet communities. A total of 20 children and young adults diagnosed with spastic CP, and 17 age and sex-matched TD controls volunteered for this study. Two participants with CP dropped out of the project: one during the control period, and the other at the beginning of the EXECP intervention, only the latter had usable pre-test data. Thus, the final sample with complete data was 18 CP and 17 TD, while for the cross-sectional studies (i.e., articles II and III) that used only pre-test data, the sample was 19 CP and 17 TD. The gross motor function classification system (GMFCS; Palisano et al. 2008) diagnostic was provided by the participant's neurologist, and confirmed by the author. None of the CP participants had lower limb surgery, serial casting, pharmacological treatments (except epilepsy medication, n = 3 and baclofen, n = 1) or had participated in a resistance-training program for the lower limbs in the past six months. All participants were able to stand with both heels touching the floor (i.e., ankle in anatomical position), had no contractures or bone deformities.

Study IV employed the full sample size, study II had 13 CP participants, and study III had 15 CP and 15 TD participants. Table 1 provides descriptive sample information for all three studies. The lower sample sizes on studies II and III were due to bad quality EMG recordings (n = 5 in article II and n = 2 in article III). Furthermore, one participant was ineligible to participate in both studies II and III due to a selective dorsal rhizotomy surgery performed one year prior to the study. Since both studies II and III have measurements related to the IA reflex arc, and the above-mentioned surgery interrupts this neural pathway, this participant could not be included. Nevertheless, this participant was eligible to take part in study IV since his scores for muscle strength, joint flexibility and gait

performance were within the range of the other CP participants. None of the TD participants had any musculoskeletal or neurological impairments.

A priori sample size calculation was performed for the project's main outcome variable, the distance walked in the 6MWT. Results from Pre-tests 1 and 2 for the first 9 participants in the project were used for sample size calculation using an online spreadsheet (www.sportsci.org; Hopkins 2006). Data was log-transformed due to the inexistence of zero and negative values, yielding a typical error of 1.06, between-subject standard deviation (SD) of 1.46 and a smallest important change of 1.08. Using the pre-post parallel groups randomized control trial (RCT) model, with a power of 0.8 and an alpha of 0.05, a sample size of 24 participants in both CP and TD groups was required. No specific sample size calculation algorithm for the present experimental design was found, thus the RCT calculation served as an upper bound for the sample size. Unfortunately, it was unfeasible to reach 24 participants in each group due to constraints of time and resources. The Covid-19 lockdown also influenced the study timetable.

Written informed consent to participate in this project was provided by participants and legal guardians of the underaged. Participants were informed that they could leave the project at any time, without any penalties.

TABLE 1. Participant characteristics in the cerebral palsy (CP) and typically developing (TD) groups in the three studies.

Participant characteristics	CP (n = 18) IV Mean ± SD (min–max)	CP (n = 15) III Mean ± SD (min–max)	CP (n = 13) II Mean ± SD (min–max)	TD (n = 17) IV & (n = 15) III Mean ± SD (min–max)
Male/female	13/5	10/5	8/5	12/5 & 10/5
Age (years)	14 ± 4 (9–22)	14 ± 4 (9–22)	13.5 ± 4 (9–22)	15 ± 4 (9–22) & 14 ± 4 (9–22)
Height (cm)	158 ± 14 (131–180)	159 ± 14 (140–180)	159 ± 12 (140–180)	161 ± 16 (133–188) & 159 ± 16 (133–180)
Body Mass (Kg)	51 ± 16 (29–81)	52 ± 16 (29–81)	52 ± 16 (29–81)	53 ± 17 (28–90) & 51 ± 15 (28–81)
LOI	Bi = 6 / Uni = 12	Bi = 2 / Uni = 13	Bi = 2 / Uni = 11	n/a
GMFCS	I = 14 / III = 4	I = 15	I = 13	n/a

CP = Cerebral palsy; TD = typically developing; SD = standard deviation; Min = minimum; Max = maximum; LOI = level of involvement; GMFCS = Gross motor function classification system; n/a = not applicable, Bi = bilateral (diplegia); Uni = unilateral (hemiplegia).

4.2 Experimental Design

The present study employed a nonconcurrent multiple-baseline design (Graham et al. 2012; Hawkins et al. 2007). The experimental CP group performed the

EXECP intervention and had four testing sessions every 3-months: 1) first pre-test (Pre1) at the beginning of the study, 2) second pre-test (Pre2) after the 3-month control period, 3) first post-test (Post1) after the 3-month EXECP intervention, 4) second post-test (Post2) after the 3-month retention period. During the control and retention periods normal physical activity and physiotherapy was permitted, however no structured physical training program was allowed. The intervention effects were assessed by comparing both pre-tests (Pre1, Pre2) with Post1. Since changes during the control period (between Pre1 and Pre2) represent the effects of normal development and activities (e.g., maturation, physiotherapy, sports), only Post1 and Post2 values that were statistically different from both pre-tests were deemed to have a significant intervention effect. Finally, Post2 was compared to Post1 and the pre-tests to verify the retention of the adaptations induced by the intervention.

The TD group performed only the two pre-tests interspaced by three months (Pre1, Pre2), and did not take part in the intervention. The Pre2 results were used in TD vs. CP group comparisons, because Pre2 had fewer missing data compared to Pre1. In articles II and III, data from the control period of both groups was used for cross-sectional comparisons. In article IV, all longitudinal data was used to study the effects of EXECP intervention on the physical capacities and motor function. Figure 8 depicts the experimental design.

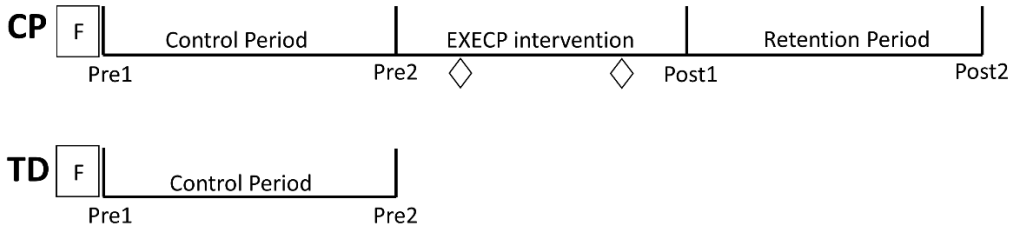

FIGURE 8 Experimental design. Within-group longitudinal analysis in the CP group had four time points for most variables (ankle and knee muscle strength and joint flexibility). The exceptions were as follows: 1) GMFM was measured only at Pre2 and Post1, and (2) trunk muscle strength was measured at the first and last intervention months (diamonds). For the TD group, GMFM and trunk strength were not assessed; thus, all variables were measured at the two time points (Pre1, Pre2). Both groups had a familiarization session (F) before Pre1. Between-group cross-sectional comparisons were done at Pre2.

The nonconcurrent multiple baseline design was selected over the RCT because the participants inside the inclusion criteria were expected to form a highly heterogeneous group regarding the studied variables (e.g., motor control, muscle strength, joint flexibility), and randomly dividing the sample into two groups was unlikely to result in two comparable groups. The chosen experimental design circumvents the expected high variability in the studied variables by allowing the subjects to be their own controls. Additionally, it allowed

performing the EXECP intervention all year-round, avoiding systematic seasonal influences to affect the study variables (e.g., vacations, seasons; Biglan et al. 2000).

The thesis author was responsible for all tests, and it was not possible to blind him to group allocation. Testing procedures were defined in detail to ensure consistency. The outcome data is quantitative and objective, with little possibility to suffer from observer bias. However, because the tester obviously hoped for better results in the Post-tests, no data analysis was performed before the data collection was done (except for the flexibility tests). Thus, the tester did not have any knowledge of the results and was focused on implementing the tests according to the protocol.

A positive ethical statement was granted by the ethics committee of the Central Finland Healthcare District (U8/2017). Furthermore, the project complied strictly with the declaration of Helsinki and was prospectively registered (ISRCTN69044459, 21/04/2017). Three minor protocol amendments were also approved (2018/2019/2021) by the ethics committee and the registered trial information was updated accordingly. The main changes were to: 1) increase the inclusion age range; 2) include the participant with selective dorsal rhizotomy, which was originally an exclusion criterion, 3) contact the EXECP participants and invite them to participate in the I-SENS sub-project. Finally, all analyses code, anonymous raw and processed data is available at the open science framework repository (https://osf.io/4kbjh/, DOI: 10.17605/OSF.IO/4KBJH).

4.2.1 EXECP project protocol article (I)

The EXECP project collected a vast amount of data from each participant, of which about 40% is presented in the current thesis, and the remainder will be published afterwards. Therefore, there was a need to publish a protocol article clearly stating the research questions, hypotheses, methods, and variables. The protocol article brought accountability and transparency to the research project, since all variables and hypotheses were declared a priori. Furthermore, since the EXECP project used a considerable amount of financial and infrastructure resources, it was important to have its benefits, risks, timeline, and procedures very well defined. Finally, by making available all raw and processed data, and analyses code, it is hoped to empower open-source, collaborative and transparent research reporting that is vital to increase quality and trust in research going forward.

4.2.2 EXECP intervention (IV)

The EXECP intervention consisted of 2–3 training sessions per week, depending on each participant's physical activity levels: those engaged in regular physical activity chose to perform 2 or 3 sessions weekly, while sedentary participants were encouraged to train three times per week. A minimum of 24 and a maximum of 36 sessions were enforced for all participants. Training sessions had 90 min duration and were separated by at least 48 h. Each session had the following components and order: treadmill walking (5–10 min), strength training

(60–75 min) and flexibility training (0–20 min; only for those with restricted flexibility). Furthermore, gait training at home was performed in addition to the participant's ongoing physiotherapy and other possible sport hobbies. All training sessions were individually supervised by a physiotherapist or strength and condition coach trained by the thesis author. At the start of each training session, the trainer asked the participant if any adverse events were experienced after the previous session. Participants were constantly reminded to provide immediate feedback about any pain or discomfort during the training sessions.

Gait training. A portable mechanical treadmill with an adjustable inclination of 6° or 7.3° (Vida XL, Venlo, Netherlands) was used for training. Participants were instructed to walk at a comfortable speed, avoid toe walking and try their best to attain heel strike. Verbal feedback was constantly given to improve gait quality, and the participant was allowed to rest at any time. In addition to 5–10 min of gait training in every session, all participants received a treadmill to take home, and were asked to walk a minimum of 10 min every day also at a comfortable speed, throughout the intervention duration. Weekly walking duration was logged on to a diary by the participants or their guardians and total gait training duration was calculated. Gait training progression was performed by increasing the treadmill incline and walking velocity and decreasing the rest periods during the training session. During the retention period, the participants chose if they wanted to keep using the treadmill at home and updating their diary or stop and return it.

Strength training. Five single-joint and five multi-joint strength exercises were divided into two training protocols performed weekly (i.e., AB or ABA/BAB) with a range of 5–10 exercises per session. This composition was chosen because single joint exercises allow greater loading control minimizing compensatory movements (Maltais et al. 2014; Verschuren et al. 2011). On the other hand, multi-joint exercises offer a mixture of strength and motor control training that may be beneficial for motor function. Table 2 describes the positioning, target muscles and kinesiology of each exercise.

Ankle plantarflexors were trained with seated and standing calf raises. Ankle dorsiflexors were trained using a rubber band resistance against the dorsiflexion movement. Additionally, dorsiflexion was also trained isometrically during a hip flexion exercise, in which the participant lay supine and flexed the hip against the rubber band resistance placed on the forefoot. Manual resistance could be used instead of the rubber band in case the trainer perceived it to be easier to apply the resistance during the entire ROM. The thigh muscles were trained using seated knee extensor and knee flexor machines. Additionally, lower limb muscles were trained using the leg press and squat. Squats were always performed with the participant holding a support for safety. Whenever necessary, a dense foam ball was used to prevent hip adduction during the lower-limb strength exercises. Exercises were done mostly unilaterally due to strength differences between limbs (80–100% of total training volume), except the squat which was mostly trained bilaterally. Bilateral training could be performed if exercise technique was correct and limb loading appeared similar; based on the

participant's perception of fatigue/effort and the trainer's visual evaluation. Trunk and hip flexors were trained isometrically with the hollow rocks exercise, in which the participant lay supine on the floor and lift the legs slightly above the floor (i.e., hip and trunk flexion, knee extension). Trunk extensors were trained isometrically using an inclined (30–45°) roman chair. To enhance autonomy, comfort and interaction within the training sessions, participants could choose the order of the exercises complying with the rule of avoiding fatigue build up, which could hinder both execution quality and intensity. More specifically, the rule was that exercises for the same muscle group always had to be separated by at least one different exercise (e.g., seated and standing calf raises could not be executed consecutively). The trainer guided this process and suggested possible sequences.

TABLE 2. Description of the strength training exercises.

	Seated Calf Raise	Standing Calf Raise	Seated Dorsiflexion	Seated Machine Knee Extension	Seated Machine Knee Flexion	Seated Horizontal Leg Press	Squat	Hip Flexion	Roman Chair Trunk Extension	Hollow Rock
Muscles targeted	Soleus, Gastrocnemius	Soleus, Gastrocnemius	Tibialis Anterior	Quadriceps femoris	Hamstrings	Gluteus maximus, quadriceps femoris, hamstrings, triceps surae	Gluteus maximus, quadriceps femoris, hamstrings, triceps surae	Iliopsoas, rectus femoris, sartorius, tensor fasciae latae, tibialis anterior	Erector spinae, multifidus	Trunk flexors, hip flexors, transversus abdominis
Initial Position	Seated with knees at 90°. Forefoot on a 10 cm step, ankle in maximum attainable dorsiflexion. Weight over the distal thigh of the training leg.	Standing with hips and knees at 0°, and the forefoot on a 10 cm step in maximal attainable dorsiflexion. Holding on parallel bars for balance*.	Hips at - 70–90°, knees at 0–20°, ankle in full plantarflexion. An elastic band on the forefoot resists the dorsiflexion movement.	Hips at -80°, knees at 115°. Knee and machine's lever arm center of rotation aligned. Lever arm positioned at the distal shank.	Hips at -80°, knees at 0–5°. Knee and machine's lever arm center of rotation aligned. Lever arm positioned at the distal shank.	Hips at -110–90°, knees at 80–100°. Feet and knees at hip width. 0–20° of hip external rotation.	Standing with hips and knees at 0°. Holding an adjustable support with both hands. Feet and knees at hip width. 10–20° of hip external rotation.	Supine position, arms laying by the side and both legs touching the mat. An elastic band on the forefoot resists hip flexion and ankle dorsiflexion.	Hips and knees at 0°, the chair is 45-90° inclined. Padded support at pelvis height. Distal posterior part of the shank locked against a padded support.	Supine position, hips and knees at 0°. Arms laying by the side and both legs touching the mat.
Kinesiologic Description	Unilateral or bilateral ankle plantarflexion.	Unilateral or bilateral ankle plantarflexion	Unilateral or bilateral ankle dorsiflexion.	Unilateral or bilateral knee extension.	Unilateral or bilateral knee flexion.	Unilateral or bilateral hip and knee extension, and ankle plantarflexion.	Bilateral hip and knee extension, and ankle plantarflexion	Unilateral or bilateral hip flexion and isometric ankle dorsiflexion.	Isometric trunk and hip extension.	Isometric trunk and hip flexion. Isometric knee extension.

Hip 0° = anatomical position (positive values = extension), Knee 0° = fully extended. *If the exercise was too hard, leaning on the bars and helping with the arms was allowed. If the exercise was too easy, it was done in the leg press machine.

The training load was adjusted monthly. In the first month, 3 sets of 8 repetitions maximum (i.e., the nineth repetition could not be attained), with a movement duration of 6 s (3 s concentric and 3 s eccentric) and 60 s of rest were performed. In the second month, the training volume was maintained while the intensity was increased by reducing the concentric movement duration to 1 s and increasing the rest to 90 s. In the third month, training volume and rest were maintained, but sets were increased to 4 while repetitions were decreased to 6, concentric muscle actions were done as fast as possible while eccentric movement duration was decreased to 2 s. Exceptionally, the squat exercise followed a different progression: 1 to 4 sets of 10 repetitions with the largest attainable range of motion were performed. Movement duration was like the other exercises, while rest started at 90 s and was decreased, when possible, to 60 s. After the entire volume could be executed with 60 s of rest, balance disks (Casall, Vantaa, Finland) were placed under the participant's feet to cause instability and increase exercise difficulty, also unilateral squats were used to further increase the exercise intensity. Table 3 provides an example for both protocols, and the load progression.

TABLE 3 Training progression (left), and an example of exercise list per training session (right)

Week	**Volume (Sets*reps)**	**Load**	**Movement Duration (s)**	**Rest (s)**	**Session A***	**Session B***
1–4	3 * 8	8 RM	3 concentric	60	1 – Seated calf raise	1 – Seated machine KF
			3 eccentric		2 – Seated dorsiflexion	2 – Seated machine KE
5–8	3 * 8	8 RM	1 concentric	90	3 – Standing calf raise	3 – Hip flexion
			3 eccentric		4 – Hip flexion	4 – Standing calf raise
9–12	4 * 6	6 RM	! concentric	90	5 – Seated LP	5 – Seated LP
			2 eccentric		6 – Roman chair TE	6 – Iso hollow rocks
					7 – Squat	7 – Squat

Reps = repetitions; RM = repetition maximum; ! = ballistic muscle action; * = minimum of 5 and maximum of 10 exercises; LP = leg press; TE = trunk extension; KF = knee flexion; KE = knee extension; Iso = isometric.

An important aspect of isoinertial strength training is that the ability to produce torque varies throughout the joint ROM, due to the sarcomere force-length curve and the joint's lever arm (Lieber and Boakes 1988). Furthermore, CP joint ROM is often reduced (de Bruin et al. 2013) and muscle weakness is present (Maltais et al., 2014). Thus, allowing CP participants to perform the strength exercises by themselves leads to a movement with a smaller joint ROM (as compared to the total possible active ROM), and the non-optimum part of the torque-angle curve is not trained. Since strength gains are generally higher at the trained angles (i.e., length-specific adaptations; Graves et al., 1989, Rhea et al., 2016) and increasing the torque production in the whole active curve seems very reasonable to allow functional benefits, an assisted training procedure was adopted. The exercise resistance was selected based on the participant's strength on the optimal joint

angles, and the trainer assisted during the concentric phase of the movement at the positions where the participant was not able to perform by himself. The eccentric phase was performed unassisted, and constant feedback about movement velocity was given.

Flexibility training. Four sets of 45 s manual passive-static stretching at the pain threshold (i.e., position where the participant acknowledges an initial stretch pain sensation) were performed for each muscle group diagnosed short in the pre-tests (please refer to section 4.2.7). The possible trained muscles were one and two-joint hip flexors, hip adductors and knee flexors. One and two-joint hip flexors were stretched in the modified Thomas test position (Harvey, 1998). The participant lay supine on a table holding one of the lower limbs in full hip flexion (assistance was provided when needed), while the other leg hung outside the table (i.e., hip extension). The only difference between the stretches was that the trainer applied the hip extension torque at the distal thigh with the knee joint positioned either in full flexion or in a relaxed position. The hip adductors were stretched with the seated butterfly stretch. The hamstrings were stretched in supine position, the trainer secured the untrained leg on the bench, then flexed the participant's hip to approximately -90° applying a knee extension torque at the posterior aspect of the shank.

4.2.3 Gross motor function (IV)

Gross motor function was assessed using the 66-item version of the Gross Motor Function Measure (GMFM; Russel et al., 2013), dimensions D (standing) and E (walking, running and jumping). The CP group was assessed at Pre2 and Post1, and the sum of the absolute scores of both dimensions were used for statistical analysis. The tests were video recorded and scored subsequently by the thesis author. Furthermore, the 6MWT was performed on an indoor 30 m track, and its result was the distance walked. The 6MWT was performed on all four timepoints (i.e., Pre1, Pre2, Post1, Post2).

4.2.4 Electromyography (II, III)

EMG activity was recorded from soleus (Sol) and medial gastrocnemius (MG) muscles with self-adhesive electrodes (Blue Sensor N, Ag/AgCl, 0.28 cm^2; Ambu, Ballerup, Denmark) following SENIAM guidelines (Hermens et al. 2000). A ground electrode was placed on the tibia. EMG signals were amplified (gain 1000) and high-pass filtered (10 Hz) by a preamplifier (NL824/NL820A; Digitimer, Welwyn Garden City, UK) and then band-pass filtered (20–195 Hz) off-line using Matlab software (v2020a, The Mathworks Inc, Natick, USA). The 20 Hz high-pass is suggested to offer the best compromise for optimizing the physiological informational content of surface EMG (De Luca et al. 2010), while the selected low-pass was chosen to eliminate high-frequency noise found in some EMG recordings (external laboratory noise).

4.2.5 Ankle and knee joint dynamometers (II, III, IV)

Two custom-built, motor-driven dynamometers (Neuromuscular Research Center, University of Jyväskylä, Finland) were used in the present thesis. The first dynamometer has a pedal with its axis aligned with the participant's ankle axis of rotation. The torque around the rotational axis of the motor was measured by a piezoelectric crystal transducer (Kistler Holding, Winterthur, Switzerland), and the ankle joint angle was monitored by a linear potentiometer. Furthermore, a small stiff metal wire attached to a spring system, located under the calcaneus, monitored heel displacement from the footplate. Torque, joint angle, and heel displacement were sampled at 1 kHz with a 16-bit A/D converter (CED power 1401, Cambridge Electronics Design, Cambridge, UK) using Spike2 software (v4, Cambridge Electronic Design, Cambridge, England). The participants were seated with hips flexed at -60° (anatomical position = 0°, positive values = hip extension), test knee fully extended (anatomical position = 0°) and test ankle joint in variable position depending on the test (details were described in the following pertinent sections). Participants were securely stabilized by an assembly of straps that fastened both shoulders and connected to a waist belt. An additional strap with a foam support prevented the tested leg knee joint from flexing. Figure 9 depicts the ankle dynamometer setup.

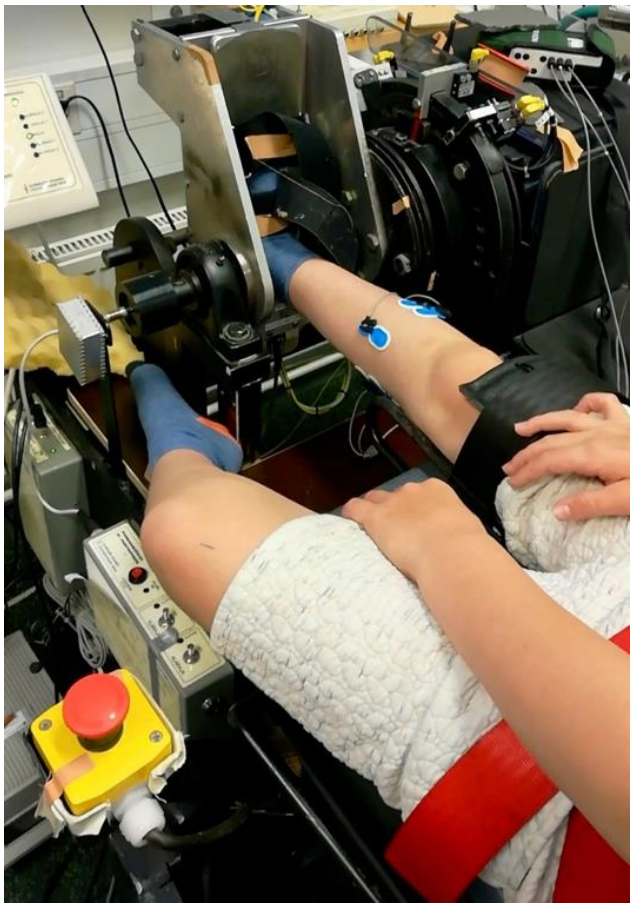

FIGURE 9 Ankle dynamometer setup.

The second dynamometer has a lever arm with its axis of rotation aligned with the participant's knee axis of rotation (Komi et al. 2000). A strain gauge capable of measuring both tensile and compressive forces is embedded on a cushioned hard plastic support that can be attached anywhere on the dynamometer's lever arm. The distal part of the participant's shank was secured with a velcro strap to the strain gauge. The distance from the dynamometer axis of rotation to the strain gauge (i.e., moment arm) was measured in each test. Torque was calculated by multiplying the moment arm by the force, and joint angle was monitored by a linear potentiometer. Both signals were sampled at 1 kHz with the same hardware and software described in the ankle dynamometer. Participants were seated with hips flexed at -80°, and the test knee joint fully extended or flexed at 105°. An assembly of straps fastened both shoulders and connected to a waist belt, securely stabilizing the participants. Figure 10 depicts the knee dynamometer setup.

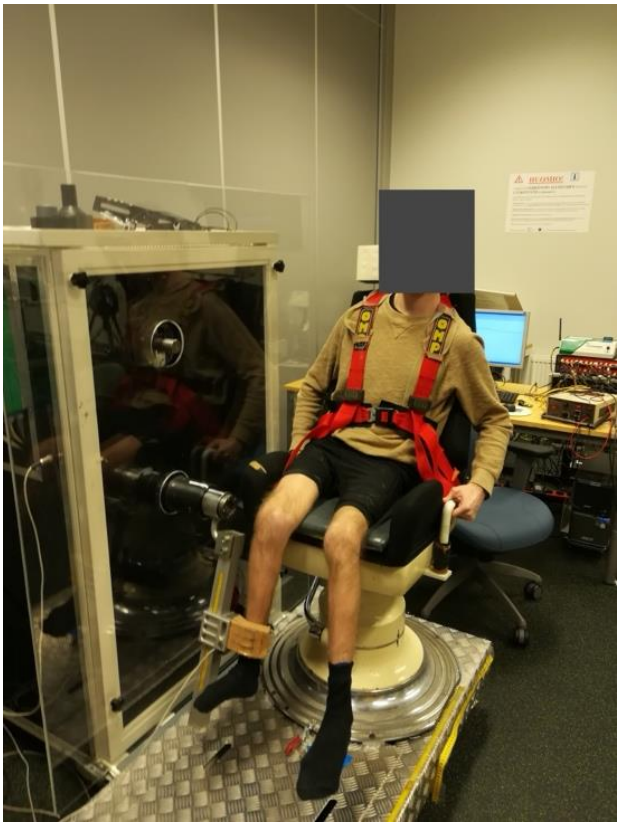

FIGURE 10 Knee dynamometer setup.

4.2.6 Muscle strength (IV)

Maximum isometric and concentric ankle plantarflexion and dorsiflexion were assessed using the ankle dynamometer. The plantarflexion test started with a 2 s maximum isometric muscle action at 0°, followed by an isokinetic ($14°\cdot s^{-1}$) concentric effort until 28°. The dorsiflexion test started with a 2 s maximum isometric muscle action at 28°, followed by an isokinetic ($14°\cdot s^{-1}$) concentric effort

until 0°. Three to five trials with 1–2 min of rest in between were performed for each test, and the highest value among all trials were used for the following variables: peak isometric torque, rate of force development, concentric angular impulse and curve width. Rate of force development was calculated from the onset of the muscle action (i.e., torque > 1 N·m) to 200 ms as delta torque divided by delta time. Concentric angular impulse was calculated as the torque-time integral. Curve width was defined as the concentric range of motion where the participant was able to exert continuously at least 50% of the peak isometric torque (Reid et al. 2010). No gravity correction was performed on torque data, since the passive delta torque between the two end positions (0° and 28°) was very small (0.5–2 N·m) due to the foot's small moment arm and weight; and because the CP participants had high variability in background muscle activity. During both plantarflexion and dorsiflexion, the maximum EMG activation, calculated as the highest RMS in a 200 ms sliding window during the entire isometric muscle action was obtained for normalization purposes.

Maximum isometric and concentric knee flexion and extension were assessed using the knee dynamometer. The knee flexion test started with a 2 s maximum isometric muscle action at 0°, followed by an isokinetic ($15°\cdot s^{-1}$) concentric effort until 75°. An examiner kept strong downward pressure at the distal part of thigh to prevent hip flexion during the trial. The knee extension test started with a 2 s maximum isometric muscle action at 75°, followed by an isokinetic ($15°\cdot s^{-1}$) concentric effort until 0°. Passive trials (i.e., passive joint movement) were used to measure torque caused by the weight of the leg, which was subtracted from the active torque curves. The same test procedures (number of trials and rest) and test variables described for the ankle joint tests were used for the knee joint tests. For all strength tests, a preparatory activity consisting of ten progressively stronger muscle actions, from 20 to 90% of the perceived maximum voluntary effort was performed before each strength test. Both ankle and knee dynamometers performed the target movement velocities with less than 2% variation throughout all trials in this study. All strength measurements were performed on the most affected leg for the CP group, and on the corresponding leg of the matching control participant. Participants were instructed to produce maximum isometric torque as fast as possible and maintain maximum effort throughout the concentric movement in each trial. Furthermore, visual feedback of the torque signal was provided in real-time, and participants received strong verbal encouragement during every trial. Figure 11 depicts the four studied variables.

Since both the trunk extension on the roman chair and hollow rocks exercises were trained isometrically, and both time and weight recorded in every training session, the best performance in the first training month (to avoid learning effects) and in the last month were used to evaluate strength gains in these muscle groups. Best performance was measured in time, as the duration of the isometric muscle action (maximum 60 s), and intensity, as the maximum weight held during the isometric muscle action. Once the participant was able to hold a given weight for the maximum duration, the intensity was increased.

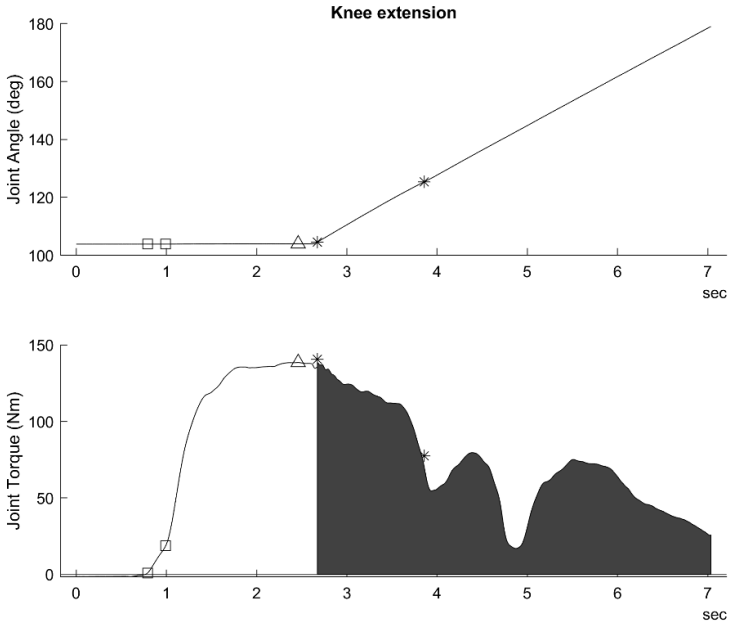

FIGURE 11 Example of the muscle strength variables analyzed in the knee extension test. From left to right: 1) rate of force development was calculated as the slope between torque onset (> 1 N·m) and torque onset plus 200 ms (two squares); 2) peak isometric torque (triangle); 3) concentric angular impulse (shaded dark area); 4) curve width is the concentric range of motion that is above 50% of the peak isometric torque (range between the two asterisks). Concentric movement onset is marked by the first asterisk.

4.2.7 Flexibility and ankle joint neuromechanical properties (III, IV)

Hip flexors flexibility was tested using the modified Thomas test position described in the flexibility training section (4.2.2). An assistant kept a goniometer with its fulcrum at the greater trochanter of the femur and held the stationary arm perpendicular to the bench. The examiner placed the goniometer's moving arm aligned to the lateral femoral condyle, and slowly pushed the distal aspect of the thigh towards hip extension and once the participant acknowledged an initial stretch pain sensation, read the resulting joint angle in the goniometer. Participants with less than 20° of hip extension were diagnosed with short hip flexors.

Knee flexors flexibility was assessed with the passive knee extension (PKE) test. PKE was performed in the same position as the hamstring stretch with the participant lying supine with the hip and knee flexed at right angles. The examiner slowly applied torque at the posterior aspect of the shank causing knee extension, while an assistant maintained strong downward pressure on the untested thigh to stabilize the pelvis. The test stopped when the participant acknowledged an initial stretch pain sensation, and the examiner read the goniometer angle (fulcrum: lateral femoral condyle, reference lines: lateral malleolus and greater trochanter of femur). Three measures per leg for each test were performed, and the mean value was used for statistical analysis.

Participants with more than 40° of knee flexion in the PKE test were diagnosed with short knee flexors.

Hip adductors flexibility was not directly assessed but inferred based on the test leg position on the modified Thomas test without the examiner pushing the hip into extension. Once the test position was attained, that is, the control leg held in full hip extension and test leg hanging outside the bench, the examiner placed the goniometer's fulcrum at the anterior-superior iliac spine, reference lines using the superior aspect of the patella and a line parallel to the bench. If the test leg presented hip adduction, hip adductors were deemed short. Similarly, the differentiation between 1-joint and 2-joint hip flexor flexibility was also assessed in the same position as the hip adductors. The knee angle was measured with a goniometer with the same position as in the PKE test. If the knee angle was smaller than 67°, two-joint hip flexors were deemed short. A limitation of the knee angle to access two-joint hip flexors flexibility is that it is affected by the sagittal hip angle. Thus, the knee angle was also measured with the thigh of the tested leg supported on the bench, instead of hanging outside of it. This second measure ensured that all participants had their hip at neutral position. Importantly, it should be noted that only the hip flexors flexibility test with the examiner pushing the leg into hip extension and the PKE test are actual joint flexibility tests, as attaining the maximum ROM requires unlimited torque and the subject acknowledging stretch sensation pain. The measurements used to assess the hip adductors and 1- and 2-joint hip flexors are the result of the interaction between gravity and the joint's passive resistance to stretch. For example, a stiffer joint will not yield much to the gravity force, and the reduced ROM cannot be mistakenly interpreted as poor flexibility. Nevertheless, substantial hip adduction and knee extension in the Thomas test position should be indicative at least to a moderate degree of low flexibility, as joint stiffness by itself is very unlikely to explain completely these deviations. Finally, the decision to use this non-optimal evaluation was based on the trade-off between usefulness of information and time spent measuring. The used knee and hip angles could be assessed within a few minutes, although with the limitations described above. An accurate flexibility measurement for hip adductors and true differentiation between hip flexors would have consumed considerably more time, required more instrumentation and assistant help.

The slow passive dorsiflexion test using the ankle dynamometer assessed ankle plantarflexors flexibility. Participants were seated with their hips flexed at -60°, test ankle at 35.5° of plantarflexion and test knee fully extended. Plantarflexors flexibility was determined by the examiner manually rotating the dynamometer ($< 10°\cdot s^{-1}$) towards ankle dorsiflexion until one of the following criteria was reached: 1) the participant acknowledged an initial stretch pain sensation; 2) there was a heel raise larger than 5 mm; 3) the maximum dorsiflexion angle of 21° was reached. This maximum angle was set to ensure participant safety during the subsequent fast cyclic dorsiflexion test. The examiner visually verified Sol and MG EMG to confirm that the participant was relaxed before starting the stretch.

The slow passive dorsiflexion test also assessed two other neuromechanical variables: passive ROM and passive torque. An EMG onset detection algorithm applying the approximated generalized likelihood principle (Lee et al. 2007; Staude et al. 2001; Staude and Wolf 1999) was used to detect muscle activation during the passive dorsiflexion. Visual inspection was used to identify false positives and negatives generated by the EMG onset detection algorithm. In these cases, muscle activation onsets were manually marked when a sustained increase (> 100 ms, > 2 SD) in the EMG-RMS signal was identified. Passive ROM was defined as the ROM from the start position (35.5° of plantarflexion) to the first EMG onset (i.e., either Sol or MG muscles). Passive peak torque was determined as the maximum joint torque in the passive ROM. Three to four trials were performed per testing session, and the trials with the largest maximum ROM and passive ROM were used for analysis. Figure 12 depicts the signals recorded during the slow passive dorsiflexion test.

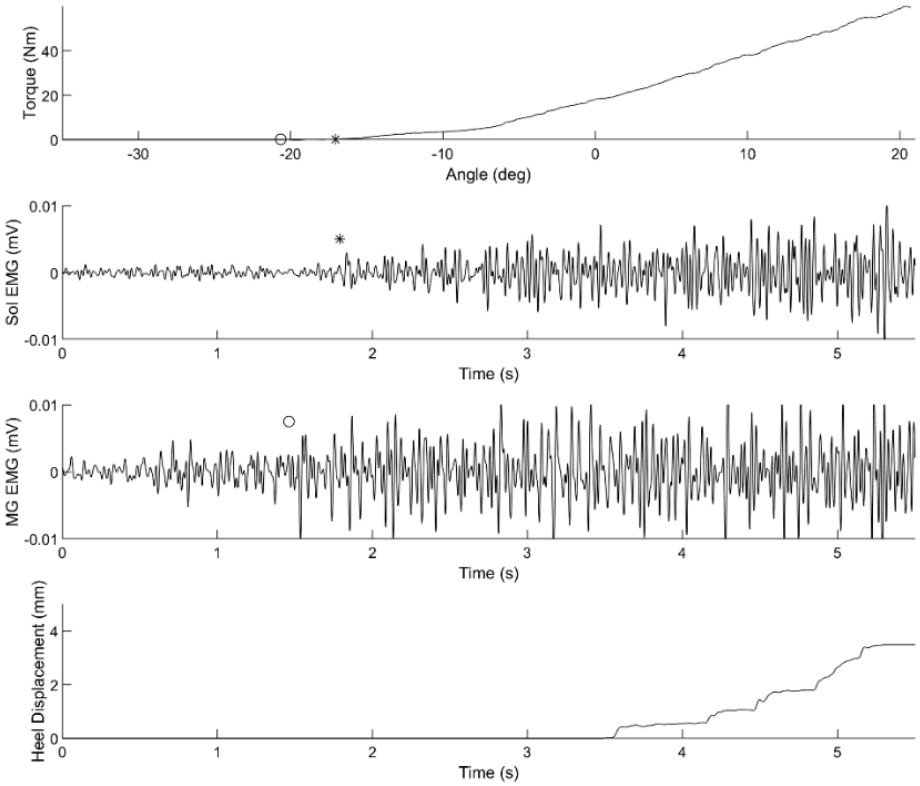

FIGURE 12 Example signals recorded during the slow passive dorsiflexion test of a participant with cerebral palsy. Top graph: ankle joint angle vs. joint torque. Plantarflexion over anatomical joint position is depicted as negative values in the x-axis, and the x-axis is not linearly related to time in the x-axes of the lower graphs. Passive range of motion (ROM) was assessed from the start of the movement until the first identified EMG onset (asterisk- soleus, circle-medial gastrocnemius-MG). Passive peak torque and peak torque were the highest torque found in the passive ROM and in the entire ROM, respectively. Middle graphs: Soleus and MG electromyography. Bottom graph: heel displacement. In this example, maximum ROM assessment could not be determined, since the end ROM was reached without the participant asking to stop and heel raise stayed below 5 mm.

The choice of neuromechanical variables took measurement complexity into account. Maximum ROM is the simplest and perhaps most used clinical measure of MTU length. Passive ROM describes the length-dependent increase in background muscle activity and requires monitoring EMG and joint angle. Passive peak torque represents the MTU's maximum resistance to the stretch while the muscle is inactive, requiring the assessment of EMG and joint torque. Given that both passive ROM and passive torque require more complex instrumentation and expertise, it is important to verify whether these variables are useful in explaining hyper-resistance and hyperreflexia.

4.2.8 Hyper-resistance (III)

HR was quantified as the peak torque during both slow passive and fast cyclic dorsiflexions. However, the fast cyclic dorsiflexions generated inertial artefacts at the beginning and end of the stretch in all velocities (~50 ms in total). Thus, due to the short stretch duration, peak torque could not be computed in the 210 and 291°·s^{-1} stretches. HR was defined as the peak torque in a common ROM of 12.5° (16–3.5° of plantarflexion, Figure 13) during stretches at 55 and 110°·s^{-1}. Mean peak torque was calculated over all stretches in each velocity. Additionally, peak torque in the same common ROM was calculated in the slow passive dorsiflexion test, for the trial with the largest passive ROM (i.e., participant's most relaxed trial).

4.2.9 Hyperreflexia (II, III)

4.2.9.1 Neuromechanical variables

Hyperreflexia was assessed in both Sol and MG muscles using three variables: the SRT, the SR EMG response amplitude (henceforth SR EMG-RMS for brevity), and the TSRT. The fast cyclic dorsiflexion test used the ankle dynamometer to induce passive ankle dorsiflexions from 20° of plantarflexion to 0° at four angular velocities: 55, 110, 210, and 291°·s^{-1}. Ten stretches in each velocity were delivered in a pseudo-randomized and balanced order every 2.6–2.9 s. Participants were instructed to relax and wore noise blocking headphones. Moreover, trials with Sol or MG EMG-RMS computed over a 200 ms sliding window exceeding 5% of the maximal isometric plantarflexion (section 4.2.6) in the 500 ms preceding the stretch were discarded. The same EMG onset detection algorithm and methodology described in the slow dorsiflexion test (section 4.2.7) was used to detect the SR EMG onset.

The SRT has been widely used in hyperreflexia research and it is defined as the joint angle at the SR EMG onset. On article II we proposed a correction to the SRT by considering the latency between the initiation of the stretch reflex at the muscle spindles and its appearance in the EMG signal (i.e., stretch reflex latency). Thus, the $SRT_{corrected}$ was calculated as the joint angle at the SR EMG onset minus the individual H-reflex latency time. For example, if the Sol SR EMG onset happened 125 ms after the stretch onset and the participant's Sol H-reflex latency

is 25 ms, SRT is the joint angle 125 ms after the stretch onset, whereas $SRT_{corrected}$ is the joint angle 100 ms after the stretch onset. The median SRT and $SRT_{corrected}$ values for each subject at each stretch velocity were calculated for statistical analysis. For group comparisons (i.e., CP vs. TD, article III), $SRT_{corrected}$ was used as an absolute joint angle and also normalized by ankle maximum ROM (i.e., SRT divided by participant´s ankle maximum ROM, section 4.2.7). Thus, while article II compares SRT and $SRT_{corrected}$, article III only uses $SRT_{corrected}$.

SR EMG-RMS was quantified as the EMG RMS in a 50 ms window from the SR onset onwards for both muscles. SR EMG-RMS was normalized by the maximum isometric plantarflexion RMS calculated in a 200 ms sliding window (section 4.2.6). Figure 13 depicts one stretch at 55° with the analyzed variables.

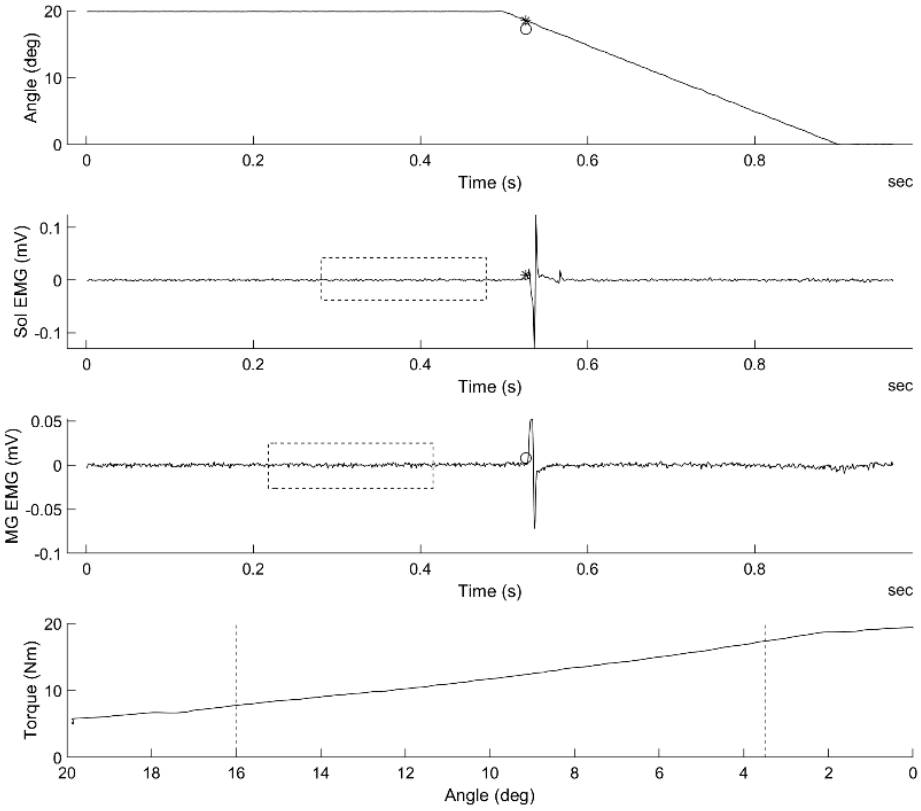

FIGURE 13 Example of signals recorded during the fast cyclic dorsiflexion stretch in a participant with cerebral palsy. Top graph: Stretch reflex threshold is the joint angle at the time of EMG onset minus H-reflex latency time (asterisk, soleus-Sol and circle, medial gastrocnemius-MG); Middle graphs: SR EMG-RMS is calculated in a 50 ms RMS window from EMG onset (asterisk and circle); pre-stretch EMG is the highest 200 ms sliding RMS window in the 500 ms preceding the stretch (dashed rectangles). Bottom: peak torque is the highest torque value found in the common range (16–3.5° of plantarflexion, dashed lines, also depicted in the top graph) of the 55 and 110°·s^{-1} stretch velocity conditions. Please note that x-axis of the bottom graph is not linearly related to time x-axes of the upper graphs.

TSRT and $TSRT_{corrected}$ were calculated as the y-intercept of the regression lines between stretch velocity and SRT or $SRT_{corrected}$, respectively. Since the stretch

velocity is the independent variable and the SRT the dependent variable, the former has been assigned to the x-axis and the latter to the y-axis, which is the opposite of how this data has been presented in previous studies (e.g., Blanchette et al. 2016; Calota et al. 2008). Thus, although the calculation of the R2 values is the same between studies, the regression slopes are different, and the TSRT in the present study is the y-, rather than x-intercept. Figure 14 depicts an example of TSRT and $TSRT_{corrected}$ calculation.

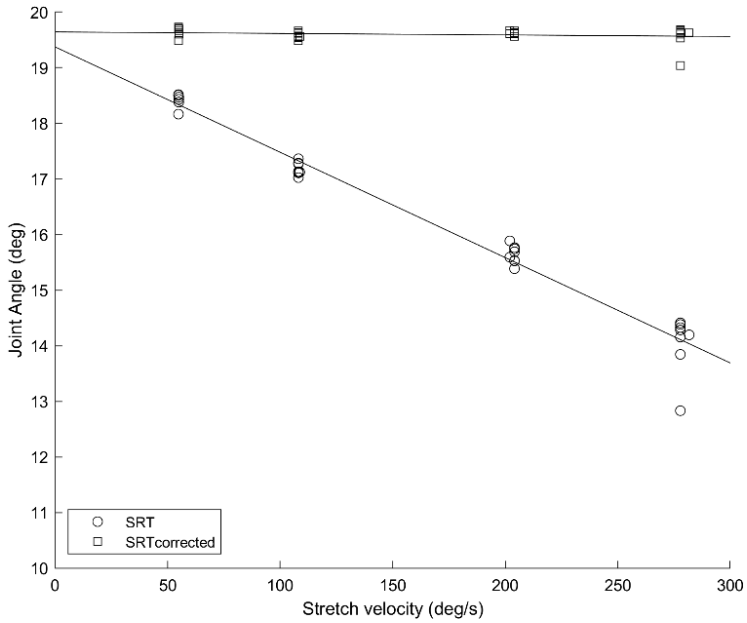

FIGURE 14 Soleus muscle SRTs (circles) and $SRTs_{corrected}$ (squares) in repeated trials at four stretch velocities for a representative participant. The error caused by not considering the SR latency increases with increasing velocity, as the amount of angular displacement between the SR onset and the SR EMG onset is increasing.

4.2.9.2 Neurophysiological variables

H-reflexes and M-waves were evoked in Sol by percutaneous electrical stimulation of the tibial nerve, with the subject lying in prone position, which was chosen to facilitate relaxation. A single rectangular pulse (1 ms) was delivered using a constant-current stimulator (DS7AH; Digitimer, UK). A circular cathode (White Sensor 4535M, Ag/AgCl; Ambu, Ballerup, Denmark) was placed over the tibial nerve on the popliteal fossa, and an anode (V-trodes; Mettler Electronics, Anaheim, USA) was placed above the patella. The full recruitment curve was attained by starting with a stimulation intensity of 3 mA and increasing in 0.5–2 mA steps at 0.1 Hz. Smaller steps were used closer to the Hmax, and stimulation intensity increased until the Mmax was reached. Peak-to-peak values of Hmax and Mmax were used to compute their ratio (Hmax/Mmax) as a measure of IA arc excitability. H-reflex latency for both muscles was visually determined as the duration between the electrical stimulus and the initial deflection of the H-reflex on the EMG signal. The PAD protocol was performed

immediately after the recruitment curve was defined, and the participant remained in the same position. First, stimulation intensity was adjusted to evoke H-reflex responses corresponding to 75% of Hmax at 0.1 Hz, then 16 stimuli at 0.1 Hz and 16 stimuli at 1 Hz were performed consecutively. These stimulation frequencies were chosen because Sol PAD has a long-lasting effect up to 10 s (Crone and Nielsen 1989; Pierrot-Deseilligny and Burke 2005). Peak to peak amplitude of H-reflexes were normalized by their preceding M-wave (H/M) and the median H/M for 0.1 and 1 Hz stimulation frequencies were calculated separately. PAD was defined as the 1 Hz/0.1 Hz ratio, with a higher ratio meaning lower PAD). Outliers in the H/M data, defined as values outside 2.5 scaled median absolute deviations were typically caused by brief muscle actions, and were automatically removed in the pre-processing stage. Two control variables were computed: the ratio between the evoked H-reflexes in the 0.1 Hz frequency and Hmax (H/Hmax), and the ratio between the preceding M-waves and Mmax for each stimulation frequency (M/Mmax). H/Hmax was used to ascertain that the H-reflexes were on the ascending limb of the recruitment curve in both groups. M/Mmax is the effective stimulation intensity for each stimulation frequency and must be the same within each group.

4.2.10 Theoretical framework – correlation matrices (III)

Figure 15 summarizes all tests and study variables on the left, and the theoretical framework linking the study variables on the right. Neuromechanical variables of hyperreflexia should have a good positive correlation with HR, since early and strong SR responses should add resistance against the stretch. Ankle neuromechanical variables should have a poor to moderate negative correlation with HR, mainly because the MTU physiological cross-sectional area and the tissue properties will act as confounding variables. Furthermore, the ankle neuromechanical variables should also have a poor to moderate negative correlation with the neuromechanical variables of hyperreflexia, since the former joint-level variables do not capture the actual muscle-tendon dynamics. Lastly, the neurophysiological variables of hyperreflexia should modulate both hyperreflexia and ankle joint neuromechanical variables (except for maximum ROM).

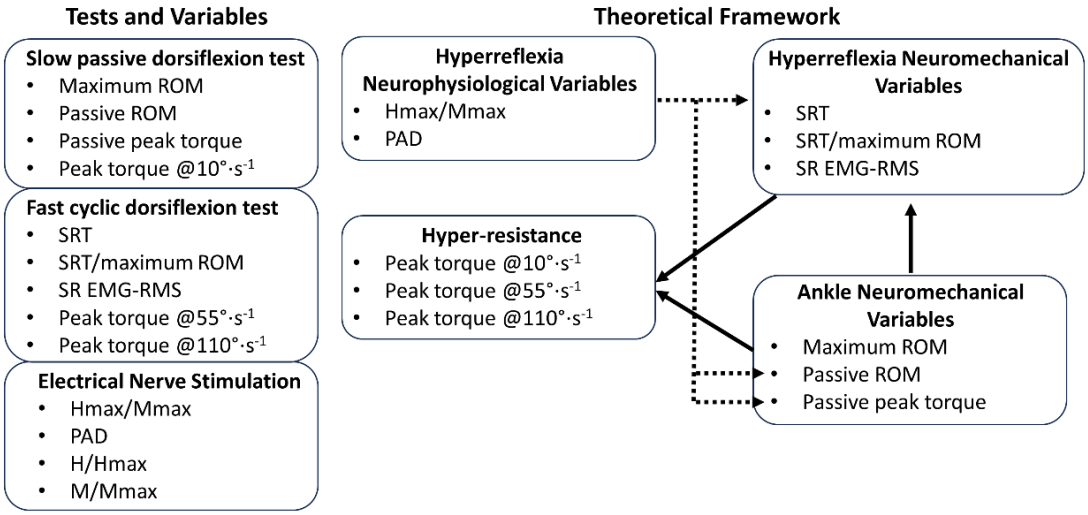

FIGURE 15 Left: tests and variables. Right: the theoretical framework for the correlations studied is depicted. ROM: range of motion; SRT: stretch reflex threshold; SR: stretch reflex; EMG: electromyography; RMS: root mean square; Hmax/Mmax: maximum H-reflex normalized by the maximum M-wave; PAD: post-activation depression ratio; H/Hmax: H-reflex normalized by the maximum H-reflex; M/Mmax: M-wave normalized by the maximum M-wave.

4.2.11 Maturation and test-retest reliability (IV)

Maturation. Due to the 9-month study duration and a sample composed mainly of children, the growth spurt could be a confounding factor in the present experimental design. The estimated time from peak height velocity (i.e., maturity offset, MO) was computed for all participants at each testing point using sex-specific equations provided by Moore et al., (2015):

Girl's MO = -7.709133 + (0.0042232 · (age · height))
Boy's MO = -7.999994 + (0.0036124 · (age · height))

For all available datapoints (i.e., participants multiplied by tests minus data loss: 100 out of 106), only in 2 tests the participants had a MO smaller than six months, in 8 tests participants had MOs of 6–12 months, and in all remaining 90 tests the participants had MOs larger than one year. Thus, for the current sample, the growth spurt was not an issue and MO was not included as a covariate in the statistical model.

Test-retest reliability. Before the first test timepoint (i.e., Pre1), all participants performed a 60 min familiarization session to experience all strength and flexibility tests, and the 6MWT (not necessarily the whole test). Whenever feasible, the familiarization session was used to duplicate tests to allow comparison with Pre1 for test-retest reliability assessment. However, due to limited time in the familiarization session, not many tests could be performed, and only variables with a minimum of 5 participants were used for this analysis.

The two-way mixed effects absolute agreement intraclass correlation coefficient (ICC 3.1/3.k, Koo and Li 2016; Weir 2005) was calculated between tests (i.e., familiarization vs. Pre1) for each group, flexibility data from both legs were pooled.

4.3 Statistical analyses

Articles II and III. Data normality and equality of variances were tested with Shapiro-Wilk and Levene's tests, respectively. The two-sided paired t-test and the non-parametric analog Wilcoxon signed rank test were used to perform within-participant comparisons (articles II and III). The two-sided independent t-test and the non-parametric analog Wilcoxon rank sum test were used to test the differences between CP and TD groups (article III). The Friedman test with a Bonferroni post hoc test was used to check differences between the four stretch velocities in the fast cyclic dorsiflexion test for each group (articles II and III). Two correlation matrices using Spearman's rank correlation coefficient were used the evaluate the linear relationship between the studied variables that were statistically different between CP and TD groups, following the theoretical framework in Figure 15. Bonferroni correction for multiple comparisons was used to adjust alpha for each matrix. Correlation coefficients were interpreted as poor (< 0.2), fair (0.21–0.41), moderate (0.41–0.6), good (0.61–0.8), and very good (> 0.8) following the guidelines from Altman (1999). Confidence intervals (95% CI) for the true population mean, and effect size between group means (Hedge's g) were calculated for parametric data.

Article IV. Generalized linear mixed models (GLMM) were used to compare the four timepoints (i.e., Pre1, Pre2, Post1, Post2) in the CP group. Time was set as a fixed factor, and the subject intercept as a random factor, thus participants had different y-intercepts but similar regression slopes. The GLMM model used the unstructured correlation structure and the identity link function, and two distributions were tested: normal and gamma. The best distribution for each dependent variable based on the Akaike information criterion and the residuals Q-Q plot was used. Theoretically, higher motor function should be associated with higher 6MWT and muscle strength performance. Thus, for these variables, the absolute Pre2 GMFM score ($GMFM_{co}$) was used as a covariate, centered and conditioned to the mean ± 1 SD. For the statistical analyses, three participant subgroups based on $GMFM_{co}$ thresholds were created: high (> mean + SD), mid (mean ± SD) and low (< mean - SD) scores. The effects of time, $GMFM_{co}$ and their interaction were tested. For the flexibility tests, in addition to time as a fixed factor, the muscle training status (i.e., trained or not trained during the intervention) was also included, and the interaction between time and training status was verified. The amount of stretching performed during the intervention was not used as a covariate because the participants were allowed to maintain their stretch routines throughout the project duration and keeping track of it through the nine months was not feasible.

The ICC_{GLMM} (i.e., subject variance divided by subject plus residual variance) was calculated for each dependent variable to verify the percentage of the total variance that was explained by the participants (i.e., random effect, different $\beta 0$ for each participant). The total number of gait training minutes, and the total number of strength and stretch exercises were tested as covariates to improve the model, since more training volume could translate into better adaptation of the dependent variables. However, none of these variables were successful in improving the models, and therefore were removed. Bonferroni post hoc and simple effect tests were used to find the specific differences between tests. Since it was hypothesized that most changes would occur between pre-tests and Post1, while most likely nothing would happen between pre-tests and between post-tests, simple effect tests for the interaction term were performed regardless of the omnibus main effect result. Only pre-post differences in which the post-tests were significantly different than both pre-tests were reported and discussed. The rationale for this decision was that only effects that were statistically different from the control period (i.e., Pre1–Pre2), which has its inherent variability, are statistically meaningful. GLMM results are presented as parameter estimates (β), 95% CI, standard error (SE), and the p-value. Descriptive data is presented as mean and standard deviation or median and interquartile range (IQR) depending on its distribution. The paired t-test or the non-parametric analog Wilcoxon signed rank was used to compare a) the two timepoints (Pre1 and Pre2) in the TD group; 2) the CP GMFM values between Pre2 and Post1; 3) the trunk strength variables between the first and last intervention month. Independent t-tests or the non-parametric analog Mann-Whitney U test was used to compare Pre2 between groups. Data analyses was performed on Matlab (v2020a, The Mathworks Inc, Natick, USA) and statistical analyses on jamovi 2.3 software (The jamovi project, https://www.jamovi.org).

5 RESULTS

5.1 Research quality overview

5.1.1 Training compliance and side effects

The participants with CP performed 24–36 (mean = 29, SD = 4) training sessions, which contained 360–1984 min (mean = 683, SD = 352) of gait training (i.e., supervised sessions plus home training), and 32–96 min (mean = 67, SD = 16) of stretching in the training sessions. Most participants also stretched the same muscles at the physiotherapy or at home. Only five participants chose to continue gait training in the retention period, reporting 600 min of training, 10 min per day as instructed.

In a total of 529 training sessions, there were 12 complaints of acute knee pain (2%) by four participants, which subsided in the same day. Furthermore, 2 participants reported moderate muscle soreness 3 times, and 1 subject reported high muscle soreness once, and had to recover for a week before restarting training. Finally, one participant reported knee pain during the first week of home inclined treadmill training, which subsided after online consulting was done to correct the gait movement pattern.

5.1.2 Data loss

There was no data loss in article II, as only participants with the full dataset were included in this study. In article III, considering all tests and participants, data loss occurred due to heel sensor malfunction (n = 2), low quality EMG (n = 4), torque mechanical artefacts (n = 5), and one participant declined to perform electrical nerve stimulation. The exact sample size of each test is displayed in the result tables. In article IV, two 6MWT in the CP group (one Pre2 and one Post1) were excluded from the analysis due to non-compliance with test execution

guidelines. Due to the Covid-19 lockdowns, six participants with CP were unable to perform Post2. Furthermore, three participants started the intervention 3 months after Pre2, and one started 5 months after Pre2. Thus, it is conceivable that this lower physical activity period lowered some study variables below the pre-test values.

5.1.3 Test-retest reliability

Figure 16 presents ICC values and 95% CI for all repeated measures between the familiarization and Pre1 testing sessions. Furthermore, table 4 presents the standard error of mean (SEM) for the same variables. The CP group had the following sample sizes: plantarflexion peak torque and rate of force development (n = 8), other plantarflexion and all dorsiflexion variables (n = 5), and PKE (n = 16). The TD group had the following sample sizes: all plantarflexion and dorsiflexion variables (n = 11), 6MWT and all knee extension and flexion variables (n = 7), and PKE (n = 27). Due to the slower familiarization process in the CP group (e.g., mobility and positioning), the 6MWT and knee extension and flexion tests could not be performed in the familiarization session due to time constraints. Most knee extension and plantarflexion variables, PKE and 6MWT had excellent to moderate reliability, while dorsiflexion and knee flexion had a wider 95% CI ranging from excellent to poor reliability (Koo and Li 2016). Curve width reliability for all four muscle actions was very poor.

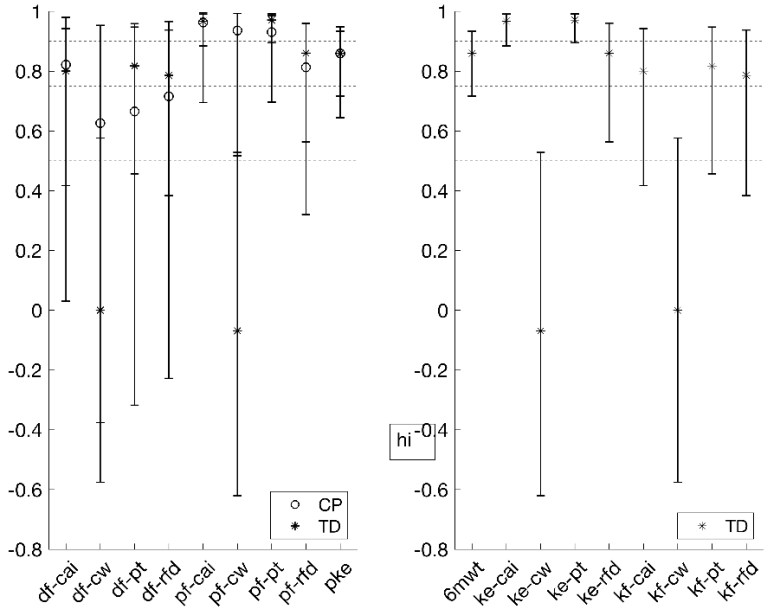

FIGURE 16 Intraclass correlation coefficient and 95% confidence intervals for successfully replicated study variables in both groups (left panel) and only in TD (right panel). The top right corner displays reliability thresholds (Koo and Li 2016). df = dorsiflexion; pf = plantarflexion; ke = knee extension; kf = knee flexion; cai = concentric angular impulse; cw = curve width; pt = peak torque; rfd = rate of force development; pke = passive knee extension; 6mwt = six minutes walking test.

TABLE 4. Standard error of mean for all replicated variables.

Muscle Action	Peak Torque CP / TD SEM (SEM/Mean)	RFD CP / TD SEM (SEM/Mean)	CAM CP / TD SEM (SEM/Mean)	Curve width CP / TD SEM (SEM/Mean)
PF	10 (13%)/12 (8%)	19 (29%)/91 (23%)	12 (20%)/20 (10%)	2 (19%)/5 (22%)
DF	6 (30%) / 8 (21%)	31 (71%) / 24 (18%)	6 (44%) / 12 (21%)	6 (54%) / 3.1 (11%)
KE	nd / 13 (11%)	nd / 74 (19%)	nd / 27 (8%)	nd / 3 (5%)
KF	nd / 6 (9%)	nd / 45 (29%)	nd / 20 (20%)	nd / 3 (7%)
Test	CP	TD		
6MWT	nd	23 (3%)		
PKE	5.5 (16%)	4.7 (36%)		

CP = cerebral palsy; TD = typically developing; RFD = rate of force development; CAM = concentric angular impulse; SEM = standard error of mean; PF = plantarflexion; DF = dorsiflexion; KE = knee extension; KF = knee flexion; 6MWT = six minutes walking test; PKE = passive knee extension test; nd = no data.

5.2 Cross-sectional analyses (II, III, IV)

5.2.1 Hyperreflexia neuromechanical variables (II, III)

5.2.1.1 Stretch reflex threshold latency correction (II)

Only participants with SRTs quantified in all four velocities were used for the statistical analysis (n = 12 for Sol, n = 11 for MG). Group Sol H-reflex latency (mean = 28 ms, SD = 3) had a range of 23–33 ms and MG H-reflex latency (mean = 28 ms, SD = 4) had range of 23–35 ms, which are in line with previous reports (Mazzocchio et al. 2001).

Regression slope between SRT and stretch velocity. Figure 17 shows the individual and group mean or median slopes for the original and latency corrected methods. In Sol, the regression slope between the original (mean = 0.014, SD = 0.012) and latency corrected (mean = 0.010, SD = 0.012) methods was statistically different ($t(11) = -19.3$, $p < 0.001$, 95%CI = -0.03 – -0.02; hedge's g = 2.0, hedge's g 95% CI = 1.0–3.0). Similarly, in MG the regression slope in the original method (median = -0.021, IQR = 0.01) was statistically different from the latency corrected method (median = 0.001, IQR = 0.01, $p < 0.001$).

Effects of stretch velocity on SRT. In the original method, SRT occurred at statistically different joint angles for both Sol ($p = 0.008$) and MG ($p < 0.001$). Bonferroni post-hoc analysis revealed that SRT in the two slowest stretch velocities occurred significantly earlier than SRT at the fastest (291°·s^{-1}) velocity for both Sol (55°·s^{-1}, $p = 0.04$; 110°·s^{-1}, $p = 0.009$) and MG (55 and 110°·s^{-1}, $p < 0.001$). With latency correction, no statistically significant differences across the stretch velocities were found for Sol ($p = 0.552$) or MG ($p = 0.315$). Table 5 shows the SRT results for the 4 stretch velocities.

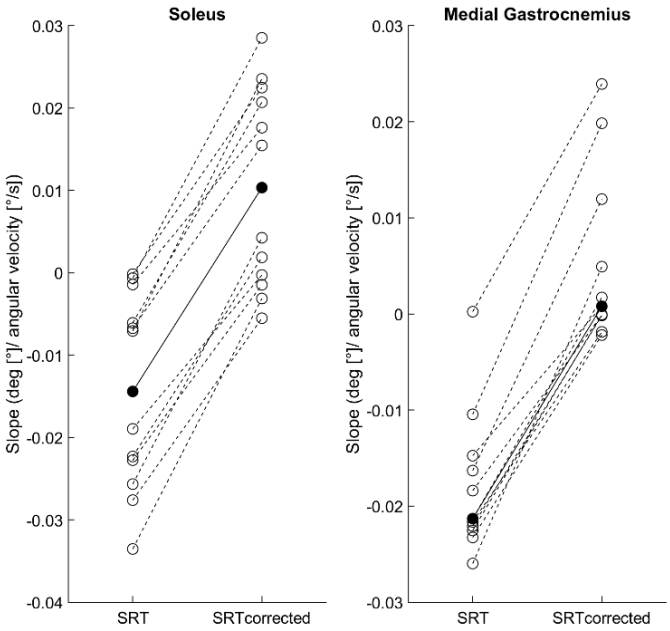

FIGURE 17 Individual SRT-velocity linear regression slopes (open circles) and soleus group mean /medial gastrocnemius median (filled circles) for both methods: original (SRT) and latency corrected ($SRT_{corrected}$).

TABLE 5. Effects of stretch velocity on Sol and MG SRT with and without latency correction.

Variables	**Median (IQR) for stretch velocities:**			
	$55°\cdot s^{-1}$	$110°\cdot s^{-1}$	$210°\cdot s^{-1}$	$291°\cdot s^{-1}$
Sol SRT (°)	14 (13)*	16 (9)*	13 (5)	11 (4)
Sol $SRT_{corrected}$ (°)	15 (13)	19 (9)	18 (5)	18 (3)
MG SRT (°)	18 (2)†	16 (1)†	14 (1)	11 (1)
MG $SRT_{corrected}$ (°)	19 (2)	19 (1)	19 (1)	19 (1)

IQR: interquartile range; Sol: soleus; MG: medial gastrocnemius; SRT: stretch reflex threshold; Stretch velocities $55°\cdot s^{-1}$ and $110°\cdot s^{-1}$ are significantly different from $291°\cdot s^{-1}$: * = $p < 0.05$, † = $p < 0.01$.

Coefficient of determination (R^2). Figure 18 shows the individual R^2 results for the SRT-velocity linear regression and group medians for both methods. In Sol the R^2 between the original (median = 0.53, IQR = 0.93) and latency corrected (median = 0.27, IQR = 0.34) methods were not statistically different ($p = 0.301$). In MG, R^2 in the original method (median = 0.91, IQR = 0.68) was statistically higher than in the latency corrected method (median = 0.08, IQR = 0.15, $p = 0.01$).

TSRT. No statistically significant difference between Sol TSRT (median = 16°, IQR = 11°) and Sol $TSRT_{corrected}$ (median = 16°, IQR = 11, $p = 0.910$) was found.

Likewise, MG TSRT (median = 18°, IQR = 2) and MG $TSRT_{corrected}$ (median = 19°, IQR = 3, p = 0.102) were not statistically different.

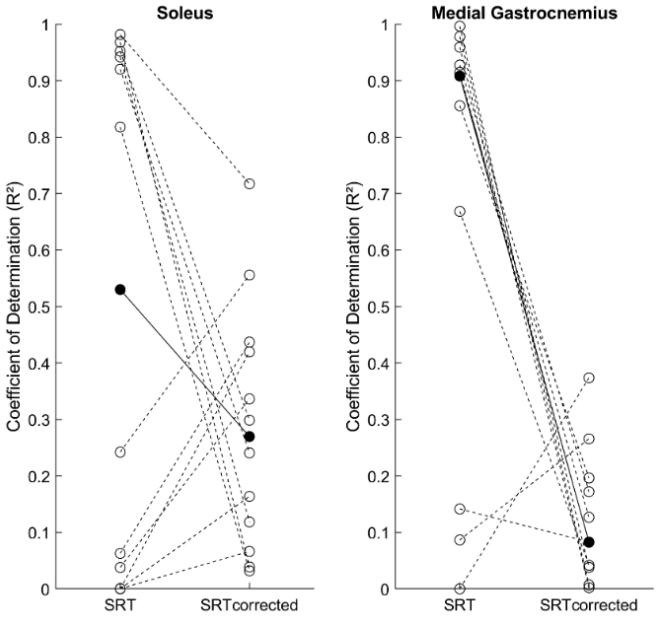

FIGURE 18 Individual SRT-velocity linear regression coefficients of determination (R^2, open circles) and group medians (filled circles) for both methods: original (SRT) and latency corrected ($SRT_{corrected}$).

5.2.1.2 Stretch reflex threshold & response magnitude (III)

Stretch reflex threshold. Participants in the CP group with SR responses in all velocities were used to check the effect of stretch velocity on SRT, and no statistically significant differences between velocities were found for Sol (n = 12, p = 0.552) or MG (n = 11, p = 0.312). TD participants had SR in less than 7% of trials in the two slowest velocities in both muscles, thus the effect of velocity on SRT could not be tested. Pooling only the two highest stretch velocities, the SRT occurred statistically earlier in the stretch (i.e., at a more plantarflexed joint angle) in the CP group compared to TD controls in both Sol (p = 0.008) and MG (p < 0.001) muscles. When the SRT was normalized by maximum ROM, no statistically significant differences between groups were found for Sol (p = 0.899) or MG (p = 0.568).

Stretch reflex EMG-RMS. In the CP group, a significant main effect for stretch velocity on SR EMG-RMS was found for both Sol (p < 0.001) and MG (p < 0.001) muscles. The Bonferroni post hoc reveled that SR EMG-RMS at the two highest stretch velocities (210 and 291°·s^{-1}) were significantly higher than at 55°·s^{-1} for both muscles (p < 0.001). Furthermore, the SR EMG-RMS at the highest stretch velocity (291°·s^{-1}) was statistically higher than 110°·s^{-1} for both Sol (p = 0.002) and MG (p = 0.003). The two fastest stretch velocities were pooled for group comparison. SR EMG-RMS was statistically greater in the CP group compared to

the TD controls in both Sol ($p < 0.001$) and MG ($p < 0.001$) muscles. Table 6 presents the results for the fast cyclic ankle dorsiflexion test variables and the sample size per variable.

TABLE 6. Comparison between CP and TD during the fast cyclic dorsiflexion test

Variables (n CP/TD)	Mean (SD) / Median (IQR)†						
	CP Group				TD Group	P value (95% CI)	Hedge's g (95% CI)
Sol SRT (°)† (13/13)	18 (7)				9 (5)	p = 0.002	n/a
MG SRT (°)† (13/12)	19 (2)				10 (5)	p < 0.001	n/a
Sol SRT (% Max ROM) # (13/12)	0.50 (0.16)				0.49 (0.08)	p = 0.899 (-0.10 – 0.11)	0.05 (-0.74 – 0.83)
MG SRT (% Max ROM)# † (13/12)	0.39 (0.20)				0.46 (0.11)	p = 0.568	n/a
Sol EMG-RMS (%MVC)#† (13/13)	0.31 (0.19)				0.06 (0.07)	p < 0.001	n/a
MG EMG-RMS (%MVC)#† (13/12)	0.19 (0.28)				0.02 (0.03)	p < 0.001	n/a
	55°·s^{-1}	110°·s^{-1}	210°·s^{-1}	291°·s^{-1}			
Sol SRT (°)† (12/NT)	15 (13)	19 (9)	18 (5)	18 (3)	NT	p = 0.552¤	n/a
MG SRT (°)† (11/NT)	19 (2)	19 (1)	19 (1)	19 (1)	NT	p = 0.315¤	n/a
Sol EMG-RMS (%MVC)† (12/NT)	0.09 (0.07)‡	0.16 (0.13)§	0.27 (0.20)	0.56 (0.29)	NT	p < 0.001¤	n/a
MG EMG-RMS (%MVC)† (11/NT)	0.04 (0.06)‡	0.09 (0.12)§	0.16 (0.23)	0.25 (0.27)	NT	p < 0.001¤	n/a

CP: cerebral palsy; TD: typically developing; Sol: soleus; MG: medial gastrocnemius; SRT: stretch reflex threshold ; ROM: range of motion; EMG: electromyography; RMS: root mean square; MVC: maximum voluntary contraction; NT: not tested due to the lack of stretch reflexes in the two slowest velocities; n/a: not applicable; † = median (interquartile range); # = group comparisons with only the two highest velocities; ¤ = within groups comparison: values given for stretch velocities; ‡ = 55°·s^{-1} is significantly (p < 0.001) different from 210 and 291°·s^{-1}; § = 110°·s^{-1} is significantly (p < 0.005) different from 291°·s^{-1}. Ankle 0° = anatomical position.

5.2.2 Neurophysiological variables of hyperreflexia (III)

The Hmax/Mmax and H/Hmax ratios were not statistically different between groups. PAD was statistically lower in the CP group compared to the TD controls ($t(27) = -2.42$, $p = 0.022$). M/Mmax was not statistically different between stimulation frequencies in both groups (within group analysis). Table 7 presents the results of the neurophysiological variables.

TABLE 7. Comparison of soleus neurophysiological variables between CP and TD groups.

	CP	**TD**		
Variables	Mean (SD) / Median (IQR)†		p value (95% CI)	Hedge's g (95% CI)
Hmax/Mmax†	0.89 (0.14)	0.80 (0.30)	p = 0.591 (n/a)	n/a
PAD	0.81 (0.21)	0.56 (0.34)	p = 0.022* (-0.47 – -0.04)	0.87 (0.1 – 1.7)
H/Hmax (0.1Hz)†	0.82 (0.25)	0.79 (0.13)	p = 0.847 (n/a)	n/a
M/Mmax (0.1 Hz)†	0.02 (0.05)	0.02 (0.04)	$CP: p = 0.194 (n/a)	n/a
M/Mmax (1 Hz)†	0.02 (0.05)	0.02 (0.02)	$TD: p = 0.639 (n/a)	

CP: cerebral palsy; TD: typically developing controls; SD: standard deviation; IQR: interquartile range; CI: confidence interval on the difference between population means; Hmax/Mmax: maximum H-reflex normalized by the maximum M-wave; PAD: post-activation depression; H/Hmax: H-reflex normalized by the maximum H-reflex at 0.1 Hz; M/Mmax: M-wave normalized by the maximum M-wave (calculated within each group); † = median (interquartile range); n/a: not applicable; $ = p-values refer to comparison of M/Mmax between the two frequencies within each group.

5.2.3 Ankle neuromechanical variables (III)

Ankle joint maximum and passive ROM were statistically lower in the CP group compared to the TD controls ($p \leq 0.002$). Passive peak torque was not statistically different between groups. Stretch velocity in the slow passive dorsiflexion test was statistically higher in the TD controls compared to CP group ($t(26) = 3.31$, $p = 0.003$). Table 8 presents the results of the slow passive dorsiflexion test variables and the sample size per test.

TABLE 8. Comparison between CP and TD groups on slow passive ankle dorsiflexions

Variables (n CP/TD)	CP Mean (SD) /	TD Median (IQR)†	p value (95% CI)	Hedge's g (95% CI)
Maximum ROM [°] (14/15)†	47 (22)	56 (1)	p = 0.001 (n/a)	n/a
Passive ROM [°] (14/14)†	29 (22)	52 (10)	p = 0.002 (n/a)	n/a
Passive PT [N·m] (14/14) †	4.6 (6.8)	5.0 (3.8)	p = 0.346 (n/a)	n/a
Stretch Velocity [°·s^{-1}] (14/14)	7.2 (1.0)	9.6 (2.5)	p = 0.003 (0.9–3.8)	1.2 (0.4–2.0)

CP: cerebral palsy; TD: typically developing controls; SD: standard deviation; IQR: interquartile range; CI: confidence interval on the difference between population means; ROM: range of motion; PT: peak torque; † = median (interquartile range); n/a: not applicable.

5.2.4 Hyper-resistance (III)

Peak torque was statistically greater in the CP group compared to the TD controls in all three stretch velocities ($p < 0.05$). In the CP group, peak torque at 110°·s^{-1} was statistically greater than at 10 and 55°·s^{-1} ($p < 0.001$). In the TD group, no statistically significant differences in peak torque were found between stretch velocities ($p = 0.115$). Table 9 presents the results for peak torque and the sample size per stretching velocity.

TABLE 9. Ankle joint hyper-resistance at three stretch velocities in CP and TD groups.

Variables (n CP/TD)	Mean (SD) / Median (IQR)†			P value (95% CI)	Hedge's g (95% CI)
	CP	TD			
PT [N·m] @ 10°·s^{-1}† (14/12)	2.0 (4.5)	0.6 (0.4)		p = 0.04	n/a
PT [N·m] @ 55°·s^{-1}† (13/14)	3.0 (5.6)	0.8 (1.6)		p = 0.005	n/a
PT [N·m] @ 110°·s^{-1} (13/14)	5.7 (4.1)	1.4 (1.2)		p = 0.003 (1.8–6.9)	1.4 (0.6–2.3)
	@10°·s^{-1}	@55°·s^{-1}	@110°·s^{-1}		
PT CP† (13)	2.1 (4.4)	3.0 (5.6)	5.2 (5.2)‡	p < 0.001¤	n/a
PT TD† (11)	0.6 (0.5)	0.7 (1.4)	0.7 (1.6)	p = 0.115¤	n/a

CP: cerebral palsy; TD: typically developing; SD: standard deviation; IQR: interquartile range; CI: confidence interval on the difference between population means; PT: peak torque; N·m: newton-meter; † = median (interquartile range); n/a: not applicable; ¤ = within groups comparison; ‡ = peak torque at 110°·s^{-1} is significantly ($p < 0.05$) larger than peak torque at 10 and 55°·s^{-1}.

5.2.5 Correlation matrices (III)

The first correlation matrix had PAD, passive ROM, SRT and SR EMG-RMS from both muscles, and the second matrix had maximum and passive ROM, SRT and

SR EMG-RMS from both muscles, and peak torque at three stretch velocities (10, 55 and 110°·s^{-1}). Additionally, the second matrix was also performed with the SRT normalized by maximum ROM, instead of the absolute SRT values. Bonferroni correction for multiple comparisons yielded a new alpha of 0.01 and 0.00625 for matrices one and two, respectively. Figure 19 depicts the correlations between studied variables.

Correlation matrix 1. The SRT of both muscles had a good positive correlation (rs = 0.74, p = 0.005), and were not correlated to SR EMG-RMS, PAD or passive ROM. The SR EMG-RMS of both muscles were not correlated to any of the studied variables, not even between muscles. Passive ROM had a good negative correlation with PAD (rs = –0.77, p = 0.003).

Correlation matrix 2. Peak torque at 55°·s^{-1} had a very good correlation with peak torque at 110°·s^{-1} (rs = .85, p < 0.001), and a good positive correlation with the Sol SRT (rs = 0.73, p = 0.006) and MG SRT (rs = 0.80, p = 0.002). Peak torque at 110°·s^{-1} had a good positive correlation with the Sol SRT (rs = 0.77, p = 0.003). The SRT normalization by the maximum ROM caused both muscles to have a very good correlation (rs = 0.91, p < 0.001), but no other significant correlations, except naturally with maximum ROM as it was the normalization parameter. Peak torque at 10°·s^{-1}, SR EMG-RMS of both muscles, maximum ROM, and passive ROM were not correlated to any of the studied variables. Figure 19 depicts the correlations between studied variables.

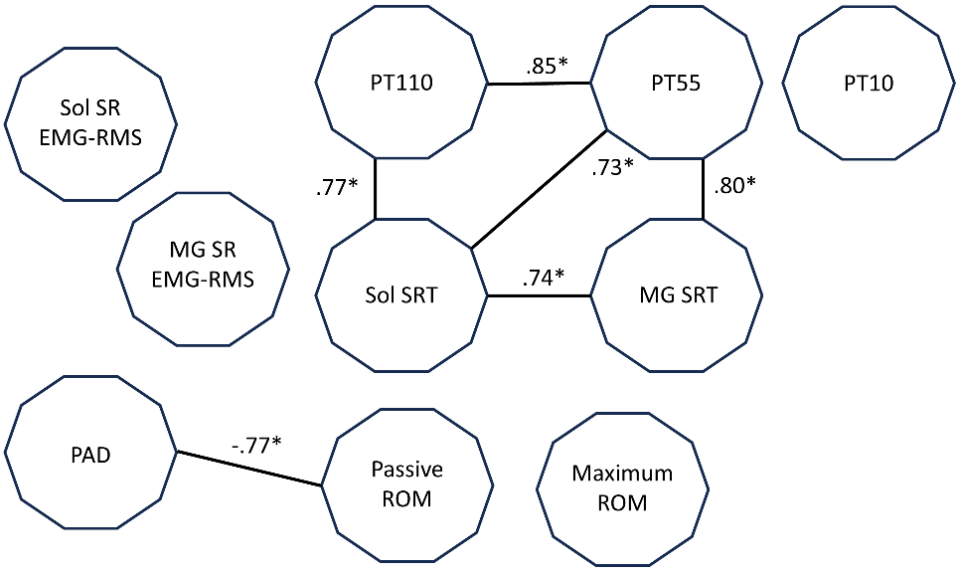

FIGURE 19 Correlations between neuromechanical and neurophysiological variables. PT110: peak torque at 110°·s^{-1}; PT55: peak torque at 55°·s^{-1}; PT10: peak torque at 10°·s^{-1}; SRT: stretch reflex threshold; Sol: soleus muscle; MG: medial gastrocnemius muscle; ROM: range of motion; SR: stretch reflex; EMG: electromyography; RMS: root mean square; PAD: post-activation depression; Hmax/Mmax: maximum H-reflex normalized by the maximum M-wave.

5.2.6 Motor function, muscle strength and joint flexibility (IV)

Table 10 shows the Pre2 comparisons between CP and TD groups, and the absolute difference in means between pre-tests for both groups. The TD group had statistically higher scores for all studied variables compared to CP, except for hip flexion flexibility.

TABLE 10. Group comparisons for all studied variables at Pre2 session. Δ Pre-tests indicates the absolute mean differences for each variable between Pre1 and Pre2 assessments for CP and TD groups.

Group	Mean (SD) / Median (IQR)†			Δ Pre-tests
Variables	CP	TD	p	CP / TD
6MWT (m)†	550 (135)	710 (106)	< 0.001	1 / 4
PKE (°)†	34 (28)	13 (20)	< 0.001	
Hip (°)	21 (8)	24 (6)	0.124	
PF torque (N·m)†	75 (53)	163 (75)	< 0.001	3 / 4
PF RFD (N·m)·s^{-1}†	67 (68)	397 (274)	< 0.001	15 / 43
PF CAI (N·m)·s†	58 (56)	208 (106)	< 0.001	11 / 9
PF CW (°)†	13 (15)	25 (4)	0.004	1 / 0.5
DF torque (N·m)†	21 (15)	38 (26)	< 0.001	2 / 3
DF RFD (N·m)·s^{-1}†	43 (22)	136 (48)	< 0.001	9 / 4
DF CAI (N·m)·s†	14 (19)	58 (23)	< 0.001	2 / 7
DF CW (°)†	12 (17)	28 (0)	< 0.001	0 / 1
KE torque (N·m)†	92 (53)	125 (104)	0.018	2 / 3
KE RFD (N·m)·s^{-1}†	109 (237)	388 (291)	0.001	33 / 13
KE CAI (N·m)·s†	202 (145)	346 (356)	< 0.001	0 / 24
KE CW (°)	37 (19)	52 (14)	0.013	7 / 7
KF torque (N·m)†	46 (46)	63 (57)	0.030	5 / 6
KF RFD (N·m)·s^{-1}†	29 (64)	155 (232)	< 0.001	6 / 41
KF CAI (N·m)·s†	40 (74)	147 (172)	< 0.001	14 / 4
KF CW (°)†	9 (21)	39 (20)	< 0.001	4 / 3

CP = cerebral palsy; TD = typically developing; SD: standard deviation; IQR: interquartile range; † = median (interquartile range); 6MWT = six minutes walking test; PKE = passive knee extension test; PF = plantarflexion; DF = dorsiflexion, KE = knee extension; KF = knee flexion; RFD = rate of force development; CAI = concentric angular impulse; CW = curve width; Δ Pre-tests = absolute difference between pre-tests means for both groups (e.g. for the 6MWT the mean difference was 1 and 4 m for the CP and TD groups, respectively).

5.3 Longitudinal analyses (IV)

5.3.1 Gross motor function

Six minutes walking test. No significant differences in the control period (i.e., Pre1 vs. Pre2) and retention period (i.e., Post1 vs. Post2) were found. Significant main effects of time (i.e., timepoints) and $GMFM_{co}$ (i.e., GMFM as a covariate) were found ($p < 0.001$), but no time x $GMFM_{co}$ interaction ($p = 0.126$). Post hoc analysis showed that Post1 had statistically higher scores than pre-tests ($\beta = 33$, 95% CI = 12–53, SE = 11, $p = 0.024$). Likewise, Post2 had statistically higher scores than pre-tests ($\beta = 43$, 95% CI = 20–67, SE = 12, $p = 0.006$). The simple effects test showed that the three $GMFM_{co}$ subgroups were statistically different in all four timepoints ($p < 0.001$) and that the pre-post differences occurred only in the two lower score $GMFM_{co}$ subgroups ($p < 0.001$).

Gross motor function measure (Pre2 vs. Post1). Four participants with CP had the maximum GMFM score at Pre2 (i.e., highly functional), and were excluded from this analysis due to the test insensitivity to measure changes induced by the intervention. The Wilcoxon signed rank test showed a statistically significant difference ($n = 14$, $p = 0.004$) between Pre2 (median = 101, IQR = 46) and Post1 (median = 104, IQR = 41) GMFM scores. Eleven participants improved a median of 3 points with a range of 1–11, and three participants had the same score on both tests.

5.3.2 Muscle strength

5.3.2.1 Control and retention periods

This section reports the pairwise comparisons between pre-tests and between post-tests performed with the Bonferroni post hoc test and the simple effects test. Main effects and other pairwise comparisons are reported in the following section (5.3.2.2).

For the four dynamic muscle actions (i.e., plantarflexion, dorsiflexion, knee extension and knee flexion) in the CP group, no statistically significant post hoc test differences for time were found between Pre1 and Pre2 in any of the studied variables (i.e., peak torque, rate of force development, concentric angular impulse and curve width). However, a few significant simple effects were statistically significant in the control period: (1) knee extension peak isometric torque was higher in Pre2 compared to Pre1 in the low $GMFM_{co}$ subgroup ($\beta = 11$, SE = 3, 95% CI = 4–17, $p < 0.001$) and the mid $GMFM_{co}$ subgroup ($\beta = 5$, SE = 2, 95% CI = 0–10, $p = 0.033$), (2) knee extension rate of force development was higher in Pre2 compared to Pre1 for the high $GMFM_{co}$ subgroup ($\beta = 52$, SE = 18, 95% CI = 15–88, $p = 0.005$) and (3) knee flexion rate of force development was higher in Pre1 compared to Pre2 for the mid $GMFM_{co}$ subgroup ($\beta = 16$, SE = 7, 95% CI = 2–30, $p = 0.027$). Thus, although no differences between pre-tests were found for the entire CP group, a few subgroups changed significantly during the control

period. In the TD group, no statistically significant differences were found between Pre1 and Pre2 in any of the studied variables, with one exception. Plantarflexion rate of force development was significantly higher in Pre2 compared to Pre1 (mean difference = 65 $(N \cdot m) \cdot s^{-1}$, t = –2.686, p = 0.017). Regarding the retention period, no statistically significant differences were found in all studied variables and dynamic muscle actions between Post1 and Post2.

5.3.2.2 EXECP intervention effects

Peak isometric torque. For the plantarflexion test a significant main effect of $GMFM_{co}$ ($p < 0.001$) was found, while there were no main effects of time or their interaction. $GMFM_{co}$ subgroups were statistically different in all four timepoints ($p \leq 0.001$). No significant main effects were found for the dorsiflexion test. In the knee extension test, a significant main effect of time ($p < 0.001$) and its interaction with $GMFM_{co}$ ($p = 0.014$) were found. Post hoc analysis showed that Post1 torque was higher than pre-tests ($\beta = 23$, SE = 3, 95% CI = 17–29, $p < 0.001$). The simple effects test showed that the post hoc differences occurred for all $GMFM_{co}$ subgroups ($p < 0.001$). In the knee flexion test, only a significant main effect for time was found ($p < 0.001$). Post hoc analysis showed that Post1 torque was higher than the pre-tests ($\beta = 16$, SE = 3, 95% CI = 9–22, $p < 0.001$).

Rate of force development. For the plantarflexion test, a significant main effect of $GMFM_{co}$ ($p = 0.011$) was found, with statistically significant differences between the three $GMFM_{co}$ subgroups in all timepoints ($p < 0.05$), except Post2. For the dorsiflexion test, a significant main effect of time ($p = 0.034$) was found. However, post hoc analysis showed that Post1 was only statistically different from the lower value pre-test, and thus was disregarded. For the knee extension test, significant effects of time ($p<0.001$) and its interaction with $GMFM_{co}$ ($p = 0.005$) were found. Post hoc analysis showed that Post 1 had higher values than the pre-tests ($\beta = 68$, SE = 17, 95% CI = 37–101, $p < 0.001$). The simple effects test showed that pre-post effects of time were significant for all three $GMFM_{co}$ subgroups ($p < 0.001$). For the knee flexion test, significant effects of time ($p = 0.12$) and $GMFM_{co}$ ($p = 0.010$) were found, however post hoc analysis and simple effects test revealed no significant pre-post differences.

Concentric angular impulse. For the plantarflexion test, a significant main effect for $GMFM_{co}$ ($p = 0.001$) was found. Simple effects test showed that $GMFM_{co}$ subgroups were statistically different in all four timepoints ($p \leq 0.011$). No significant main effects were found for dorsiflexion concentric angular impulse. In the knee extension test, significant effects of time ($p < 0.001$) and its interaction with $GMFM_{co}$ ($p = 0.009$) were found. Post hoc analysis showed that Post1 concentric angular impulse was statistically higher than the pre-tests ($\beta = 46$, 95% CI = 22–70, SE = 12, $p = 0.001$). The simple effects test showed that pre-post differences occurred only in the two lower score $GMFM_{co}$ subgroups ($p < 0.001$), and that $GMFM_{co}$ subgroups were statistically different only in the pre-tests ($p < 0.05$). For the knee flexion test, significant effects for time ($p = 0.005$) and $GMFM_{co}$ ($p = 0.033$) were found. Post hoc analysis showed that Post1 concentric angular impulse was statistically higher than the pre-tests ($\beta = 45$, 95% CI = 19–71, SE =

13, p = 0.01). Simple effects test showed that the pre-post differences were only present in the two higher score $GMFM_{co}$ subgroups ($p \leq 0.005$), and that $GMFM_{co}$ subgroups were statistically different in Pre1 and Post1 ($p < 0.05$).

Curve width. No significant effects in plantarflexion, dorsiflexion and knee flexion curve width were found. In the knee extension test, a significant effect of $GMFM_{co}$ ($p < 0.001$) was found, while no effects of time or their interaction. Simple effects analysis showed that only in Pre1, the $GMFM_{co}$ subgroups had statistically different curve width values ($p < 0.001$), with higher $GMFM_{co}$ scores having higher curve width.

Trunk isometric strength. A statistically significant difference in trunk extension weight between the first (median = 0.5, IQR = 5, p = 0.001) and last (median = 5, IQR = 7) months was found. There was no difference between the duration of the trunk extension exercise in the first (median = 60, IQR = 7) and the last (median = 60, IQR = 0) month. For trunk flexion, the opposite happened: no difference in trunk flexion strength between the first (median = 0, IQR = 0) and last (median = 0, IQR = 0) months was found, while the duration of the trunk flexion exercise was statistically lower in the first (median = 45, IQR = 30, p = 0.013) compared to the last (median = 60, IQR = 15) month.

5.3.3 Joint Flexibility

Passive knee extension test. No statistically significant differences in the control and retention periods were observed for the passive knee extension test. For the passive knee extension test, significant main effects of time (p = 0.001) and training status ($p < 0.001$) were found. Post-hoc analysis showed that the legs chosen for flexibility training during the intervention had statistically lower flexibility compared to the untrained legs ($\beta = 14$, SE = 4, $p < 0.001$), and the simple effects test further revealed that the differences were present in all four timepoints ($p \leq 0.004$). The second post-hoc analysis (i.e., merged training status) showed that both post-tests had statistically higher joint flexibility compared to the pre-tests ($p < 0.05$). Furthermore, the simple effects test revealed that pre-post differences were present only in the trained legs (p = 0.003). Post1 had significantly higher joint flexibility than both pre-tests ($\beta = 5$, SE = 2, 95% CI = 1–8, p = 0.008), and so did Post2 ($\beta = 4$, SE = 2, 95% CI = 0–8, p = 0.037).

Hip extension test. No statistically significant differences in the control and retention periods were observed for the hip extension test. No main effects of time, training status and their interaction were found for the hip extension test. Additionally, post hoc tests showed no differences. Oppositely, the simple effects test found a significant effect for time only in the trained legs (p = 0.019) and pairwise comparisons showed that Post1 flexibility was statistically higher than the pre-tests ($\beta = 5$, SE = 2, 95% CI = 1–9, p = 0.018). Table 11 presents the estimated marginal means for all longitudinal variables (except GMFM) at the best pre-test, Post1 and Post2 timepoints.

TABLE11. Estimated marginal means (EMM), standard error (SE) and GLMM model intraclass correlation coefficient (ICC_{GLMM}) for all longitudinal variables at the best pre-test and both post-tests for CP participants.

Variable		Pre-test	Post1	Post2
	ICC_{GLMM}	EMM (SE)	EMM (SE)	EMM (SE)
6MWT (m)	0.87	502 (21)	535 (21)*	546 (21)*
PKE trained (°)	0.73	39 (5)	35 (5)*	35 (5)*
PKE untrained (°)		24 (4)	21 (4)	22 (4)
Hip trained (°)	1.00	19 (3)	24 (3)*	18 (4)
Hip untrained (°)		26 (3)	24 (3)	24 (4)
PF torque (N·m)	0.74	74 (6)	81(6)	81(7)
PF RFD (N·m)·s^{-1}	0.49	93 (15)	119 (15)	95 (17)
PF CAI (N·m)·s	0.83	68 (10)	77 (9)	81 (10)
PF CW (°)	0.99	17 (2)	16 (2)	17 (2)
DF torque (N·m)	1.00	25 (4)	25 (4)	26 (4)
DF RFD (N·m)·s^{-1}	1.00	56 (9)	60 (10)	61 (10)
DF CAI (N·m)·s	0.84	15 (4)	18 (4)	19 (4)
DF CW (°)	0.66	14 (2)	13 (2)	14 (2)
KE torque (N·m)	1.00	95 (11)	113 (11)*	103 (11)
KE RFD (N·m)·s^{-1}	1.00	218 (36)	286 (37)*	264 (39)
KE CAI (N·m)·s	0.79	190 (21)	236 (21)*	233 (23)
KE CW (°)	0.23	40 (4)	41 (3)	39 (4)
KF torque (N·m)	1.00	52 (6)	68 (7)*	62 (7)
KF RFD (N·m)·s^{-1}	1.00	86 (13)	79 (12)	102 (15)
KF CAI (N·m)·s	0.64	49 (16)	94 (16)*	73 (18)
KF CW (°)	0.55	18 (5)	20 (5)	18 (5)

* = Different from pre-tests ($p < 0.05$). CP = cerebral palsy, 6MWT = six minutes walking test, PKE = passive knee extension test, PF = plantarflexion, DF = dorsiflexion, KE = knee extension, KF = knee flexion, RFD = rate of force development, CAI = concentric angular impulse, CW = curve width.

6 DISCUSSION

The main purpose of this thesis was to design (article I) and verify the effects of the multicomponent EXECP intervention on muscle strength, joint flexibility and motor function (article IV). Furthermore, this thesis performed a neuromechanical and neurophysiological comparison between participants with cerebral palsy and their typically developing age and sex-matched controls (article III). Finally, this thesis also sought to verify the effects of correcting the stretch reflex latency on two methods of assessing hyperreflexia, namely the stretch reflex threshold and the tonic stretch reflex threshold (article II).

The main findings of this thesis were that:

1. The stretch reflex latency correction significantly changed the SRT-velocity slopes and rendered the group-level CP SRT for both Sol and MG muscles velocity independent. Thus, the lack of linear relationship between SRT and stretch velocity invalidates the use of a linear regression to find the TSRT. (article II).

2. The CP group had higher hyper-resistance in all three tested stretch velocities, and higher hyperreflexia measured by both SRT and SR EMG-RMS compared to the TD group. At high stretch velocities (i.e., 210–291 $deg \cdot s^{-1}$), the SRT in absolute joint angles was found significantly earlier in CP compared to TD. However, when the SRT was normalized by joint flexibility, no differences between groups were found. Furthermore, the SR EMG-RMS was greater in CP compared to TD, and had a positive correlation with stretch velocity. (article III)

3. Ankle joint neuromechanical variables that were significantly different between groups, were not correlated to both hyperreflexia and HR. Furthermore, theoretically important neurophysiological measures of hyperreflexia were not correlated to both neuromechanical variables of

hyperreflexia and ankle joint. Finally, HR measured at high stretch velocities had a good positive correlation with the SRT. (article III)

4. The EXECP intervention was safe, feasible and effective in enhancing gait performance and overall gross motor function. Furthermore, knee and trunk muscle strength, and hip and knee joint flexibility also improved. However, at the ankle joint, no significant effects of the intervention were found. (article IV)

5. Almost all significant improvements brought upon by the EXECP intervention regressed back to baseline values after the retention period, highlighting the need of constant training to gain and maintain optimal levels of strength and motor function. (article IV)

6.1 Cross-sectional analyses (II, III, IV)

6.1.1 Stretch reflex threshold latency correction (II)

Study II sought to verify the effects of the SR latency correction on the SRT and TSRT methods of stretch hyperreflexia assessment. Participants with CP had their triceps surae muscles stretched at four velocities by the motor-driven dynamometer (i.e., fast cyclic dorsiflexions test), and SRT was calculated in the original and latency corrected method. To account for the SR latency (i.e., time between SR onset at the muscle spindles and the SR EMG onset), the H-reflex latency was subtracted from SR EMG onset, yielding the joint angle at the SR onset. The main findings were that latency correction significantly changed the SRT-velocity slopes and rendered the group-level SRT for both Sol and MG velocity independent. Thus, the lack of linear relationship between SRT and stretch velocity invalidates the use of a linear regression to find the TSRT.

Regression slopes. To group individual SRT-velocity slopes, a 'near zero slope' was defined as having a modulus value smaller than 0.01, which would result in a maximum of 2.5° difference between the slowest and fastest stretch velocities used in this study. Since within participant and velocity SRT median range was 1.5° (min–max range: 0.1–16°), a slope smaller than 0.01 would cause changes in SRT that are indistinguishable from the subject's natural variability. At the individual level, with SR latency correction, six participants had near zero slopes in Sol (i.e., velocity independent) while the other six had positive slopes (i.e., earlier SRT at higher velocities). In MG, nine participants had near zero slopes and two had positive slopes. Interestingly, all participants that showed velocity independent SRT, had an early SRT within the first 2° of the stretch, whereas positive slopes were present in participants with SRT later in the range of motion. These individual differences suggest that stretch velocity has a negligible effect on participants with early SRT. A possible explanation may be that the MTU is already under tension and/or the IA arc is highly excitable (Nielsen et al. 2005).

The changes in the regression slopes caused by the SR latency correction towards positive values were expected since the correction shifts the SRT of higher velocities to earlier angles. This means that when the stretch velocity is increased, SRT without the latency correction occurred progressively later in the range of motion, whereas the corrected SRT occurred progressively earlier. Only the latter is an expected phenomenon of the velocity-dependent nature of hyperreflexia, and is also expected due to the viscoelastic behavior of the MTU (i.e., increased stretch resistance at higher velocities; Taylor et al. 1990; Wu et al. 2010).

Coefficient of determination. Although the regression slope changes with SR latency correction were unidirectional (i.e., towards positive slope values), its effect on R^2 was bidirectional among the participants: 1) negative slopes shifting towards near zero slopes reduced R^2 (50% of participants in Sol and 82% in MG); 2) near zero slopes shifting towards positive slopes increased R^2 (50% of participants in Sol and 18% in MG). This explains why the statistically significant effect of SR-latency correction on R^2 was observed only in MG. The lower SRT variability in MG was probably due to the extended knee testing position, which placed the biarticular MG under considerably more tension than Sol. Overall, the low median latency corrected R^2 values for both muscles (Sol = 0.27, MG = 0.08) and high variability among participants, argues against the use of the linear regression to calculate the TSRT, at least in the present sample. Nevertheless, previous studies have reported positive slopes without the SR latency correction for the same muscles that were studied here (Blanchette et al. 2016; Germanotta et al. 2017). These studies would have had steeper slopes with the latency correction, and if a high R^2 was found, the TSRT method would be justified. Even though latency correction increased the slopes significantly in the current study, as expected, no differences in TSRT (i.e., y-intercept) were found between methods in both examined muscles. Since latency correction had a minimal effect on the SRTs at slow velocities (e.g., 28 ms · 55°·s $^{-1}$ = 1.5° correction), even considerable changes in the regression slope had a small effect on the TSRT.

Methodological remarks (II). Several important aspects of article II require further clarification. Firstly, a powerful motor-driven ankle-joint movement actuator was used to induce the stretches, whereas most of the aforementioned studies applied manual stretches. It took only 20–40 ms to achieve the target velocity in the actuator used in the present study, which seems unlikely in manual stretches or even in the mechatronic device used by Germanotta et al., (2017), which had a maximum torque output of 7.1 N·m. Thus, it is possible that although mean joint velocities are comparable between studies, the joint acceleration profiles were very different (Sloot et al. 2021). Notably, during clinically applied manual tests such as the Modified Tardieu Scale, the stretch velocity is unknown making it impossible to perform the latency correction to the catch angle. Secondly, the stretch range of motion in the present study was shorter than other studies assessing the same joint (Blanchette et al. 2016; Germanotta et al. 2017). This was due to the extended knee testing position and the use of a motor driven dynamometer with end stop limits set for safety reasons.

Fortunately, even in the slowest stretch velocity, consistent SRs were evoked within this range of motion. Thirdly, the H-reflex bypasses the muscle spindle and is evoked at the popliteal fossa, thus a small systematic error underestimating the SR latency by a few milliseconds is unavoidable, causing a small error in the SRT calculation at high stretch velocities. Nevertheless, the distance from the SR onset location in the tested muscles to the popliteal fossa could not be more than 15% of the entire IA arc pathway, thus for the current dataset it would represent a maximum of 4.2 ms (i.e., 15% of the mean latency) or an error of 1.2° in the fastest stretch velocity, still inferior to the within-subject and velocity SRT variability. Since using electrical nerve stimulation to assess SR latency is not feasible in most clinical setups, it would be very helpful to create easy measures using for example height and limb length that could estimate the SR latency of different muscles. Furthermore, since there is already considerable amount of data published on the subject, an effort to re-analyze it correcting for reflex latency would be of immense help for the scientific community. Finally, the chosen EMG onset method performed very well in the fast stretches since the signal-to-noise ratio was very high. However, in the two slowest stretch velocities, many false positives and negatives were identified by visual inspection, and manual onset correction was extremely time-consuming. All onset corrections were logged, and the information will be available at the project's repository. The lower SRT variability and better automatic EMG onset detection at higher stretch velocities strongly suggests designing SRT testing protocols with higher minimum stretch velocities.

6.1.2 Group comparisons (III, IV)

Study III used the fast cyclic dorsiflexion test and the passive dorsiflexion test to assess neuromechanical variables in CP and TD groups. Furthermore, electrical nerve stimulation was used to assess IA arc reflex excitability and the PAD mechanism in the Sol muscle of both groups. The main variable HR (i.e., peak torque), behaved as theoretically expected, velocity-dependent and consistently higher in the CP group, with TD controls displaying very low resistance to the stretch. There were four main findings in study III. First, the SRT was not velocity-dependent in the CP group and could not be tested in the TD controls due to the lack of SRs at the slow stretch velocities. Second, at high stretch velocities, when SRT were present in both groups, earlier SRT in absolute joint angles were found in the CP group compared to the TD controls. However, when the SRT was normalized by maximum ROM, no differences between groups were found. Third, SR EMG-RMS was greater in the CP group compared to the TD controls and was velocity-dependent in the CP group. Fourth, the neurophysiological measures of the IA reflex arc (i.e., Hmax/Mmax and PAD) were not associated with the neuromechanical variables of hyperreflexia (i.e., SRT and SR EMG-RMS). Furthermore, a good correlation between HR in high stretch velocities and SRT for both muscles was found.

Neurophysiological variables of hyperreflexia. Soleus Hmax/Mmax was considerably variable in both groups, as already shown by previous research

(Koelman et al. 1993; Willemse et al. 1994). Similar to the present results, Achache et al. (2010) did not find differences in the Hmax/Mmax ratio between CP and TD groups, when the recruitment curve was built at 0.16 Hz (Achache et al. 2010). At higher stimulation frequencies, the PAD mechanism affects the H-reflex excitability (i.e., confounding variable), and thus the results are difficult to interpret. The lower PAD values in the CP group compared to the TD controls found in the present study are consistently corroborated by other studies comparing participants with spasticity and TD controls (Grey et al. 2008; Lamy et al. 2009; Yang et al. 2015). To the best of the author's knowledge, the present study is the first that simultaneously reported two important control variables: 1) H/Hmax was similar among groups and the test was done on the ascending limb of the recruitment curve and 2) the M/Mmax remained constant between the two stimulation frequencies (i.e., constant effective stimulation intensity). Therefore, differences in H/M between groups can be attributed to the PAD mechanism.

Neuromechanical variables of hyperreflexia. Both neuromechanical variables of hyperreflexia (i.e., SRT and SR EMG-RMS) were significantly different between groups, with the CP group displaying earlier and stronger SR responses. The SRT in both Sol and MG was not velocity-dependent in the CP group, while SR EMG-RMS was clearly velocity-dependent. The SRT could only be recorded at higher velocities in the TD controls (i.e., 210–291°·s^{-1}) whereas they were present in all velocities in the CP group. This suggests that stretch reflex sensitivity was different between groups. Since muscle fascicle behavior was not measure in the present study, it is not possible to rule out that this happened, at least partially, due to differences in muscle-tendon dynamics. Furthermore, there were no differences between groups in the normalized SRT, suggesting that the SRT was not elicited earlier in the relative ROM in the CP group compared to the TD controls. Finally, the SRT has been extensively used to assess hyperreflexia (e.g., Bar-On et al. 2014; Blanchette et al. 2016; Germanotta et al. 2017), unfortunately, due to lack of latency correction in the SRT measure of these studies, the results are not comparable to those of the present study.

Ankle neuromechanical variables. Lower maximum and passive ROM in the CP group compared to the TD controls were found even with a large heterogeneity in the CP group. Despite these kinematic differences, passive peak torque was not statistically different between groups. This result is reasonable since the shape of the torque-angle relationship was different between groups with passive resistance starting earlier and rising faster in the CP group, compared to a lower slope and greater ROM in the TD controls. The differences in the torque-angle relationship are likely due to differences in muscle-tendon morphology and/or the size of the muscle-tendon physiological cross-sectional area (Chleboun et al. 1997; Lieber and Fridén 2019).

Limitations and methodological remarks (III). Study III had the following limitations: firstly, the small sample size coupled with a high within group variability in CP resulted in some underpowered tests. Secondly, maximal ROM could not be measured in seven TD participants due to the dynamometer movement restriction at 21° of dorsiflexion. Based on TD normative data

(Moseley et al. 2001, n = 300), mean passive dorsiflexion is 18° (SD = 7) and maximum 45°, thus it is possible that a higher maximum ROM in these participants could have pushed the SRT/maximum ROM ratio lower, towards a significant difference between groups. Finally, although ankle joint angle and heel raise were measured with precision, it was not possible to control or measure foot pronation, which is a known compensation in CP to increase ankle dorsiflexion (Weide et al. 2020). However, controlling foot pronation would only have made the findings of lower maximum ROM in the CP group more significant.

A few methodological remarks are important to point out: 1) the SR detection method used in the present study was extremely sensitive, and a small increase of 1% maximum voluntary contraction EMG-RMS could be enough to trigger it. Thus, by present definition, the muscle was either very relaxed (i.e., < 5% MVC EMG-RMS) or it was active, even though the activation may have been physiologically unimportant; 2) although mean stretch velocity during manually performed slow stretches was statistically higher in the TD group, it is argued that the difference in group mean stretch velocity (CP: 7.2°·s^{-1}, TD: 9.6°·s^{-1}) was irrelevant, as it was well below the TD SR velocity threshold, demonstrated by the very low number of SRs at 55°·s^{-1}; 3) as occurred in the study II, SRT was more constant and easier to identify both visually and using the detection algorithm at higher stretch velocities, and since the SRT was not velocity dependent, methodologically it makes sense to perform assessments at higher stretch velocities.

Strength, flexibility and motor function. Study IV performed a cross-sectional comparison between CP and TD groups regarding motor function (6MWT), muscle strength for knee and ankle muscles (peak torque, concentric angular impulse, rate of force development and curve width) and joint flexibility (hip and knee flexors). The TD group had superior performance in all strength variables, which corroborates with Hanssen et al. (2021) that reported lower isometric peak torque in the same muscle actions as study IV. Hip extension flexibility was the only dependent variable similar among groups. Only two participants with CP had low flexibility (< 20°), and both group medians were very similar, thus in the present sample hip extension flexibility was not a problem for CP participants. The group differences found in the 6MWT were expected, as it has been well documented by other researchers (e.g., Fitzgerald et al. 2016). Lastly, although the passive knee extension deficit in CP is widely known anecdotally, the thesis author could not find any well documented comparisons between people with CP and TD controls.

6.1.3 Correlation matrices (III)

The second aim of study III was to verify the correlations between the studied variables that were significantly different between CP and TD groups, and could be potentially used clinically to assess hyperreflexia and HR.

Hyper-resistance. The very good positive correlation between peak torque at 55 and 110°·s^{-1} was expected as stretch resistance increases with stretch velocity

(Taylor et al. 1990). The lack of correlation between peak torque at $10°\cdot s^{-1}$ with the peak torque at the higher stretch velocities and with the SRT of both muscles was expected as the slow stretch did not evoke SRs. While peak torque at $110\cdot s^{-1}$ was significantly higher than peak torque at $10\cdot s^{-1}$ for the CP group, there was a very large within-group variability (coefficient of variation of delta peak torque between both velocities = 0.69). Since the increase of peak torque at higher velocities is attributed to hyperreflexia and to the viscoelastic behavior of the muscle, the present data suggests a variable and individual contribution of hyperreflexia to HR.

Neuromechanical variables of hyperreflexia. The present results suggest that the SRT was an appropriate measure of hyperreflexia: it was significantly different between groups, had a good correlation between muscles and with HR at the two highest stretch velocities. A good positive correlation between Sol and MG SRT was expected due to their mechanical linkage, while a good positive correlation between SRT and HR was theoretically expected, as early stretch reflexes should increase the muscle's resistance to the stretch. While SR EMG-RMS was significantly different between groups, it had no correlation between muscles, and it was not correlated to HR or SRT. Estimating force production from surface EMG is unavoidably challenging due to signal amplitude cancellation and the inability to control confounding factors such as thickness of subcutaneous tissues and muscle fiber spatial distribution (Farina et al. 2004, 2014). Furthermore, the normalization procedure using the maximum isometric root mean square EMG may have introduced some error since the amount of muscle co-activation in the CP group during the isometric strength test could have been highly heterogeneous. Finally, the lack of correlation between both measures of hyperreflexia in CP is not surprising since different interactions between several spinal circuits and the Ia afferent volley could confound this relationship. For example, presynaptic inhibition, PAD and recurrent inhibition could theoretically attenuate the SR EMG-RMS without altering the SR timing, thus not affecting the SRT. Thus, the present results cast doubt on the usefulness of the SR EMG-RMS as a clinical measure of hyperreflexia.

Neurophysiological variables of hyperreflexia. The PAD variable reflects the rate-dependent presynaptic attenuation in the amplitude of the H-reflex (Hultborn et al. 1996; Voerman et al. 2005). In theory, PAD should be negatively correlated to the neuromechanical variables of hyperreflexia, as less frequency-dependent signal attenuation should result in earlier and/or stronger stretch reflexes if all other aspects are kept constant. However, PAD was negatively correlated only with passive ROM, which makes sense as more frequency-dependent attenuation in the Ia afferent inputs should assist in keeping the alpha motoneuron poll inactive (with the caveat that only Sol PAD was assessed). It is believed that the lack of correlation between PAD and neuromechanical variables of hyperreflexia was due to the massive number of possible intervening variables (e.g., muscle-tendon dynamics, excitability of several other spinal circuits modulating the Ia reflex arc). Thus, exploring specific neurophysiological

pathways for clinical diagnostic purposes seems unwarranted, although it may continue to be highly useful in basic research.

Ankle neuromechanical variables. The lack of correlations between maximum ROM and the neuromechanical variables of hyperreflexia and HR is understandable since it does not consider muscle-tendon dynamics or tissue mechanics. Ultimately, it is the mechanical tension in the intrafusal muscle fiber that causes the stretch reflex, which is dependent on muscle-tendon dynamics and on the excitability of the muscle spindle. Furthermore, variables such as the muscle's physiological cross-sectional area and the tissue properties should have much bigger effects on HR than the MTU's extensibility per se (Chleboun et al. 1997; Lieber and Fridén 2019). Thus, although joint maximum ROM is a very useful clinical measure, by itself it does not seem to offer advantages in understanding HR and hyperreflexia. The passive range of motion reflects the extent to which the MTU can be slowly lengthened in a relaxed state. While TD participants could remain with their muscles relaxed throughout most of the stretch, that was not the case in most participants with CP. Thus, the reasoning for this variable was that a participant with very low passive flexibility should respond to a fast stretch with an early and strong stretch reflex, as his alpha motoneuron pool was already somewhat excitable even in the slow stretches. The lack of correlation between passive ROM and hyperreflexia, and passive ROM and HR may have been due to the rigid criteria of how a relaxed muscle is classified (i.e., less than 5% of the maximum isometric activation). It is entirely possible that some participants with CP had a short passive ROM, maintaining a very low muscle activation throughout the stretch, which had no meaningful impact on the excitability of the alpha motoneuron pool and on force production, and thus no impact on hyperreflexia and HR. The results suggest that none of the joint-level neuromechanical variables were useful in gauging hyperreflexia and HR.

6.2 Longitudinal analyses (IV)

The main aim of study IV was to verify the effects of the EXECP intervention by comparing the best performance in the pre-tests with Post1. Furthermore, both pre-tests and Post1 were compared to Post2 to verify the effects of the cessation of the EXECP intervention following the retention period.

Motor Function. A group mean increase in 6MWT distance of 33 and 44 m comparing pre-tests with Post1 and Post2 was found, respectively. Maher et al. (2008) performed the 6MWT with a similar sample (CP, same mean age and GMFCS levels, n = 41) and found a mean test-retest group difference of 1 m, and a 95% confidence interval for individual test-retest variability of ± 43 m. Thus, the present intervention effect seems clinically significant as it is very unlikely that the group mean would shift towards the upper test-retest variability bound per chance. Interestingly, Gillet et al. (2018) induced similar changes in the 6MWT (mean difference = 48 m) with a combined strength training for ankle muscles

and anaerobic training program for slightly older participants with CP (mean age 20 years). Oppositely, Kirk et al. (2016) performed a strength training program for lower limbs and trunk muscles for adults with CP (mean age 36 years), and although their gait kinematics and muscle strength improved, no changes in the 6MWT was found. In all the three studies above, participants had similar GMFCS distributions and a baseline 6MWT performance of 480–502 meters, the interventions had adequate strength training load distribution and other than the age differences, the only major difference was in the chosen training protocol structure. While Gillet et al., (2018) used exercises very relevant to motor function (e.g., stair climbing, changing direction), and gait was trained on the treadmill, the protocol from Kirk et al., (2016) may have failed to increase 6MWT because of the lack of a more generalized motor function training to enhance coordination. Regarding the GMFM scores, merging GMFCS I–III and GMFM dimensions D and E, Oeffinger et al. (2008) reported a minimum clinically important difference of 1.2–1.6 and 1.8–2.6 for medium (0.5) and large (0.8) effect sizes, respectively. In the present study, absolute GMFM score changes (n = 11, median = 3) were equal or above two points for 9 participants, and one point for two participants. It is worth considering that a one-point increase may or may not be functionally relevant. For example, five participants were able to climb stairs with alternating feet after the intervention, instead of stepping twice on the same step as they did before the intervention. Other one-point increases, for example being able to keep balance with one foot up from 2 to 4 s, may not be so functionally relevant. Interestingly, two intervention studies were able to increase both lower limb strength and GMFM using multi-joint functional exercises (e.g., sit and stand, front and lateral step-ups, walking and running exercises; Nikolaos et al. 2019; van Vulpen et al. 2017), while two studies achieved similar results using single-joint knee exercises (MacPhail and Kramer 1995; Moreau et al. 2013). These results demonstrate that different exercise and load configurations can induce motor function gains without adverse side effects, and future research is needed to understand the potential differences, especially for longer term training.

Muscle strength. Thigh muscles responded well to the EXECP intervention. Knee extension had an increase of 19% in peak torque, 31% in rate of force development and 24% in concentric angular impulse, while knee flexion had an increase of 31% in peak torque and 92% in concentric angular impulse. Acknowledging that previous studies had different training loads, testing procedures and CP populations, it seems that all studies discussed below had adequate training loads and testing procedures, and thus will be cautiously compared to the present study. Previous studies reported similar gains for knee extension peak torque (12–27%; Hanssen et al. 2022; Scholtes et al. 2010; Taylor et al. 2013), concentric knee extension and flexion peak torque (25%) and work (21%; MacPhail and Kramer 1995). Kirk et al., (2016) reported a one repetition maximum (1RM) increase of 82% for knee flexion and 45% for knee extension, which is fairly comparable to the present study's concentric angular impulse variable (i.e., assuming a similar repetition duration), and has similar magnitude strength gains. The increase in knee flexion concentric angular impulse was

exceptionally high, and it is probably not a result of only increased muscle strength, but also enhanced motor control to maintain higher torque output throughout the concentric muscle action, even though, curve width did not increase significantly after the intervention.

The present intervention did not cause significant changes in the strength of the shank muscles, which is in contrast with other studies that successfully induced adaptations in plantarflexion peak torque (25–77%; Gillett et al. 2018; Hanssen et al. 2022; van Vulpen et al. 2017), plantarflexion and dorsiflexion 1RM (137% and 87%, respectively; Kirk et al., 2016). Gillet et al. (2018) had a similar training load as the present study, however most of their exercises (4 out of 5) trained plantarflexion, while one trained dorsiflexion. Thus, it is reasonable that they successfully increased plantarflexion peak torque, while no changes were reported for dorsiflexion. Kirk et al. (2016) also had a similar training load compared to the present study and reported a large increase in plantarflexion (137%) and dorsiflexion (87%) 1RM test, in the same device that training occurred. However, no changes in dorsiflexion peak torque and rate of force development were found when measured by a stationary dynamometer (increases in rate of force development in shorter time windows were found), suggesting that specificity between testing and training is an important factor. In the present study, the plantarflexion testing position had the knee and ankle at the anatomical position, which placed both hamstrings and gastrocnemius muscles in a lengthened and often slightly uncomfortable position (participants with CP consistently asked to flex their knee during rest). Since the weight used for exercising these muscles consistently increased for all participants during the intervention, it may be that the testing position may have blurred the strength gains for plantarflexion. Lastly, dorsiflexion was the only muscle action trained with manual resistance and rubber bands, highly dependent on the ability of the trainer to keep the appropriate stimulus. Therefore, these aspects may reduce the efficiency of training these ankle muscles, and a higher training load may be required for adaptations to be verified.

The EXECP intervention was not able to increase curve width in any of the four muscle actions, not even in the thigh muscles which had a significant increase in concentric angular impulse, demonstrating an inability to maintain steady torque output during the movement. This result is reasonable, since only the first training month of the intervention had an optimum training load configuration (i.e., slow and controlled movement) for curve width enhancement. Increases in trunk muscles strength followed an expected pattern: a) for trunk extensors, maintaining the maximum exercise duration (60 s) was already possible in the first month of the intervention for 12 participants, thus increasing the weight (900%) was the main procedure for increasing the training load; b) for trunk flexors, only 5 participants were able to achieve the maximum exercise duration (60 s) in the first month, thus increasing the hold duration (33%) was the main way to increase the training load. Additionally, a slight increase of weight in the trunk flexion exercise (i.e., weight on the distal shank and held above the head) causes a large increase in difficulty due to the very long

resistance arm. Finally, since the participants were not used to progressive resistance training, increases of muscle strength may be affected by psychological aspects (e.g., willingness to exert maximum efforts), as the ten-fold increase in trunk extension weight suggests.

Joint flexibility. The EXECP intervention caused a mean joint flexibility increase of 5° for both passive knee and hip extension. Although statistically significant, the change magnitude seems functionally insignificant, although it was achieved with a low training volume (mean of 6 min per week), and it is unclear if a higher training volume could have yielded a better outcome. Furthermore, it is also unclear if the enhanced flexibility was due to sensorimotor (i.e., increased tolerance to stretch) or structural adaptations (i.e., morphological changes in the muscle-tendon structure), as this topic is still under active investigation (Chagas et al. 2016; Weppler and Magnusson 2010). It seems unlikely that flexibility training with a feasible training load can induce mechanical alterations in most participants with CP. This claim is based on the following arguments: 1) for clinical populations there is an overall lack of evidence for stretching effectiveness (Harvey et al. 2017), and specifically for CP (Pin et al. 2006; Walhain et al. 2021; Wiart et al. 2008), 2) morphological alterations in CP muscle, such as a lower number of satellite cells and ribosomes, should mechanistically hinder muscle growth response (Gough and Shortland 2012; Lieber and Fridén 2019; Von Walden et al. 2018), 3) sarcomerogenesis is hindered in CP, evidenced by the usual reduced number of lengthened sarcomeres found in CP muscle (Lieber and Fridén 2019). Given the widespread use of stretch as a treatment for CP, and the lack of evidence of its effectiveness, current therapeutic practices should focus on other interventions that have been shown successful, such as strength and gait training. Finally, the data shows that the intervention had no negative effect on joint flexibility, which has been an anecdotal concern of many health practitioners.

Covariates. Overall, $GMFM_{co}$ was very useful in improving the GLMM models. $GMFM_{co}$ was expected to affect the 6MWT outcome since it includes many items related to gait, but $GMFM_{co}$ also worked very well for plantarflexion and knee extension (torque, rate of force development and concentric angular impulse), and knee flexion (rate of force development and concentric angular impulse). Interestingly, $GMFM_{co}$ was not useful to stratify participants in any of the dorsiflexion variables, meaning that participants with high GMFM scores had similar performance than participants with low scores.

Participants with higher GMFM were able to perform more exercises during the training session due to improved training logistics (e.g., faster locomotion, easier setup attainment). Interestingly, training load variables such as total number of training sessions and strength exercises, amount of gait training and number of stretching exercises had no effect on the statistical models. Confounding variables such as individual physiology (e.g., hormones, nutrition) and the extent to which the participant was willing to push himself (i.e., exercise intensity) could have blurred the logical correlation between the training load variables and the induced adaptations.

Control and Retention Periods. Maturation and normal daily activities did not affect the studied variables in both groups during the control period, except for plantarflexion rate of force development in the TD group. A few CP $GMFM_{co}$ subgroups had significant changes between pre-tests for knee extension (peak torque and rate of force development) and knee flexion (rate of force development), although non-significant for the whole group. While not significant, a clear declining trend towards pre-test values was found in Post2, which was expected since the intervention stopped at Post1. It is well document in TD people that once the training stimulus cease, the induced adaptations regress towards pre-training status (Häkkinen et al. 2022; Mujika and Padilla 2000; Psilander et al. 2019). Furthermore, detraining after 6–12 weeks in CP has also been reported, corroborating with the present findings (MacPhail and Kramer 1995; Scholtes et al. 2010; Taylor et al. 2013). This result suggests that a life-long change in behavior is needed for people with CP, physical training must be continued throughout the person's life spam.

Study limitations (IV). The main limitation of study IV was the sample size; it was unfeasible to reach 24 participants due to constraints of time and resources. The ICC_{GLMM} displayed in table 11 shows that the within group variability was very large and allowing the model to have a $\beta 0$ (i.e., y-intercept) for each participant was very helpful. A higher sample size would have allowed the use of the participants as a random effect, making it possible to find clusters of participants with different slopes (e.g., responders and non-responders). Test-retest reliability (i.e., ICC) was adequate for PKE, 6MWT, and most knee extension and plantarflexion strength variables, while the lower 95% CI bounds of dorsiflexion and knee flexion strength variable reached the poor reliability threshold. Furthermore, some variables had very high SEM values, especially the dorsiflexion variables for the CP group. The SEM represents the random variation in a measure, and high values associated with the low sample size surely negatively affected the present study's power. This result suggests that similar intervention studies should perform longer familiarization sessions and at least two pre-tests before an intervention, not only to verify maturation effects, but also to evaluate the test-retest variability. Since Maher et al. (2008) had reliability data for the 6MWT with a similar sample, it was decided to use the available time to duplicate other measures. Overall, curve width for both groups had very poor reliability even with a familiarization session, clear instructions, and multiple trials per test, suggesting that this measurement is not reliable and should be modified in future studies. Another important inherent limitation of training studies is that not all training sessions were optimal: participants were sometimes tired, in a bad mood or unmotivated. Thus, optimizing training for good execution and attitude, nutrition and resting, is a process which surely demands more than three months. Lastly, the criterion for diagnosing hip flexors shortness was corrected from 0° in the protocol article to 20° of hip extension, as it is evident that hip extension is necessary during the late stance phase of gait.

6.3 Practical and ethical issues

6.3.1 Testing sessions

The peripheral nerve stimulation was challenging in the young participants. The progressive increase in stimulation intensity to build the recruitment curve caused anxiety in several participants, and the help from parents to comfort and calm their children was instrumental. Care was taken to not let the participant be aware of the stimulation timing, as it generally led to muscle activation. One participant with CP refused to perform the procedure, while the other tried and received a lot of support from parents, however the examiner chose to discontinue the test since the participant was visibly in distress. In the case of the latter participant, the parents wanted the test to be performed as they thought that it could bring important diagnostic information. The thesis author explained that while that could be true, the fact that the participant was under discomfort would alter and invalidate the test. Furthermore, and more importantly, even with the approval and support from the parents, the test could not continue due to distress to the participant. As stated in the declaration of Helsinki (World Medical Association. 2001): ”Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject”.

6.3.2 EXECP intervention

The intervention was carried out smoothly and the communication between the trainer, families and the thesis author happened at least weekly. Unfortunately, there was one exception, one 9-year-old participant chose to drop out because he thought that the combination of school, other activities and the intervention was too much for him to handle. After discussing with all involved parts, the author believes that the combination of the parents being excited and incentivizing the participant, with the trainer not recognizing the demotivation cues during the training sessions, caused a dissatisfaction buildup that led to the drop out. Various load adjustments were successfully made during the intervention for many participants, which were made possible by effective communication. Thus, especially for young participants, it seems that instructing parents and trainers to not only motivate, but also listen to the complaints and actively try to solve them, could solve this type of drop out.

The participants, especially the young ones, very much enjoyed the ability to choose the exercise order in the training sessions. It gave them a sense of autonomy and control over their training, which was reported by many to make the training sessions more enjoyable. Only benefits were identified from this procedure as it did not negatively affect the training load or the training sessions in any way. Thus, this simple and yet beneficial procedure could be adopted in other training settings.

In the current sample, a patent difference between physical educators and physiotherapists was perceived by the thesis author. Generally, physiotherapists were very careful with positioning and execution of exercises, however they often failed to comply with the overload and load progression training principles. Most physiotherapists tended to be very sensitive to participant's complaints and would stop pushing them to train prematurely. The physical educators, on the other hand, were clearly paying more attention to these training principles, however some lacked kinesiological understanding for optimal subject positioning and adjustment. Furthermore, physical educators generally do not receive training on how to deal with different participant populations other than typically developing people, and the thesis author had to thoroughly explain these to them. In conclusion, neither of the two pools of professionals were completely prepared to perform an ideal intervention, and a lot of extra information and monitoring from the thesis author was necessary for ensuring acceptable (not optimal) execution quality. This aspect needs more attention in future interventions, as ensuring a high quality of training is paramount for a successful intervention. Lastly, due to the above-mentioned aspects, home-based and community-based interventions are unlikely to produce good outcomes. At least in the first months of training, the participants must receive clear instructions about how to execute each exercise, and later they could train well with less or no assistance, depending on the level of impairment.

6.3.3 Informed consent

The results of blood exams, body composition and flexibility tests were provided and discussed with the participants after the pre-tests. Additionally, after the EXECP project, a follow-up training program was provided to the participants so that they could exercise with their families. Many of the participants and parents stayed in contact with the thesis author for years, and asked various questions, which were always promptly replied. From this sample in Finland, throughout the years of 2017–2020, it became very evident that there is a notable lack of information provided to individuals with CP and their families. Usually, a team of health professionals decide the "best course of action" and send a letter to the parents explaining the next phase of the treatment. It is understandable that medical professionals want to present a confident and united message, and that the subject is not easily understood by a person without specific medical training. However, current treatment knowledge is far from settled, all procedures carry risks and benefits, thus time must be spent given full information before these treatments are agreed upon. For example, tendon lengthening surgery and selective dorsal rhizotomy can have very desirable or catastrophic consequences. Therefore, it seems appropriate that the person and their guardians fully understand and choose accordingly, as they will be the ones ultimately dealing with the consequences of their choices.

6.4 Project limitations

Besides the specific study limitations already discussed, the EXECP had other important limitations. The recruitment process resulted in an unbalanced sample both in GMFCS (78% level I, no level II, 22% level III) and level of involvement (67% unilateral, 33% bilateral). Furthermore, the age range (9–22) was wide, including young adults (n = 5, 19–22 years) and young children (n = 8, 9–12 years). In addition to the small, wide and unbalanced sample, most of the measurements were lab-based, therefore, caution is necessary for the generalizability of the results. Another limitation was that the EXECP project had no participant reported outcome variables, which would be important to assess improvements considered important by the participants and not measured in your testing battery. Due to the large number of tests, training sessions and home training, it was unfeasible to add further work to the participants.

6.5 Clinical considerations

The present thesis offers several important clinical considerations for the treatment and evaluation of individuals with CP. Firstly, the SRT as a measure of hyperreflexia is promising when the latency correction is performed, because it had a high correlation with the hyper-resistance measures and differed between CP and TD groups. Oppositely, the H-reflex and PAD neurophysiological variables had no correlation with hyperreflexia, casting doubt into their clinical usefulness. The joint maximum range of motion is an important functional variable, but it had not correlation with hyper-resistance and hyperreflexia, therefore being insufficient for clinical diagnosis.

Unfortunately, no easy assessment method to distinguish between the components of HR is available. Currently, the best strategy seems to be measuring HR accurately with a torque sensor, while using IMUs to measure joint angular velocity and acceleration. With the above-mentioned setup, the clinician can perform stretches at different velocities to infer about the velocity-dependent contribution on hyperreflexia. Interestingly, the present study found a very large within-group variability in the delta peak torque between $10 \cdot s^{-1}$ and $110 \cdot s^{-1}$, suggesting that the contribution of each of the three HR components varies considerably among people with CP.

Study IV confirmed previous studies showing that muscle strength and motor function can be enhanced with progressive resistance training without any adverse effects. Although no side effects were found in the present thesis, the next aim is to evaluate if the intervention provoked any changes in the neuromechanical properties of triceps surae muscle. By providing clear evidence of no adverse effects, it is hoped that strength training for CP is fully embraced by physiotherapists and clinicians. The stretching protocol did not seem to provoke any meaningful alterations in the participant's MTU, corroborating with

a growing list of studies questioning its therapeutic use. Finally, it is important to reflect that all intervention studies discussed in this thesis had a very short duration (≤ 3 months), considering that training in CP must be a life-long endeavor. Longer duration studies, tackling participant motivation, nutrition, sleep, and cycling through different training load configurations are vital to push current understanding about training adaptation in CP.

7 MAIN FINDINGS AND CONCLUSIONS

The main findings and conclusions of the present thesis are summarized as follows:

1. It is vital to consider SR latency when assessing the SRT and consequently the TSRT. To the best of the author's knowledge, most if not all current research using the SRT as a measure of hyperreflexia has incurred in this error, thus a careful re-examination of data is important to update understanding of this promising assessment method.

2. Compared with the TD controls, participants with CP had a smaller maximum and passive ankle joint ROM, higher SRT and SR EMG-RMS, higher peak torque in all three studied velocities and lower PAD. Furthermore, neurophysiological and neuromechanical variables of hyperreflexia assessed from the soleus muscle were not correlated. Finally, ankle neuromechanical variables were not correlated to HR or the neuromechanical variables of hyperreflexia.

3. The EXECP intervention was effective in enhancing gait performance and overall gross motor function. Furthermore, the training program containing strength, flexibility and gait training was found safe and feasible for children and young adults with CP.

4. Following the retention period, most adaptations induced by the EXECP intervention regressed back towards pre-test values. This result suggests that a life-long change in behavior is needed for people with CP, physical training must be continued throughout the person's life spam.

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ORIGINAL PUBLICATIONS

I

EXERCISE INTERVENTION PROTOCOL IN CHILDREN AND YOUNG ADULTS WITH CEREBRAL PALSY: THE EFFECTS OF STRENGTH, FLEXIBILITY AND GAIT TRAINING ON PHYSICAL PERFORMANCE, NEUROMUSCULAR MECHANISMS AND CARDIOMETABOLIC RISK FACTORS (EXECP)

by

Valadão, P., Piitulainen, H., Haapala, E. A., Parviainen, T., Avela, J & Finni, T. (2021).

BMC Sports Science, Medicine and Rehabilitation 13:17.

https://doi.org/10.1186/s13102-021-00242-y

Valadão et al. BMC Sports Science, Medicine and Rehabilitation (2021) 13:17
https://doi.org/10.1186/s13102-021-00242-y

BMC Sports Science, Medicine and Rehabilitation

STUDY PROTOCOL Open Access

Exercise intervention protocol in children and young adults with cerebral palsy: the effects of strength, flexibility and gait training on physical performance, neuromuscular mechanisms and cardiometabolic risk factors (EXECP)

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Abstract

Background: Individuals with cerebral palsy (CP) have problems in everyday tasks such as walking and climbing stairs due to a combination of neuromuscular impairments such as spasticity, muscle weakness, reduced joint flexibility and poor coordination. Development of evidence-based interventions are in pivotal role in the development of better targeted rehabilitation of CP, and thus in maintaining their motor function and wellbeing. Our aim is to investigate the efficacy of an individually tailored, multifaceted exercise intervention (EXECP) in children and young adults with CP. EXECP is composed of strength, flexibility and gait training. Furthermore, this study aims to verify the short-term retention of the adaptations three months after the end of the EXECP intervention.

Methods: Twenty-four children and young adults with spastic CP will be recruited to participate in a 9-month research project with a 3-month training intervention, consisting of two to three 90-min sessions per week. In each session, strength training for the lower limbs and trunk muscles, flexibility training for the lower limbs and inclined treadmill gait training will be performed. We will evaluate muscle strength, joint flexibility, neuromuscular and cardiometabolic parameters. A nonconcurrent multiple baseline design with two pre-tests and two post-tests all interspaced by three months is used. In addition to the CP participants, 24 typically developing age and sex-matched participants will perform the two pre-tests (i.e. no intervention) to provide normative data.

(Continued on next page)

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Discussion: This study has a comprehensive approach examining longitudinal effects of wide variety of variables ranging from physical activity and gross motor function to sensorimotor functions of the brain and neuromuscular and cardiometabolic parameters, providing novel information about the adaptation mechanisms in cerebral palsy. To the best of our knowledge, this is the first intervention study providing supervised combined strength, flexibility and gait training for young individuals with CP.

Trial registration number: ISRCTN69044459, prospectively registered (21/04/2017).

Keywords: Cerebral palsy, Strength, Flexibility, Gait, Training, Neuromuscular, Cardiometabolic

Background

Cerebral palsy

Cerebral palsy (CP) describes a group of permanent disorders of the development of movement and posture, that are attributed to nonprogressive disturbances that occurred in the developing fetal or infant brain [1]. The spastic-type CP accounts for approximately 80% of all CP cases [2–4] and is characterized by hyper-resistance [5] which has three components: stretch hyperreflexia (i.e. velocity dependent involuntary activation), involuntary background activation and altered muscle-tendon mechanical properties. Muscle-tendon unit mechanical alterations [6–8] can cause severe reduction in joint range of motion (i.e. contractures) and lead to bone deformation [9–11]. The development of contractures does not seem to be caused solely by hyperreflexia [12, 13]; rather muscle inactivity and impaired muscle growth seems to be important factors [14, 15]. Furthermore, another debilitating symptom in CP is a decreased muscle strength [6, 16–18] that severely hinders the ability to perform tasks such as walking and climbing stairs [19–21]. These secondary clinical symptoms induce lower physical activity (PA) levels in people with CP [22, 23] and lead to higher levels of body adiposity and other cardiometabolic risk factors [16, 24, 25]. Therefore, although the initial brain lesion is nonprogressive in CP, secondary symptoms such as reduced muscle strength and joint range of motion (ROM) typically further deteriorate with time, increasing sedentarism and creating a cycle of inactivity and loss of function [26, 27]. Thus, well targeted therapeutic interventions to reduce these secondary problems and maintain motor function are in pivotal role to enable independent mobility in individuals with CP and thus support their lifelong health and wellbeing.

Strength training for CP

Cross-sectional studies demonstrated that muscle strength had a high shared variance with gait and motor function in CP (r^2 = 50–60% [28, 29];). Furthermore, the effectiveness of strength training complying with known training guidelines [25, 30, 31] to increase muscle strength in individuals with CP has been well demonstrated [32–40]. Nevertheless, these interventions have reported no changes [33, 35–38] or improvement in motor function [32, 34, 35]. This controversy seems to arise from two main reasons. Firstly, functional tasks involve a complex interaction between muscle strength, joint flexibility (i.e. maximum joint ROM) and motor coordination. Secondly, the neural and morphological adaptations are highly specific to the strength training methods [35, 41], which varied in these studies. A final remark is that every day functional tasks involve activation of several muscle groups spanning across joints. Thus, an adequate intervention should train all relevant muscles with naturalistic patterns of neural activation.

A major yet unproven concern is that strength training could increase hyper-resistance and thus would be an unadvisable intervention [42]. Only few strength training studies have measured components of hyper-resistance longitudinally and reported no changes after the intervention [33, 34, 37, 38]. Furthermore, the term "spasticity" is often used under varied definitions and assessment methods [43]. For example, the modified Ashworth Scale [44] utilized in two of these studies [33, 34] is a subjective assessment of resistance to passive movement [45]. Scholtes et al. [37, 38] evaluated hyperreflexia by assessing the presence of a catch (i.e. sudden increase in muscle tone) in response to a single fast (< 1 s) passive stretch, being also a subjective measure of the velocity-dependent resistance to passive movement. Both measurements fail to differentiate between the different components of hyper-resistance. Therefore, although it is generally accepted that strength training does not increase "spasticity" [46], no longitudinal study has reported an objective, valid and reliable measurement of hyper-resistance.

Flexibility training for CP

Stretching is often utilized to prevent or treat loss of joint flexibility, however a recent systematic review pooling different stretch modalities (e.g. splint, serial casting, manual therapy) for different patient groups (e.g. CP, stroke, spinal cord injury) reported high-quality evidence that it does not have clinically relevant effects [47]. Passive stretching has the advantage of not restricting joint

movement for a prolonged time as opposed to serial casting or splints, however, its effectiveness to increase joint flexibility in people with CP is unclear [48, 49]. Theis et al. [50] demonstrated that passive dorsiflexion stretching caused an acute increase in flexibility due to the elongation of both muscle and tendon. However, comprehensive longitudinal data is scarce. Theis et al. [51] showed an increase in dorsiflexion flexibility after a 6-week passive stretching intervention (4 days per week, 15 min per day). Zhao et al. [52] demonstrated that passive dorsiflexion stretching and active ankle joint movement within a 6-week intervention was able to increase fascicle length of both soleus (Sol) and medial gastrocnemius (MG). However, the study did not have a control group and the sample was composed of children, thus maturation may have been an intervening variable. The disparity between the limited scientific understanding of stretching effectiveness for CP and its widespread utilization must be addressed swiftly.

Gait training for CP

The inability to dorsiflex the ankle joint during the stance phase (i.e. toe walking) and swing phase of gait (i.e. foot drop) are common problems in CP [53]. A recent literature review has concluded that gait training is safe, feasible and effective to improve walking ability in children and young adults with CP [54]. The incline treadmill setup seems particularly effective as it forces a greater ankle dorsiflexion in the swing phase and serves as a dynamic passive stretch for the ankle plantarflexors in the stance phase [55, 56]. Daily gait training with an inclined treadmill has been demonstrated to increase walking speed, dorsiflexion strength and active ROM, and reduce ankle stiffness in only four to six weeks [57, 58]. No changes in ankle passive flexibility were found in these studies, however the decrease in passive stiffness suggests that the muscle-tendon unit is adaptable, but further improvements in ROM may require a longer period of training. It is noteworthy that altering gait pattern and automatizing it for use in everyday over ground walking may require intense individualized feedback, so the person is aware or is at least guided towards the correct pattern (e.g. verbal feedback, biofeedback/augmented reality; [54]).

Study purpose

The present study has three aims: 1) to investigate the effects of the EXECP intervention on the following variables: gait performance (primary outcome), physical activity level, lower limb muscle strength and joint flexibility, gross motor function, cardiometabolic risk factors, neuromuscular parameters for triceps surae muscle, cortical processing of proprioceptive stimuli evoked by passive ankle dorsiflexions, tibialis anterior (TA) cortico-muscular and intramuscular coherence; 2) to evaluate the retention of the adaptations induced by the intervention after three months; 3) to compare CP and typically developing (TD) individuals in all studied variables using group and matched (age/sex) individual data. The EXECP intervention is composed of a three-month individually tailored exercise intervention containing strength training targeting lower limb and trunk muscles, combined with stretching of diagnosed short muscles and gait training. The primary hypothesis is that the EXECP intervention will enhance gait performance by: a) increasing walking distance in the 6 min walking test; b) increasing ankle dorsiflexion during the swing and stance phase of gait; c) increasing maximal walking velocity, joint net moments and ranges of motion in the lower limb joints. The secondary hypothesis are that the EXECP intervention will: d) increase habitual PA; e) increase maximal isometric and concentric torque and rate of force development for the trained muscles; f) not affect cardiometabolic risk factors such as arterial stiffness, insulin resistance, blood lipids and body fat content; g) increase gross motor function measure (GMFM; [19]) score; h) increase lower limb joint flexibility of the trained muscle groups; i) decrease ankle plantarflexors average joint stiffness and increase joint energy (i.e. area under the torque-angle curve) during slow passive stretching; j) decrease antagonist muscle electromyography (EMG) during maximal voluntary isometric and concentric plantarflexion and dorsiflexion; k) not change spinal mechanisms related to hyperreflexia in the Sol muscle (i.e. Hoffmann-reflex (H-reflex), post-activation depression (PAD) ratio, tonic stretch reflex threshold (TSRT)); l) increase amplitude of cortical responses to proprioceptive stimulation, recorded with magnetoencephalography (MEG) in the primary somatosensory cortex; m) increase cortico-muscular coherence between cortical MEG and TA EMG signals; n) increase intramuscular coherence within TA muscle during a submaximal isometric dorsiflexion and during the swing phase of the gait cycle. No changes in any of the studied variables are expected during the 3-months control period, except if the participant is going through the growth spurt. After the 3-month maintenance period, the values of the studied variables are expected to be in between the control and post intervention values (project aim B). All study variables are hypothesized to show differences between the TD and CP groups (project aim C). It is expected that the comprehensive assessment battery of neuromuscular, brain, cardiometabolic and functional parameters will add valuable information about how people with CP adapt to exercise.

Methods

Study design

The present study utilizes a nonconcurrent multiple-baseline design [59, 60] composed of two pre-tests interspaced by three months (i.e. control period), followed by the three-month EXECP-intervention, and two post-tests performed immediately after and three months after the intervention (i.e. maintenance period; Fig. 1). The TD age and sex-matched control group will be tested in the two pre-tests without the intervention. The nonconcurrent multiple baseline design was selected over the randomized controlled trial (RCT) because the participants inside the inclusion criteria are expected to form a highly heterogeneous group regarding the studied variables (e.g. motor control, muscle strength, joint flexibility and gait mechanics), and randomly dividing the sample into two groups is not likely to result in two comparable groups. The chosen experimental design circumvents the expected high variability in the studied variables by allowing the subjects to be their own controls. Additionally, it allows performing the EXECP intervention all year-round, avoiding systematic seasonal influences to affect the study variables (e.g. vacations, seasons [61];). During the participation in the project, both groups will perform their normal activities (i.e. physiotherapy and/or sport activities), with the instruction of maintaining the number of sessions and activity type as stable as possible. Exceptionally during the intervention period, a reduction in the volume of the activities may be necessary to avoid overloading the participants. Any important deviations from the activities done during the control period will be reported.

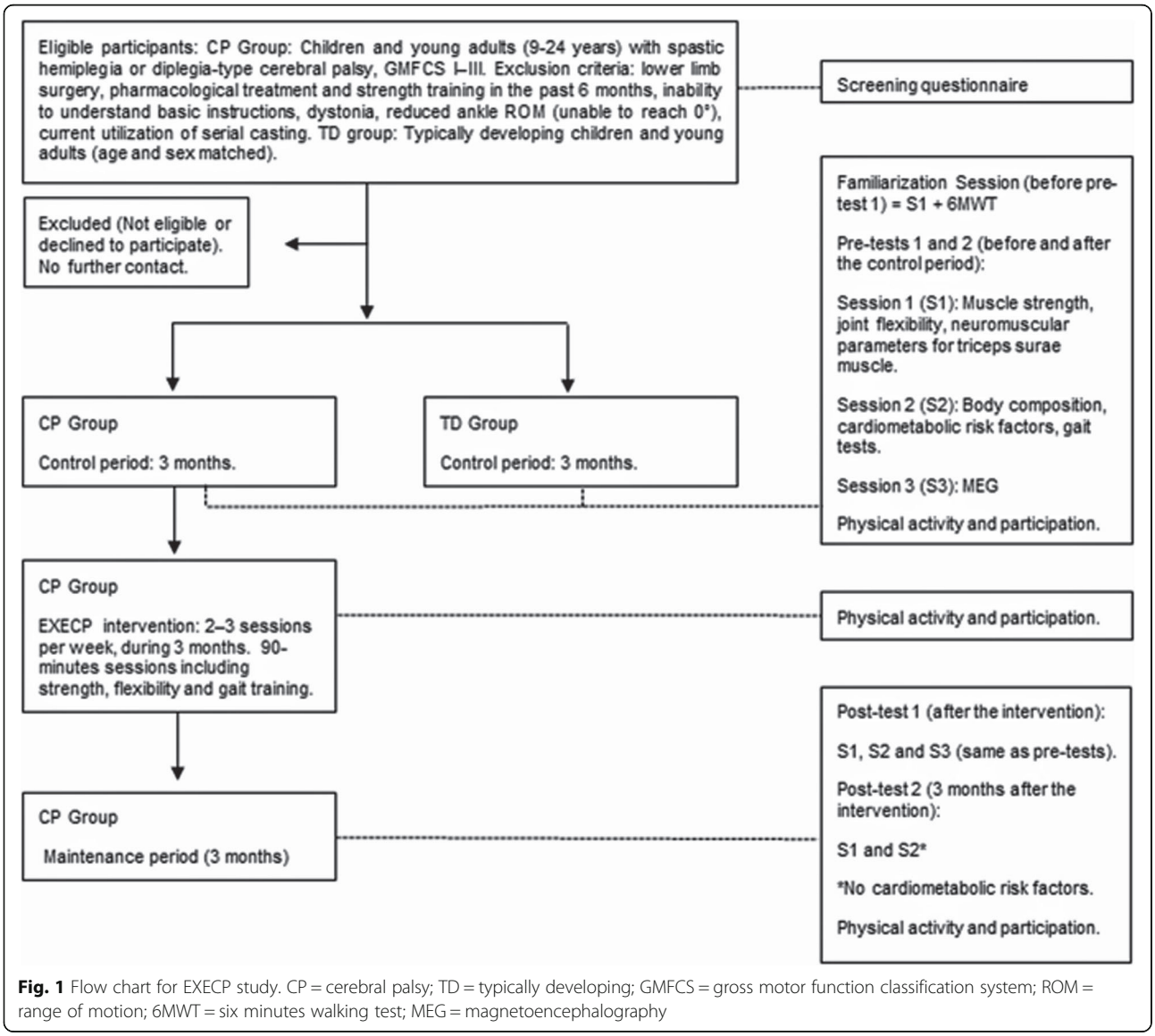

Fig. 1 Flow chart for EXECP study. CP = cerebral palsy; TD = typically developing; GMFCS = gross motor function classification system; ROM = range of motion; 6MWT = six minutes walking test; MEG = magnetoencephalography

The first author will be responsible for all tests, and it is not possible to blind him to group allocation. Testing procedures were defined in detail to ensure consistency. The outcome data is quantitative and objective, with little possibility to suffer from observer bias. However, because the tester will obviously hope for better results in the Post-tests, no data analysis will be performed before the data collection is done (except for the flexibility tests). Thus, the tester will not have any knowledge of the results and will be focused on implementing the tests according to the protocol.

Study sample and recruitment

Twenty-four individuals aged 9–24 years with spastic-type CP will be recruited predominantly in the city of Jyväskylä, Finland. Hospitals, physiotherapy clinics and CP associations will be contacted in search of suitable participants. Acquiring the whole sample size in Jyväskylä was not possible, so the recruitment of participants was extended to nearby cities. Furthermore, the coronavirus caused a three-month lockdown in spring 2020, which paused the participation of some participants and delayed recruitment. Twenty-four typically developing age and sex-matched controls will also be recruited from local schools and the University of Jyväskylä. The recruitment has been ongoing since May of 2017 and it is expected to end in December of 2020.

Inclusion criteria

The project will accept male and female participants who are aged 9–24 years, with a confirmed diagnosis of unilateral or bilateral spastic CP and are classified as level I to III in the Gross Motor Function Classification System (GMFCS [62];).

Exclusion criteria

The project will not accept participants who: a) have had lower limb surgery and/or pharmacological treatments (e.g. intrathecal baclofen, botulinum toxin) in the past six months; b) have had selective dorsal rhizotomy; c) are utilizing serial casting on the lower limbs; d) have participated in a resistance training program for the lower limbs in the last six months; e) are unable to provide sufficient cooperation in the intervention and testing sessions; f) are unable to stand with both heels touching the floor (i.e. ankle in anatomical position). Exceptionally, participants within exclusion criteria a) and b) may be accepted as case studies.

Sample size

Power calculation was performed for the project's primary hypothesis (i.e. gait performance), specifically for the effect of the EXECP intervention on walking performance measured by the six minutes walking test (6MWT [63];). The distance covered in the 6MWT is reported to reflect functional capacity and has been recommended as a submaximal exercise test for children with CP of GMFCS I to III [64, 65]. Results from Pre-tests 1 and 2 for the first 9 participants in the project were utilized for sample size calculation utilizing an online spreadsheet (www.sportsci.org [66];). Data was log-transformed due to the inexistence of zero and negative values, yielding a typical error of 1.06, between-subject SD of 1.46 and a smallest important change of 1.08. Utilizing the pre-post parallel-groups RCT model, with a power of 0.8 and an alpha of 0.05, a sample size of 24 participants in both CP and TD group is required. No specific sample size calculation algorithm for our experimental design was found, thus the RCT calculation served as an upper bound for the sample size.

Study procedures

The EXECP intervention will mainly take place at the NMRC, although it can be performed in other gyms having all required training equipment. It consists of two to three training sessions per week for 12 weeks. The number of weekly sessions will depend on the level of PA of each participant: those engaged in regular weekly PA may choose to perform 2 or 3 sessions, while sedentary participants will train 3 times per week. Training sessions are 90 min in duration and are separated by at least 48 h. Each training session starts with an inclined treadmill walking (5–10 min), followed by strength (60–75 min) and flexibility training (10–20 min). To ensure optimum training quality all sessions are performed individually and supervised by a strength and conditioning coach or a physiotherapist with full understanding of the training protocol.

Gait training

A portable mechanical treadmill with an inclination of 6° or 7.3° (Vida XL, Venlo, Netherlands) will be utilized for training. Participants are instructed to walk at a comfortable speed, avoiding toe walking and trying their best to attain heel strike. A non-motorized treadmill was chosen because belt movement is attained by pushing it with hip extension and ankle plantarflexion, reinforcing a correct pattern of muscle activation. Verbal feedback will be constantly given during the warm-up to improve gait quality, and the participant will be allowed to stop and rest whenever necessary. In addition to 5–10 min of gait training in every session, all participants will receive a treadmill to take home, and are asked to walk a minimum of 10 min per day every day for the duration of the intervention. An exercise diary will be filled by the participants or their guardians to document the weekly walking durations. During the maintenance period, the participants will choose if they want to keep using the treadmill at home and updating their diary or stop and return it.

Strength training

Five single joint and five multi-joint exercises were selected for the intervention (Table 1). This composition was chosen because single joint exercises allows greater loading control minimizing compensatory movements [24, 64], whereas multi-joint exercises offer a mixture of strength and motor control training that may be beneficial for motor function. The strength training program has two protocols (A/B), with a minimum of 7 exercises targeting lower limb and trunk muscles. However, depending on the speed that exercises can be performed, which varies considerably between GMFCS levels, 7 to 10 exercises may be performed in every session. The protocols are trained weekly in the order AB (2 sessions per week) or an alternating pattern of ABA/BAB (3 sessions per week).

Ankle plantarflexors will be trained with seated and standing calf raises. Ankle dorsiflexors will be trained utilizing a rubber band resistance against the dorsiflexion movement. Additionally, TA will also be trained isometrically during a hip flexion exercise, in which the participant laying supine must flex the hip against the rubber band resistance placed on the forefoot. Manual resistance may be used instead of the rubber band in case the trainer perceives it as easier to apply the resistance during the entire ROM. The thigh muscles will be trained utilizing seated knee extensor and knee flexor machines. Additionally, lower limb muscles will be trained using the leg press and squat holding with both hands an adjustable support. A dense foam ball may be placed between the participant's knees to prevent hip adduction during leg press and squat. Exercises will be done mostly unilaterally due to strength differences between limbs (80–100% of total training volume), except the squat which will mostly be trained bilaterally. Bilateral training may be performed if exercise technique is correct and limb loading appears similar; based on the participant's perception of fatigue/effort and the trainer's visual evaluation. Trunk and hip flexors will be trained isometrically with the hollow rocks exercise, in which the participant lies supine on the floor and must lift the legs slightly above the floor (i.e. hip and trunk flexion, knee extension). Trunk extensors will be trained isometrically using an inclined (30–45°) roman chair. Table 1 describes the positioning, target muscles and kinesiology of each exercise.

To enhance autonomy, comfort and interaction within the sessions, participants can choose the order of the exercises if this complies with the general rule of avoiding fatigue build up, which could hinder both execution quality and intensity. More specifically, this rule states that exercises for the same muscle group always must be separated by at least one different exercise (e.g. seated and standing calf raises cannot be executed consecutively). The trainer will guide this process and suggest possible sequences. Table 2 provides an example for both protocols.

The training load was devised to increase muscle strength and mass, complying with the American College of Sports Medicine and National Strength and Conditioning Association guidelines [30, 31] and specific guidelines for CP [25]. The intervention is divided in 3 blocks of 4 weeks: the first block consists of 3 sets of 8 repetitions maximum (i.e. the ninth repetition cannot be attained due to fatigue), movement duration of 6 s (3 s concentric and 3 s eccentric), 60s of rest and no muscle relaxation between repetitions. This training load has been shown adequate to increase muscle strength and mass, and it is very safe as the intensity is approximately 50% of 1 repetition maximum [67]. Furthermore, people with CP usually perform strength exercises with fast concentric bursts and have difficulty in controlling slow eccentric muscle actions. Thus, this protocol also tackles an important motor control deficit in CP. In the second block, the volume is maintained while the intensity is increased by reducing the concentric movement duration to 1 s and increasing the rest to 90 s. In the third block, training volume and rest are maintained, but the sets are increased to four while the repetitions are decreased to 6, concentric muscle actions are now done as fast as possible while eccentric muscle action duration is reduced to 2 s. Thus, concentric power is trained, and the modifications permits further increases in exercise intensity. Table 2 depicts the training load progression during the intervention.

Exceptionally, the squat exercise will follow a different progression because of its inherent higher intensity (due to body weight): one to four sets of 10 repetitions with the largest attainable ROM will be performed. Movement duration is similar to the other exercises, while the rest starts at 90 s, and will be decreased when possible to 60 s. After the entire volume is executable with 60 s of rest, balance disks (Casall, Vantaa, Finland) will be placed under the participant's feet to cause instability and increase exercise difficulty, also unilateral squats may be utilized to further increase the exercise intensity.

An important aspect of isoinertial strength training is that the ability to produce torque varies throughout the joint ROM, due to the sarcomere force-length curve and the joint's lever arm [68]. Furthermore, CP joint ROM is often reduced [69] and muscle weakness is present [24]. Thus, allowing CP participants to perform the strength exercises by themselves leads to a movement with a smaller joint ROM (as compared to the total possible active ROM), and the non-optimum part of the torque-angle curve is not trained. Since strength gains are generally higher at the trained angles (i.e. length-specific adaptations [70, 71];) and increasing the torque production

Table 1 Description of the strength training exercises

	Seated Calf Raise	Standing Calf Raise	Seated Dorsiflexion	Seated Machine Knee Extension	Seated Machine Knee Flexion	Seated Horizontal Leg Press	Squat	Hip Flexion	Roman Chair Trunk Extension	Hollow Rock
Muscles targeted	Soleus, Gastrocnemius	Soleus, Gastrocnemius	Tibialis Anterior	Quadriceps femoris	Hamstrings	Gluteus maximus, quadriceps femoris, hamstrings, triceps surae	Gluteus maximus, quadriceps femoris, hamstrings, triceps surae	Iliopsoas, rectus femoris, sartorius, tensor fasciae latae, tibialis anterior	Erector spinae, multifidus	Trunk flexors, hip flexors, transversus abdominis
Initial Position	Seated with knees at 90°. Forefoot on a 10 cm step, ankle in maximal attainable dorsiflexion. Weight over the distal thigh of the training leg.	Standing with hips and knees at 0°, and the forefoot on a 10 cm step in maximal attainable dorsiflexion. Holding on parallel bars for balance[a].	Hips at 70–90°, knees at 0–20°, ankle in full plantarflexion. An elastic band on the forefoot resists the dorsiflexion movement.	Hips at 80°, knees at 115°. Knee and machine's lever arm center of rotation aligned. Lever arm positioned at the distal shank.	Hips at 80°, knees at 0–5°. Knee and machine's lever arm center of rotation aligned. Lever arm positioned at the distal shank.	Hips at 110–90°, knees at 80–100°. Feet and knees at hip width. 0–20° of hip external rotation.	Standing with hips and knees at 0°. Holding an adjustable support with both hands. Feet and knees at hip width. 0–20° of hip external rotation.	Supine position, arms laying by the side and both legs touching the mat. An elastic band on the forefoot resists hip flexion and ankle dorsiflexion.	Hips and knees at 0°, the chair is 30–45° inclined. Padded support at pelvis height. Distal posterior part of the shank locked against a padded support.	Supine position, hips and knees at 0°. Arms laying by the side and both legs touching the mat.
Kinesiologic Description	Unilateral or bilateral ankle plantarflexion.	Unilateral or bilateral ankle plantarflexion	Unilateral or bilateral ankle dorsiflexion.	Unilateral or bilateral knee extension.	Unilateral or bilateral knee flexion.	Unilateral or bilateral hip and knee extension, and ankle plantarflexion.	Bilateral hip and knee extension, and ankle plantarflexion	Unilateral or bilateral hip flexion and isometric ankle dorsiflexion.	Isometric trunk and hip extension.	Isometric trunk and hip flexion. Isometric knee extension.

Hip 0° = anatomical position (positive values = flexion), Knee 0° = fully extended

[a]If the exercise is too hard, leaning on the bars and helping with the arms will be allowed. If the exercise is too easy, it will be done in the leg press machine

Table 2 Training progression over 12 weeks (left), and example of exercise list per training session (right)

Week	Volume	Load	Movement Duration (s)	Rest (s)	Session A[a]	Session B[a]
1–4	3 sets of 8 repetitions	8 RM	3 concentric 3 eccentric	60	1 – Seated calf raise 2 – Seated dorsiflexion 3 – Standing calf raise 4 – Hip flexion 5 – Seated horizontal leg press 6 – Roman chair trunk extension 7 – Squat	1 – Seated machine knee flexion 2 – Seated machine knee extension 3 – Hip flexion 4 – Standing calf raise 5 – Seated horizontal leg press 6 – Isometric hollow rocks 7 – Squat
5–8	3 sets of 8 repetitions	8 RM	1 concentric 3 eccentric	90		
9–12	4 sets of 6 repetitions	6 RM	! concentric 2 eccentric	90		

! = ballistic muscle action; *RM* repetition maximum; [a] = each session has a minimum of 7 exercises and a maximum of 10 (i.e. all exercises)

in the whole active curve seems very reasonable to allow functional benefits, an assisted training procedure will be adopted. The trainer will actively help the participant on the concentric phase of the movement in the positions in which the participant is not able to perform by himself. The exercise resistance will be selected based on the participant's strength on the optimal angles, thus the trainer will always be assisting on non-optimal angles, and never increasing the resistance. The eccentric phase will be performed unassisted, but constant feedback about movement velocity will be given. Due to the assistance during every repetition, no 8 or 6 repetition maximum test will be performed to adjust the weight, rather the trainer will frequently ask the participant to try and perform one more repetition, if successful, the weight will be increased for the next set. Whenever a participant is unable to perform a minimum concentric ROM, the protocol will be adjusted to include an isometric muscle action of 3 s in each repetition. Thus, it will be a small concentric movement, followed by the isometric hold phase and then the eccentric phase. If during the intervention the concentric movement increases, the isometric part will be removed. At the start of each training session, the trainer will ask the participant if any adverse events were experienced after the previous session. Participants will be constantly reminded to provide immediate feedback about any pain or discomfort during the training sessions.

Flexibility training

Four sets of 45 s passive-static stretching at the pain threshold (i.e. position in which the participant acknowledges an initial stretch pain sensation) will be performed for each muscle group diagnosed short in the pre-tests. One and two-joint hip flexors will be stretched in the Modified Thomas Test position [72]. The participant lies supine on a table holding one of the lower limbs in full hip flexion (assistance may be provided), while the other leg hangs outside the table (i.e. hip extension). The only difference between the stretches is that the trainer will apply the hip extension torque at the distal thigh with the knee joint positioned either in full flexion (two-joint stretch) or in a relaxed position (one-joint stretch). The seated butterfly stretch will be utilized to stretch the hip adductors. The participant will have his back supported on a wall, hips externally rotated, knees flexed and the soles of the feet in contact. The trainer will use his knees to keep the participant's feet in position while pressing down the thighs causing hip abduction. Hamstring stretch will be performed in supine position, the trainer will secure the untrained leg flat against the floor, then flexes the participant's hip approximately to 90° and applies a knee extension torque at the posterior aspect of the shank. No flexibility training will be performed for the triceps surae muscle group because of the following reasons: 1) lack of feasibility to execute the selected stretching protocol bilaterally for all tested muscles (session duration would greatly exceed 90 min); 2) the inclined gait training forces the ankle joint into dorsiflexion during the stance phase, working as a passive dynamic stretch. To check if the gait intervention by itself can change flexibility, it seemed reasonable to avoid stretching triceps surae and to focus on the other muscle groups.

Testing sessions

Participants will attend the NMRC and the Centre for Interdisciplinary Brain Research (CIBR) at the University of Jyväskylä for all testing sessions. Each testing point (pre-test 1 and 2, post-test 1) consist of three testing sessions (S1, S2 and S3) performed 4 h to seven days apart. Exceptionally, post-test 2 is composed only of S1 and a modified S2. Session 1 (S1) consists of the following tests: joint flexibility, electrostimulation (H-reflex and PAD), muscle strength and tonic stretch reflex threshold. Session 2 (S2) consists of the following tests: blood sampling, body composition and height, arterial stiffness, instrumented gait analysis, six minutes walking test. In session 3 (S3) magnetoencephalography measurements are performed. The modified S2 performed in post-test 2 consists only of the gait tests and height measurement. Table 3 describes the timeline of each testing session. The TD group will only perform the pre-tests 1 and 2. A familiarization session will be performed before the pre-test 1 to get the participants acquainted with the tests.

Table 3 Testing sessions timetable and procedures

Testing session 1 (S1)	Testing session 2 (S2)	Testing session 3 (S3)
08.00 – Flexibility tests (Hip and Knee)[b];	08.00 – Blood sampling;	08.00 – Participant preparation;
08.15 – H-reflex/PAD protocol for Sol[a] at rest;	08.10 – Body composition, height and arterial stiffness;	08.45 – Resting-state;
08.40 – Flexibility test (ankle)[b];	08.30 – Breakfast;	09.05 – Proprioceptive stimulation for TS[a];
08.50 – Preparatory activity PF[a]/DF[a];	09.00 – Participant preparation;	09.30 – Preparatory activity DF[a];
09.00 – MVC (PF[a]/DF[a]);	09.50 – Gait preparatory activity;	09.40 – Isometric DF MVC[a];
09.20 – TSRT protocol (TS[a]);	09.55 – 6 × 1 min gait test;	09.50 – Cortico-muscular coherence protocol[a];
09.30 – Preparatory activity KE[a]/KF[a];	10.10 – Rest;	10.01 – Session ends.
09.40 – MVC (KE[a]/KF[a])	10.25 – 6 min walking test;	
10.00 – Session ends.	10.31 – Session ends.	

NMRC Neuromuscular Research Center, *CIBR* Centre for Interdisciplinary Brain Research, *PAD* post-activation depression, *Sol* soleus muscle, *MVC* maximum voluntary contraction, *PF* plantarflexion, *DF* dorsiflexion, *TSRT* tonic stretch reflex threshold, *TS* triceps surae muscle, *KE* knee extension, *KF* knee flexion; [a]most affected limb is tested; [b] both limbs are tested

Additionally, this session will be utilized to duplicate some tests of S1 and S2 for a repeatability study.

Primary outcome measure

The six minutes walking test will be performed on an indoor 30 m rubber track, and its primary result is the distance that the participant is able to walk in 6 min. Heart rate (Polar Electro Oy, Kempele, Finland) will be measured to quantify exercise intensity (i.e. mean, maximum and minimum). An inertial measurement unit (NGIMU, X-IO Technologies, UK) will be firmly strapped at the participant's lower back slightly above the posterior superior iliac spine. Gait variability will be calculated with refined composite multiscale entropy [73, 74] and refined multiscale permutation entropy [75, 76]. Three-dimensional (3D) raw acceleration signals and the resultant acceleration will be utilized for this calculation. Following the approach by Ihlen et al. [77], entropy will be calculated with coarseness scales of $\tau = 1$ to 20, the length of the template for entropy evaluation equals four and the tolerance for refined composite multiscale entropy will be set at 0.3 times the standard deviation of the entire test. Raw signals will be sampled at 100 Hz, and stored on a SD card for later offline analysis utilizing a freely available java implementation (https://github.com/tjrantal/javaMSE). Cross-sample entropy [78, 79] will be utilized to compare the pre and post-tests for each participant.

Secondary outcome measures

Gait analysis (kinematics, kinetics, gait variability)

3D lower limb kinematics will be captured utilizing a Vicon system (Vicon Motion Systems, Oxford, UK), consisting of 8 cameras (MXT40) with a sampling frequency of 200 Hz. Reflective markers will be placed at: (1) medial and lateral malleolus; (2) second metatarsal head; (3) heel; (4) lateral aspect of the shank; (5) medial and lateral femoral epicondyles; (6) three non-collinear markers at the lateral aspect of the thigh; (7) anterior superior iliac spine; (8) posterior superior iliac spine. This marker set was chosen to allow calculation of both direct kinematics with the Vicon Nexus 2.5 lower limb gait plug-in [80, 81] and inverse kinematics with the free open-source OpenSim software (http://opensim.stanford.edu/; [82]). Ground reaction forces will be simultaneously acquired at 1 kHz using two force plates (51 cm × 46 cm, AMTI OR6-6-2000, Watertown, USA) on a 7.4 m × 0.6 m rubber walking path. Inverse dynamics will be utilized to calculate joint moments using both OpenSim and Vicon Nexus for comparison purposes. Gait variability will be calculated utilizing the same procedures previously explained in the 6MWT. Gait cycles will be identified using footswitches (Noraxon, Scottsdale, USA) with two sensors placed bilaterally on the heel and forefoot.

The test consists of 6 trials of 1 min, in which participants must walk back and forth at a maximum safe and controlled walking speed. Participants are instructed to disregard the force plates (i.e. not modify the gait to step on them) and to inform in case they are feeling tired. A preparatory activity of 2 min with progressively higher walking speeds will be performed before the test. Rest periods of 1–2 min between trials based on the participant's feedback will be given. Each trial can be stopped at any time for adjustments or resting, nonetheless a total of 6 min of walking will be recorded.

Muscle strength

Maximal isometric and concentric ankle plantarflexion and dorsiflexion will be assessed using a custom-build motor driven dynamometer (NMRC, University of Jyväskylä, Finland). Participants will be seated with the knee joint fully extended, hip joint flexed at 60° (anatomical position = 0°), and the ankle joint at an initial position of 0° (i.e. the sole of the foot at right angles to the tibial axis) or 28° into plantarflexion. The foot will be firmly attached to a footplate mounted on the rotation

platform so that the rotation axes of the ankle joint and the motor-driven platform coincide. Participants will be securely stabilized by an assembly of straps that fastened both shoulders and connected to a waist belt. An additional strap with a foam support prevents the knee joint of the tested leg from flexing. The torque around the rotational axis of the motor will be measured by a piezoelectric crystal transducer (Kistler Holding, Winterthur, Switzerland), and the ankle joint angle will be monitored by a linear potentiometer. Furthermore, a small stiff metal wire attached to a spring system, located under the calcaneus, will continuously monitor heel displacement from the footplate. Torque, joint angle, and heel displacement signals will be sampled at 1 kHz with a 16-bit A/D converter (CED power 1401, Cambridge Electronics Design, Cambridge, UK). The plantarflexion test starts with a 2 s maximal isometric muscle action at 0°, followed by an isokinetic (14°/s) concentric effort until 28°. The dorsiflexion test starts with a 2 s maximal isometric muscle action at 28°, followed by an isokinetic (14°/s) concentric effort until 0°. The highest attained torque at 0°, 5.5°, 11°, 16.5°, 22°, and 27.5° among the trials will be used for analysis. This approach will be used because force fluctuations during the trials (i.e. short moments of relaxation) often happen when testing the CP group, thus it may be that one single trial will not have all the peak torque values for all joint angles.

Maximal isometric and concentric knee flexion and extension will be assessed using a custom-build motor driven dynamometer (NMRC, University of Jyväskylä, Finland). Participants will be seated with the knee joint fully extended (0°) or at 75° of flexion and hip joint flexed at 80°. The distal part of the shank will be secured with a velcro strap to a strain gauge (NMRC, University of Jyväskylä, Finland) capable of measuring both tensile and compressive forces. The position of the strain gauge on the lever arm is adjustable and the distance from the axis of rotation will be individually recorded and reproduced for all following measurements. The rotation axis of both dynamometer and knee will be carefully aligned during a submaximal voluntary muscle action, to take into account the system's compliance. Participants will be securely stabilized by an assembly of straps fastening both shoulders and connected to a waist belt. The knee flexion test starts with a 2 s maximal isometric muscle action at 0°, followed by an isokinetic (15°/s) concentric effort until 75°. An examiner will keep strong downward pressure at the distal part of thigh to prevent hip flexion during the trial. The knee extension test starts with a 2 s maximal isometric muscle action at 75°, followed by an isokinetic (15°/s) concentric effort until 0°. Torque will be obtained by multiplying the moment arm by the force, and joint angle will be monitored by a linear potentiometer. Both signals will be sampled at 1 KHz with the same hardware and software utilized for the ankle torque tests. The highest attained torque in non-overlapping 5° steps among all trials will be used for analysis. For all four strength tests, rate of force development will be calculated as the maximum slope during the 0–200 ms time period in the isometric part. All measurements will be performed on the most affected leg for the CP group, and on the corresponding leg on the matching control participant. Test isokinetic velocity was chosen based on the slowest intervention training velocity (3 s muscle action for the ankle joint with a ROM of ~ 45°), and because slower velocities are easier to be well performed by the CP participants (observed in the pilot experiment). A preparatory activity consisting of ten progressively stronger efforts from 20 to 90% of the maximum voluntary contraction (MVC) will be performed before each test. Visual torque feedback will be provided in real-time and participants will receive strong verbal encouragement during every trial. Three to five trials with 1–2 min of rest for each test will be performed. In addition to the specific torque-angle analysis described, joint angles in which torque is greater than 90% of the peak concentric torque (i.e. optimum angle; [83]) and greater than 50% of the peak concentric torque (i.e. curve width; [84]) will also be calculated.

Electromyography

Three EMG setups will be utilized in the project. The first setup will be used for the strength, ankle flexibility and neural tests (i.e. H-reflex, PAD and tonic stretch reflex threshold). EMG activity will be recorded from Sol, MG and TA muscles with self-adhesive electrodes (Blue Sensor N, Ag/AgCl, 0.28 cm^2; Ambu, Ballerup, Denmark), and a ground electrode placed on the tibia. Electrode placement and skin preparation will be performed according to SENIAM [85]. The electrodes will be placed on the muscle belly in accordance with the underlying fiber direction (20-mm-interelectrode distance). EMG signals will be amplified and high-pass filtered (1000X, 10 Hz) by a preamplifier (NL824; Digitimer, Welwyn Garden City, UK) and then band-pass filtered (10 Hz to 1 kHz) by a differential amplifier (NL900D/NL820A; Digitimer Ltd., UK). The signals will be acquired on a personal computer at a rate of 5 kHz via a 16-bit A/D converter (CED power 1401; Cambridge Electronics Design, Cambridge, UK). EMG during the maximal voluntary plantarflexion and dorsiflexion trials will be quantified in 200 ms root mean square (RMS) windows preceding the joint angles of 0°, 5.5°, 11°, 16.5°, 22° and 27.5°. TA coactivation during plantarflexion will be expressed as % of the maximal RMS EMG obtained on the dorsiflexion trial at the same joint position. The same procedure will be performed to express Sol and MG coactivation during ankle dorsiflexion.

The second setup utilizes a wireless EMG system (Noraxon, Scottsdale, USA) that will acquire EMG activity during the instrumented gait analysis. Data acquisition configuration, muscles tested, and electrode placement are identical to setup 1, except for two differences: 1) sampling frequency is 1.5 kHz; 2) two sets of bipolar electrodes will be placed in TA muscle, 8–10 cm apart depending on the subject's tibia length.

The third EMG setup is built-in the MEG System (Elekta Neuromag TRIUX, Elekta Oy, Helsinki, Finland). The same bipolar electrode placement as in setup 2 will be utilized, with a MEG compatible electrode (Neuroline 720, Ag/AgCl, 0.28 cm2; Ambu, Ballerup, Denmark). EMG signal will be sampled at 1 KHz and band-pass filtered 1–1000 Hz.

Neural parameters

H-reflexes and M-waves will be evoked in Sol by percutaneous electrical stimulation of the tibial nerve, while the participant lies in prone position. A single rectangular pulse (1 ms) will be delivered from a constant-current stimulator (DS7AH; Digitimer, UK). A circular cathode with a pickup area of 0.77 cm^2 (Unilect4535M, Ag/AgCl; Unomedical, Redditch, UK) will be placed over the tibial nerve on the popliteal fossa, and an oval-shaped, 5.1 × 10.2 cm anode (V-trodes; Mettler Electronics, Anaheim, CA) will be placed above the patella. The stimulation site providing the greatest amplitude of evoked responses in Sol will first be located by a hand-held cathode electrode that will be later replaced by a self-adhesive electrode. The full recruitment curve will be attained by starting with a stimulation intensity of 3 mA and increasing in 0.5–2 mA steps (i.e. smaller steps closer to the maximum H-reflex) at 0.1 Hz until the Maximal M-wave is reached. Peak-to-peak values for maximum H-reflex and M-wave will be computed.

The PAD protocol will be performed after the recruitment curve with the participant in the same position. Stimulation intensity will be first adjusted to evoke H-reflex responses corresponding to 75% of maximum H-reflex at 0.1 Hz, then sixteen stimuli at two frequencies (0.1 and 1 Hz) will be performed. H-reflexes will be normalized by the preceding M-wave, and PAD will be computed as the ratio between 1 Hz and 0.1 Hz (i.e. greater values means less PAD). This normalization procedure is important because the H-reflex is directly influenced by the preceding M-wave (i.e. effective stimulation intensity). However, since the stimulation intensity is constant and supposedly so is the preceding M-wave, previous studies [86, 87] have chosen to report the ratio between the absolute peak-to-peak H-reflex values. Thus, for comparison purposes the absolute PAD ratio will also be calculated.

Stretch hyperreflexia of Sol and MG will be assessed utilizing the TSRT [88, 89]. The participant will be seated with the knee joint fully extended and the ankle dynamometer previously described will induce stretches (from 20° of plantarflexion to 0°) on triceps surae at four different velocities: 50, 100, 200 and 300°/s. The stretch velocities will be delivered in a pseudo-randomized and balanced order, yielding 10 stretches in each velocity, with an interval of 2.6–2.9 s. EMG data will be used to determine the joint angle in which the stretch reflex onset occurred (i.e. dynamic stretch reflex threshold, DSRT). A regression line based on a first-order linear equation using all DSRT will be calculated on a stretch velocity-joint angle plot. From the equation, the coefficient of determination, slope and intercept with the x-axis will be attained. The TSRT is the angle at which the regression line intersects with the x-axis, larger values indicate a high level of stretch hyperreflexia. TSRT will be calculated separately for each muscle. In addition to the already established TSRT calculation, we propose using the latency of the H-reflex to take into account the time between the stretch reflex onset and the EMG burst onset. A typical H-reflex latency of 35 ms and a stretch velocity of 300°/s can cause a difference of 10.5° between the stretch reflex and EMG onset, provoking a systematic error towards higher velocities. Thus, all variables in the TSRT will be calculated with and without the latency correction, and the differences will be discussed. Participants will be instructed to relax and will be wearing noise blocking headphones. Trials with EMG exceeding 5% of the isometric plantarflexion MVC in the 500 ms preceding the stretch will be discarded. The EMG burst onset will be defined as a 3SD increase in the RMS of a 20 ms moving average for at least 20 ms compared to the pre-stretch state. Visual inspection of false positives due to small brief muscle activations will be performed and automated onset detection algorithms may also be utilized. H-reflex, PAD and TSRT will be assessed on the most affected limb of the CP group and in the same leg of the age/sex matched control.

Frequency domain analysis of the EMG will be performed in the following conditions: A) intramuscular TA coherence during the swing phase of gait; B) corticomuscular and intramuscular TA coherence during a sustained isometric ankle dorsiflexion. Condition A will be collected with EMG setup 2, during the instrumented gait analysis test (S2), and B will be collected in the MEG session (S3), using EMG setup 3. Surface EMG will be preprocessed using full-wave rectification as it has been shown to be appropriate for low force muscle actions [90–92]. The finite Fourier Transform using the method of disjoint sections will be utilized [93], in which the complete EMG record R (i.e. approximately 60 s for A, and 480 s for B) is divided in L non-overlapping

disjoint sections of length T, where R = LT. For A, T will be selected to reach a spectral resolution ≤5 Hz. If possible, the swing phase will be divided into two or three parts, depending on the actual length of these phases and the achieved frequency resolution. For B, R = 480 s and T = 1 s, yielding a frequency resolution of 1 Hz. The coherence between two rectified EMG signals (x and y) is defined as their cross-spectrum normalized by the auto-spectra for a given frequency (λ), according to the following equation:

$$\left|R_{xy}(\lambda)\right|^2 = \frac{\left|f_{xy}(\lambda)\right|^2}{f_{xx}(\lambda)f_{yy}(\lambda)}$$

Coherence measures how closely two signals are related by a linear transformation [93, 94], providing a bounded and normative measure of association, taking values between 0 and 1. A perfect linear relationship has a value of 1, while full independence has a value of 0. Pooled coherence estimates will be calculated to characterize both control and experimental groups [95]. The estimation of pooled coherence joins separate signals from different subjects into a single longer signal, and then calculates the coherence of this record using the periodogram approach. Pooling the EMG signal of different participants may result in a non-constant standard deviation (i.e. nonstationarity) between the different sections, in this case signals will be normalized to have unit variance [96].

Joint flexibility and triceps surae mechanics

Lower limb joint flexibility will be assessed using three tests: the modified Thomas test, the passive knee extension test and a passive ankle dorsiflexion test. In the modified Thomas test, three measurements are performed at the hip joint and 2 at the knee joint [72]. The first hip measure has the goniometer fulcrum at the greater trochanter of femur, reference lines using lateral femoral condyle and a line perpendicular to the bench. The second hip measurement is like the first, however the researcher will apply a hip extension torque at the distal thigh and will record the angle at which the subject reports initial stretch pain sensation. The third hip measure has the goniometer fulcrum at the anterior superior iliac spine, references lines using the superior aspect of the patella and a line parallel to the bench. The first knee measure has the goniometer fulcrum at the lateral femoral condyle, reference lines using lateral malleolus and greater trochanter of femur. A limitation of the knee angle to access two-joint hip flexors flexibility is that it is directly affected by the hip angle 1 (one-joint hip flexors). Thus, the second knee angle is measured with the participant in the same position, except that the thigh of the tested leg is supported on the bench (instead of hanging outside of it), thus hip angle will be 0°. It should be noted that only hip measurement 2 is a flexibility test, because attaining maximum ROM requires unlimited torque and the subject acknowledging stretch sensation pain. On the other four measurements, the joint angle attained is a result of the interaction between gravity and the joint's passive resistance to stretch. Thus, a stiffer joint will not yield much to the gravity force, and the reduced ROM cannot be mistakenly interpreted as poor flexibility. Although not true flexibility tests, these measurements provide important information about how different muscles affects the lower limb joints.

The passive knee extension test is performed in the same position as the hamstring stretch with the participant lying supine with hip and knees at 90°. The non-tested thigh is strapped to the bench to avoid pelvic retroversion during the test. The examiner slowly applies torque at the posterior aspect of the shank causing knee extension, while another examiner maintains the hip position. The test stops when the participant acknowledges an initial stretch pain sensation. A similar knee angle measurement used in the modified Thomas test is performed. A manual goniometer is utilized to perform the both tests.

The passive dorsiflexion test will be performed using the same motor-driven dynamometer utilized for the ankle strength tests. The participant will seat with the hip joint flexed at 60°, ankle at 35.5° of plantarflexion and knee on two possible positions: 90° or 0°. The examiner will slowly (< 5°/s) move the device causing ankle dorsiflexion until the participant acknowledges an initial stretch pain sensation. Another criterion to stop the test is a heel raise higher than 5 mm. Since the motor-driven dynamometer will be also utilized subsequently to perform very fast dorsiflexions for the TSRT test, for safety reasons the maximum attained dorsiflexion angle is 21°. Thus, it is possible that maximal ROM is not measurable for some participants in the extended knee position. Three to five trials in each knee position will be performed and the maximum ROM for each position and leg will be reported. From the passive dorsiflexion torque-angle curve, in the region encompassing 20–80% of the participant's peak torque [51], average joint stiffness (i.e. first derivative), energy (i.e. integral) and hysteresis (difference between loading and unloading integral) will be calculated. Additionally, these variables will be calculated utilizing absolute joint angles. Trials with triceps surae EMG RMS higher than 3 SD of resting levels will be discarded (EMG setup 1). Table 4 presents the criteria for each joint flexibility measurement based on TD normative data [97–103].

Table 4 Joint flexibility tests and references from typically developing controls attained in other studies

Reference Values and Diagnostic
Modified Thomas test
Hip measurement 1: Hip angle > 0° = short and/or stiff one-joint hip flexors (i.e. iliopsoas, hip adductors).
Hip measurement 2 (examiner applies hip extension torque): Hip angle > 0° = short one-joint hip flexors.
Hip measurement 3: Reference value = 0° (i.e. ASIS and patella aligned on the sagittal plane). Positive values = short and/or stiff hip abductors.
Knee measurement 1: Knee angle < 67° = short and/or stiff two-joint hip flexors (i.e. rectus femoris, tensor fascia latae, sartorius).
Knee measurement 2 (supported hip): Knee angle < 67° = short and/or stiff two-joint hip flexors.
Note: Tightness of the anterior capsule and ligaments may also influence the test.
References: [72, 97–99]
Passive knee extension test
Knee angle > 40° (with the hip at 90°) = short hamstring muscles.
References: [100, 101]
Passive ankle dorsiflexion test
Ankle angle < 18° with knee at 0° and 90° = short Soleus muscle.
Ankle angle < 18° with knee at 0° and ankle angle > 18° with knee at 90° = short Gastrocnemius muscle.
Ankle angle > 18° in with knee in 0° and 90° = good triceps surae flexibility.
References: [102, 103].

ASIS anterior superior iliac spine. Hip measurements 1–2: 0° = anatomical position, positive values = hip flexion. Knee measurements: 0° = knee in full extension. Ankle measurements: 0° = sole of the foot at right angles to the tibial axis, positive values = dorsiflexion

Neuromechanical responses during the TSRT protocol (i.e. 40 stretches at 4 velocities) will also be analyzed. The higher stretch velocities in this protocol will result in reflex muscle activation, thus involving both passive and active elements. First, mechanical artifacts at the beginning and end of each stretch will be removed, then peak and mean torque, joint stiffness and energy will be calculated for each stretch. Additionally, maximum EMG RMS (EMG setup 1) will be calculated with a 50 ms moving window for each stretch. Comparisons between velocities and between the first and last group of 8 stretches will be performed to gain insight into the differences in neuromechanical responses to dynamic repetitive stretching.

Physical activity

PA will be assessed using questionnaires and device-based measurements. The questionnaire from Jackson et al. [104] with a scale from 0 to 7 describing the overall PA level is complemented by questions about the amount of moderate-to-vigorous PA during a week. PA will also be measured with a tri-axial waist-worn accelerometer (X6-1a, Gulf Coast Data Concepts Inc., Waveland, MS, USA) with a dynamic range of ±6 g and a sampling frequency of 40 Hz. The accelerometer will be utilized during seven typical days at three time points: control, intervention and maintenance periods. A minimum of 3 days with 8 h of measurement will be accepted for analysis as it has been shown to provide adequate reliability for children and adolescents with CP GMFCS I [105]. Raw acceleration data will be transformed to Actigraph counts [106], in order to utilize validated GMFCS specific thresholds for different intensity zones for children with CP [107].

Specialized shorts with textile electrodes (Myontec Ltd., Kuopio, Finland, 108) will be utilized to record EMG bilaterally from quadriceps and hamstring muscles during an entire typical day. A small recording electronic module is able to collect muscle activity data for 8–12 h and can be analyzed offline afterwards. EMG signals will be acquired with a sampling frequency of 1 KHz and band-pass filtered (50–200 Hz, − 3 dB). The raw signals will be full wave rectified and averaged over 100 ms non-overlapping windows and stored into a portable module. Daily muscle inactivity, light, moderate and vigorous activity time of the participant will be extracted [108, 109]. Muscle inactivity will be defined as < 90% of the standing EMG activity for each muscle group. Moderate activity threshold is the preferred walking EMG activity, and vigorous activity is twice the amplitude of walking [110]. The participants will receive a diary to record times when the accelerometer and EMG pants are worn, facilitating the removal of non-wear time in the analysis.

Gross motor function

Gross motor function will be assessed in the CP group using the 66-item version of the Gross Motor Function Measure (GMFM-66; [111]). GMFM-66 is a valid, reliable and responsive observational instrument, based on interval scaling to evaluate changes in gross motor function in participants with CP [19]. Dimensions D (standing) and E (walking, running and jumping) will be utilized. The test will be video recorded and scored subsequently by the same evaluator. Absolute and relative (i.e. percentage of maximum) scores for each dimension will be reported.

Biological maturity

To control for the confounding effect of maturation on the dependent variables during the study, estimated time from peak height velocity (i.e. maturity offset) will be computed using the sex-specific equation provided by Moore et al. [112]:

$$\text{Girl's maturity offset} = -7.709133 + (0.0042232*(\text{age}*\text{height})); R^2 = 0.898; \text{SEE} = 0.528;$$

$$\text{Boy's maturity offset} = -7.999994 + (0.0036124 \text{ x } (\text{age x height}), R^2 = 0.896; \text{SEE} = 0.542.$$

Body composition, height and cardiometabolic risk factors

Body height will be measured with the head positioned in the Frankfurt plane with a wall-mounted stadiometer and body composition with a bioelectrical impedance device (InBody 720, Seoul, South Korea; [113]). Venous blood samples will be drawn after standard overnight (12 h) fast. Serum insulin will be measured by electrochemiluminescence immunoassay (Immulite 2000, Siemens, Germany). Fasting plasma glucose, total plasma cholesterol, plasma high and low-density cholesterol and plasma triglycerides will be measured by enzymatic colorimetric assays (Konelab 20XTi, Thermo, USA). Insulin sensitivity estimated by the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) will be calculated using the formula: fasting serum insulin x fasting plasma glucose/22.5 [114]. White and red blood cells, hematocrit and hemoglobin will be measured by an automated analyzer (Sysmex XP300, Sysmex, Japan). Systolic and diastolic blood pressure, aortic pulse wave velocity, and augmentation index will be measured in supine position with a non-invasive oscillometric device after 10 min of rest (Arteriograph, Tensiomed Ltd., Budapest, Hungary; [115]). Participants will be allowed to drink water and take their usual medications during fastening.

Magnetoencephalography (MEG)

Participants will be seated inside a magnetically shielded room (VACOSHIELD, Vacuumschmelze GmbH & Co. KG, Hanau, Germany) with their heads positioned within the sensor array of the whole-head-MEG System (Elekta Neuromag TRIUX, Elekta Oy, Helsinki, Finland), consisting of 204 planar gradiometers and 102 magnetometers. MEG data will be acquired at 1000 Hz and band-pass filtered (0.1–330 Hz).

Subject preparation Five head position indicator (HPI) coils will be attached to the participant's scalp to measure and follow head position with respect to the MEG sensors. Positions of the HPI coils, three anatomical landmarks (nasion, left and right preauricular points), and ~ 150 additional points on the head surface will be determined with a Fastrak 3D digitizer (Polhemus, Vermont, USA). Vertical and horizontal electro-oculograms will be recorded with two pairs of electrodes. The first pair is placed above and below the right eye and the second pair lateral to the outer canthi of the eyes. Electrocardiography will be recorded with a pair of electrodes placed 3–5 cm bellow right and left clavicle and with 8–12 cm inter-electrode distance. The ground electrode will be placed on the right clavicle. EMG activity will be recorded from Sol, MG and TA muscles (EMG setup 3).

Equipment Two custom-made MEG-compatible devices (University of Jyväskylä, Jyväskylä, Finland) were utilized in the study: a pneumatic ankle-movement actuator and a dynamometer capable of measuring isometric ankle plantarflexion and dorsiflexion forces. The chair was modified by removing its shank and foot support and installing a metallic rail (aluminum and brass) to allow adjustable attachment of both equipment to it.

The pneumatic ankle-movement actuator was adapted from the device made by Piitulainen et al. [116]. The operating principle of the MEG compatible pneumatic actuators is reported in Piitulainen et al. [117]. In brief, the actuator is composed of four pneumatic muscles (DMSP-10-120 N-AM-CM, Festo, Vantaa, Finland) embedded into a plastic and aluminum frame with a rotating platform that supports the participant's foot. The rotation occurs in the sagittal plane (i.e. ankle dorsiflexion) when the internal pressure of the pneumatic muscles is increased (from 1 to 7 bar) and returns to the initial position when the air pressure is released. Rotation axes of the actuator and ankle joint can be aligned. The foot is fixed to the rotating platform by a strap passing firmly around the forefoot.

The isometric dynamometer composed of a metallic plate in L format, with a hard foam support for the posterior distal shank and for the heel. An adjustable metal attachment is positioned on the person's forefoot. The shank-support is embedded with a load cell and has a strap to fasten the shank, enabling it to measure isometric knee extension and flexion forces. Another load cell is embedded in a support part holding the adjustable metal attachment. A strap firmly secures the participant's forefoot and thus the load cell can measure both isometric ankle plantarflexion and dorsiflexion forces.

MEG protocol Before the participant enters the shielded room, a 5-min empty room measurement will be recorded to estimate intrinsic noise levels of the MEG sensors for the needs of later data analysis. Three experimental MEG tasks will be performed in the following order: (1) resting state, (2) proprioceptive stimulation and (3) isometric muscle action. The resting-state task consists of two 5-min recordings, in which the participant will remain relaxed fixating on a cross on a screen positioned 1 m away from the participant's eyes. In the proprioceptive stimulation, the pneumatic ankle-movement actuator will evoke a

total of 100 dorsiflexions (movement range from 0° to ~ 15°) with a moderate velocity (mean: 15°/s; peak: 35°/s) every 4 s with ±0.2 s random jitter between the consecutive stimuli. Participants will be instructed to relax and not to resist the movement, fixate on the fixation cross, and avoid excessive eye and head movements. The view of the foot and actuator is blocked, and Brownian noise will be played through earplugs in an individually adjusted volume to mask the noise generated by the movement actuator. In the third task, first a preparatory activity of ten 2-s isometric dorsiflexions with increasing intensity (i.e. ~ 20 to 90% of MVC) at 0° ankle angle in the ankle dynamometer will be performed. Then, in the same position, three to five maximum isometric dorsiflexions (~ 3 s each) will be performed, followed by four sets of submaximal isometric dorsiflexions at 10% of MVC with 120 s of duration each. Visual torque feedback will be provided in real-time, and participants will be instructed to maintain as stable as possible torque throughout each submaximal trial. To avoid fatigue, 1–2 min of rest periods will be enforced between trials (except in the preparatory activity). If the participant is unable to perform dorsiflexion adequately, plantarflexion will be used. Continuous HPI tracking will be performed for later correction of head movements.

MEG data preprocessing First, visual data inspection will be performed to identify bad channels and periods with intense noise. Maxfilter (ver. 3.0, Elekta Neuromag Oy, Helsinki, Finland) with spatiotemporal signal space separation method [118] will be used to reduce endogenous and exogenous sources of noise in the MEG signals. Additionally, MaxFilter will be utilized for head movement compensation during MEG recording and to align the head coordinates across the different longitudinal recording sessions (pre-tests 1 and 2, and post-test 1). The coordinate transformation will be done to the participant individual mean reference head position within each MEG task separately. Artifacts caused by eye movements and cardiac activity will be identified and removed using the electro-oculograms and electrocardiography signals in combination with independent component analysis implemented in MNE-Python software package [119, 120].

Data analyses All further analyses of the MEG data will be processed and analyzed with the MNE-Python software package. The data will be averaged across proprioceptive stimuli in order to examine the passive-movement-evoked fields, and the respective modulation of the rhythmic activity in primary sensorimotor cortex at alpha, beta and possibly also at gamma frequency bands. Cortico-muscular and intramuscular coherence for TA will be computed as described in the EMG section. Resting state data will be analyzed with a special focus on the sensorimotor cortical network. Torque steadiness during the 8 min of submaximal effort will be calculated utilizing the standard deviation and coefficient of variation.

Statistical analyses

Mean/median values, standard deviations and 95% confidence intervals will be reported. Normality of data will be tested using the Shapiro-Wilk test. General linear mixed model analysis will be utilized to study the differences between time (2 pre-tests and 1 or 2 post-tests) in the CP group. Independent t-tests will be used to compare groups (i.e. CP vs. TD) and paired t-tests to compare the two TD testing times (pre-test 1 and 2). Pearson correlation analysis will be performed to check association between dependent variables. The interference of variables such as PA levels or maturity offset will be controlled if they have a strong linear relationship with other dependent variables. Nonparametric tests may be utilized if necessary. Significance level will be set at $P < 0.05$.

Discussion

The current training intervention protocol combines different training modalities in an attempt to enhance motor function in people with CP. The individually tailored aspects are: 1) flexibility training for muscles diagnosed short in the Pre-tests; 2) the number of training sessions and strength exercises in each session will be adjusted depending, e.g., on the participants' motivation, stamina and schedule; 3) introduction of a short isometric hold (3 s) in case that the joint range of motion is limited; 4) stabilization procedures to ensure proper execution of the movements, e.g., using a foam ball to prevent hip adduction during leg press.

The major limitation in this study is the effect of the growth spurt on the dependent variables. If it happens during the control period, results between pre-test 1 and 2 may be considerably different, making it harder for any intervention related changes to be statistically different. If the growth spurt happens during the intervention it may increase its effectiveness, whereas if it happens during the maintenance period it may slow down the adaptation loss. By carefully monitoring the maturity indicators it will be possible to understand and estimate how each participant was affected by the maturation process. A second limitation regards the study's external validity: due to its length and great number of testing and training sessions, only participants and families that are already inclined towards higher levels of PA will likely choose to participate. Since PA levels are being measured objectively, it will be possible to characterize and compare this study with others and check this assumption.

Abbreviations
3D: Three-Dimensional; 6MWT: Six Minutes Walking Test; CIBR: Centre For Interdisciplinary Brain Research; CP: Cerebral Palsy; DSRT: Dynamic Stretch Reflex Threshold; EMG: Electromyography; GMFCS: Gross Motor Function Classification System; GMFM: Gross Motor Function Measure; HPI: Head Position Indicator; H-Reflex: Hoffman Reflex; MEG: Magnetoencephalography; MG: Medial Gastrocnemius; MVC: Maximum Voluntary Contraction; NMRC: Neuromuscular Research Center; PA: Physical Activity; PAD: Post-Activation Depression; RCT: Randomized Controlled Trial; RMS: Root Mean Square; ROM: Range of Motion; S1: Testing Session One; S2: Testing Session Two; S3: Testing Session Three; Sol: Soleus; TA: Tibialis Anterior; TD: Typically Developing; TSRT: Tonic Stretch Reflex Threshold

Acknowledgements
We would like to thank all personnel involved in building the two equipment utilized in the magnetoencephalography measurements. The force dynamometer was designed by Simon Walker and Janne Avela, and built by Sirpa Roivas, Jouni Tukiainen and Markku Ruuskanen, all from NMRC. The pneumatic actuator was built by Viki-Veikko Elomaa (CIBR). Technical support to design the pneumatic actuators was provided by Helge Kainulainen and Veikko Jousmäki both from Aalto NeuroImaging, Aalto University.

Authors' contributions
PV, HP, EAH, TP, JA and TF defined the study protocol. PV and TF developed the training protocol and PV is responsible for implementing it. PV is responsible for data collection. TF is the chief investigator for the study. All authors will be involved in preparing the manuscripts for publication according to the Vancouver protocol. All authors have read and approved the final manuscript.

Funding
This work was supported by the Olvi Foundation, University of Jyväskylä, Academy of Finland (grants #296240, #307250 and #327288) and Jane and Aatos Erkko Foundation. None of the funding organizations participated in the creation of the study design, data collection, data analyses or writing the manuscript.

Availability of data and materials
Not applicable.

Ethical approval and consent to participate
Ethical approval has been granted by the ethics committee of the Central Finland Healthcare District (U8/2017) and the study has been registered prospectively in the International Standard Randomized Controlled Trial (ISRC TN69044459). Two minor protocol amendments were also approved (2018/2019) and the registered trial information was updated accordingly. Prospective participants will come to the Neuromuscular Research Center (NMRC) at the University of Jyväskylä, where a detailed overview of the project will be given. All individuals who are willing to participate, and who meet the inclusion criteria (assessed by a screening questionnaire), will sign the informed consent to participate in the study. Legal guardians will be asked to sign the informed consent for children under 18 years, and written assent from children will also be required. Participation to this study is voluntary, all participants are informed that they can withdraw from the study at any time without consequences. The study will comply with the Declaration of Helsinki. Any adverse effects caused by the intervention will be reported to the ethics committee within 15 days, and later reported in the publications.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 23 July 2020 Accepted: 5 February 2021
Published online: 26 February 2021

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Publisher's Note

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REVISING THE STRETCH REFLEX THRESHOLD METHOD TO MEASURE STRETCH HYPERREFLEXIA IN CEREBRAL PALSY

by

Valadão, P., Bar-On, L., Cenni, F., Piitulainen, H., Avela, J & Finni, T. (2022).

Frontiers in Bioengineering and Biotechnology 10:897852.

https://doi.org/10.3389/fbioe.2022.897852

frontiers | Frontiers in Bioengineering and Biotechnology

TYPE Original Research
PUBLISHED 23 November 2022
DOI 10.3389/fbioe.2022.897852

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Biomechanics, a section of the journal Frontiers in Bioengineering and Biotechnology

RECEIVED 16 March 2022
ACCEPTED 09 November 2022
PUBLISHED 23 November 2022

CITATION
Valadão P, Bar-On L, Cenni F, Piitulainen H, Avela J and Finni T (2022), Revising the stretch reflex threshold method to measure stretch hyperreflexia in cerebral palsy. *Front. Bioeng. Biotechnol.* 10:897852. doi: 10.3389/fbioe.2022.897852

Revising the stretch reflex threshold method to measure stretch hyperreflexia in cerebral palsy

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Hyper-resistance is an increased resistance to passive muscle stretch, a common feature in neurological disorders. Stretch hyperreflexia, an exaggerated stretch reflex response, is the neural velocity-dependent component of hyper-resistance, and has been quantitatively measured using the stretch reflex threshold (i.e., joint angle at the stretch reflex electromyographic onset). In this study, we introduce a correction in how the stretch reflex threshold is calculated, by accounting for the stretch reflex latency (i.e., time between the stretch reflex onset at the muscle spindles and its appearance in the electromyographic signal). Furthermore, we evaluated how this correction affects the stretch reflex threshold in children and young adults with spastic cerebral palsy. A motor-driven ankle dynamometer induced passive ankle dorsiflexions at four incremental velocities in 13 children with cerebral palsy (mean age: 13.5 years, eight males). The stretch reflex threshold for soleus and medial gastrocnemius muscles was calculated as 1) the joint angle corresponding to the stretch reflex electromyographic onset (i.e., original method); and as 2) the joint angle corresponding to the electromyographic onset minus the individual Hoffmann-reflex latency (i.e., latency corrected method). The group linear regression slopes between stretch velocity and stretch reflex threshold differed in both muscles between methods ($p < 0.05$). While the original stretch reflex threshold was velocity dependent in both muscles ($p < 0.05$), the latency correction rendered it velocity independent. Thus, the effects of latency correction on the stretch reflex threshold are substantial, especially at higher stretch velocities, and should be considered in future studies.

Abbreviations: CP, cerebral palsy; EMG, electromyography; H-reflex, Hoffmann-reflex; IQR, interquartile range; MG, medial gastrocnemius muscle; R2, coefficient of determination; SD, standard deviation; Sol, soleus muscle; SR, stretch reflex; SRT, stretch reflex threshold; TSRT, tonic stretch reflex threshold.

10.3389/fbioe.2022.897852

KEYWORDS

hyperreflexia, neurophysiology, stretch reflex, electromyography, cerebral palsy

Introduction

Hyper-resistance is defined as an increased resistance to passive muscle stretch, commonly reported in people with the upper motor neuron syndrome. Three main contributors to hyper-resistance have been identified: non-neural tissue properties, neural velocity-dependent stretch hyperreflexia and neural non-velocity dependent involuntary background activation (Gracies, 2005; Trompetto et al., 2014; van den Noort et al., 2017). Correctly assessing all components of hyper-resistance is crucial for treatment decision making and monitoring individual changes through life (e.g., effects of aging or a intervention; Tedroff et al., 2009; Tedroff et al., 2011). Stretch hyperreflexia is often characterized by the occurrence of the stretch reflex (SR) at abnormally lower stretch velocities and earlier joint angles (i.e., earlier in the stretch) compared to typically developing muscles. In clinical practice, manual stretch hyperreflexia assessment scales, such as the Modified Tardieu Scale (Boyd and Graham, 1999) have been widely used due to their ease of implementation without complex instrumentation requirements. The Modified Tardieu Scale dynamic range of motion test attempts to measure the joint angle at the SR torque onset (i.e., SR EMG onset plus electromechanical delay). The test consists of the examiner performing a fast passive stretch on the target muscle and measuring the angle of catch (i.e., angle at which muscle activity abruptly increases and stops the movement). Although simple to execute, this method is limited by the lack of stretch velocity and amplitude standardization, and inaccuracies related to subjectively measuring the angle of catch (van den Noort et al., 2009).

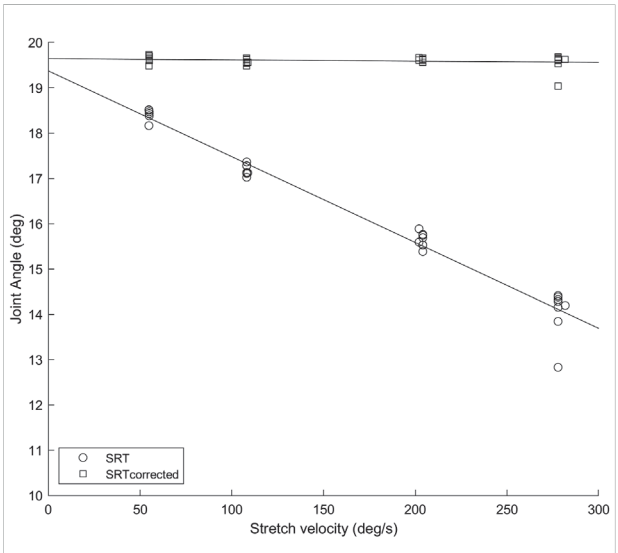

FIGURE 1
Soleus muscle SRTs (circles) and $SRTs_{corrected}$ (squares) in repeated trials at four stretch velocities for a representative participant. The error caused by not considering the SR latency increases with increasing velocity, as the amount of angular displacement between the SR onset and the SR EMG onset is increasing.

To improve validity and reliability of stretch hyperreflexia assessments, quantitative methods utilizing recordings of joint kinematics and muscle electromyographic (EMG) activity have been developed, allowing more accurate assessment of the joint angle at the SR EMG onset, also termed stretch reflex threshold (SRT). The SRT can be measured at different stretch velocities, and it is generally assumed that higher stretch velocities will result in earlier onset joint angles (Levin and Feldman, 1994). Furthermore, the Tonic Stretch Reflex Threshold (TSRT) proposed by Levin and Feldman (Levin and Feldman, 1994) estimates a joint angle in which involuntary muscle activity would hypothetically start in the absence of joint movement. The TSRT is the y-intercept of the linear regression line through the SRTs with stretch velocity, thus representing the joint angle at zero velocity (Figure 1). Several studies have reported a moderate to high coefficient of determination (R^2) for the linear regression between the SRTs and stretch velocity (Calota et al., 2008; Blanchette et al., 2016; Germanotta et al., 2017; Frenkel-Toledo et al., 2021), which is vital for the validity of the TSRT (i.e., extrapolating the linear regression to zero velocity).

In the present study, we argue that the SRT and TSRT measures are influenced by a systematic error due to the lack of SR latency correction. SR latency is the duration between the SR being mechanically initiated at the muscle spindles (i.e., SR onset) to its appearance in the EMG signal (i.e., SR EMG onset). For a given SR latency, the difference between the joint angle at the SR onset and at the SR EMG onset (i.e., SRT) will have a positive linear relationship with the stretch velocity. For example, if we consider a SR latency of 30 ms and two stretches performed at 50°/s and 300°/s, the errors of using the angle at the SR EMG onset are 1.5° and 9° respectively, simply because the EMG onset is delayed by the ~30 ms monosynaptic SR latency. Thus, without the SR latency correction, the SRT is progressively overestimated to later angles as velocity increases. While the study by Levin & Feldman (Levin and Feldman, 1994) acknowledged the SR latency problem in calculating the SRT and proposed subtracting 30 ms as a mean latency for the SR, later studies implementing the method did not make any correction. Since SR latency mainly depends on body dimensions associated with the axon pathway to the target muscle, subtracting an average value of 30 ms produces an unknown subject-specific systematic error. Therefore, the aim of the present study was twofold: 1) to correct the SRT calculation by considering the SR reflex latency and evaluate the effect of the correction on the linear relationship between SRT and stretch

velocity; 2) to verify the validity of the TSRT method once the SRT is latency corrected. SR latency was estimated using the soleus (Sol) and medial gastrocnemius (MG) Hoffmann-reflex (H-reflex) latency. Thus, the SRT was computed as the joint angle at the SR EMG onset time (i.e., original method), and as the joint angle at SR EMG onset time minus the individual H-reflex latency time (i.e., latency correction method). We hypothesized that a significant change in the stretch velocity-SRT regression slope will occur due to the latency correction, as it will necessarily shift higher velocity SRT to earlier joint angles. Although the change in the regression slope is predictable, it is impossible to predict how the regression line R^2 will change, and thus the adequacy of the TSRT method. Furthermore, we hypothesized that the TSRT angle will not significantly change as the latency correction will have only a small effect on the lower velocity SRTs, and consequently will not change the y-intercept of the regression line considerably.

Materials and methods

Subjects

Thirteen children and young adults diagnosed with spastic cerebral palsy (CP), aged between 9 and 22 years participated in this study. None of the participants had lower limb surgery, serial casting, pharmacological treatments (except for oral medication) or had participated in a resistance-training program for the lower limbs in the past 6 months. All participants were able to stand with both heels touching the floor (i.e., ankle in anatomical position). Table 1 presents participant characteristics.

TABLE 1 Participant characteristics (n = 13).

Male/female	8/5
Mean (SD) age (years)	13.5 (4)
Mean (SD) height (cm)	159 (12)
Mean (SD) weight (Kg)	52 (16)
Level of involvement	Bilateral = 2/Unilateral = 11
GMFCS	I = 13

Data presented as mean (SD). GMFCS, gross motor function classification system.

Study design

The present study is part of a larger nonconcurrent multiple-baseline research project called EXECP (Valadão et al., 2021), prospectively registered in the International Standard Randomized Controlled Trial (ISRCTN69044459). Data collected in Pre-tests 1 and 2 was utilized in this study.

Experimental protocol

Detailed information about the testing procedures can be found in the research protocol (Valadão et al., 2021). EMG activity was recorded from Sol and MG muscles with self-adhesive electrodes (Blue Sensor N, interelectrode distance = 2 cm; Ambu, Ballerup, Denmark) following SENIAM (Hermens et al., 2000), and a ground electrode was placed on the tibia. EMG signals were amplified (gain 1,000) and high-pass filtered (10 Hz) by a preamplifier (NL824/NL820A; Digitimer, Welwyn Garden City, United Kingdom) and then band-pass filtered (20–195 Hz) off-line using Matlab software (v2020a, The Mathworks Inc, Natick, United States). The 20-Hz high-pass is suggested to offer the best compromise for optimizing the physiological informational content of surface EMG (De Luca et al., 2010), while the selected low-pass was chosen to eliminate high-frequency noise found in some EMG recordings (external laboratory noise). The H-reflex recruitment curve for Sol was elicited by percutaneous electrical stimulation of the tibial nerve at the popliteal fossa. H-reflex latency for both Sol and MG was determined by visual inspection as the duration between the electrical stimulus and the initial deflection of the H-reflex on the EMG signal. The H-reflex monosynaptic pathway is almost identical to the SR, except that the former is evoked by an electrical stimulus at the popliteal fossa, and the latter is generated by muscle spindles in the muscle. Subsequently, a motor-driven dynamometer (Neuromuscular Research Center, University of Jyväskylä, Finland) induced passive ankle dorsiflexions from 20° of plantarflexion to 0° at four angular velocities (55, 110, 210, and 291°/s). Ten stretches in each velocity were delivered in a pseudo-randomized and balanced order every 2.6–2.9 s. Participants were instructed to relax and wore noise blocking headphones. Moreover, trials with Sol or MG EMG root mean square computed over a 200 ms sliding window exceeding 5% of the maximal isometric plantarflexion test [see (Valadão et al., 2021)] in the 500 ms preceding the stretch were discarded. An EMG onset detection algorithm applying the approximated generalized likelihood principle (Staude and Wolf, 1999; Staude et al., 2001; Lee et al., 2007) was used to detect the SR EMG onset. Visual inspection was used to identify false positives and negatives generated by the algorithm, and manual onsets were set based on the criteria of the EMG signal reaching two standard deviations (SD) for a minimum of 100 ms. Since the stretch velocity is the independent variable and the SRT the dependent variable, the former has been assigned to the x-axis and the latter to the y-axis, which is the opposite of how this data has been presented in previous studies [e.g., (Calota et al., 2008; Blanchette et al., 2016)]. Thus, although the calculation of the R^2 values are the same between studies, the regression slopes are different, and the TSRT in the present study is the y-, rather than x-intercept.

Data analysis

Data analysis was performed in Matlab (v2020a, The Mathworks Inc, Natick, United States). SRT was calculated in the original method as the joint angle at SR EMG onset time and with latency correction as joint angle at SR EMG onset time minus the individual H-reflex latency time for each muscle ($SRT_{corrected}$). For example, if the Sol SR EMG onset happened 125 ms after the stretch onset and the participant's Sol H-reflex latency is 25 ms, SRT is the joint angle 125 ms after the stretch onset, whereas $SRT_{corrected}$ is the joint angle 100 ms after the stretch onset. The median SRT and $SRT_{corrected}$ values for each subject at each stretch velocity were calculated for statistical analysis. TSRT and $TSRT_{corrected}$ were calculated as the y-intercept of the regression lines between stretch velocity and SRT or $SRT_{corrected}$, respectively.

Statistical analysis

Data normality and equality of variances was tested with Shapiro-Wilk and Levene's tests, respectively. The two-sided paired *t*-Test and the non-parametric analog Wilcoxon signed rank test were used to test differences between variables with and without latency correction. The Friedman test with the Bonferroni post hoc test was used to check the effect of stretch velocity on SRT and $SRT_{corrected}$. All statistical analyses were performed in Matlab. Effect size between group means was calculated using Hedge´s g. Significance level was set at $p < 0.05$.

Results

Results are presented as mean ± standard deviation for normally distributed data and median (interquartile range) otherwise. Figure 1 depicts an example of how the SRT data was used to calculate TSRT in the original method and with SR latency correction ($TSRT_{corrected}$). Only participants with SRTs quantified in all four velocities were used for the statistical analysis (n = 12 for Sol, n = 11 for MG). Group Sol H-reflex latency was 28 ± 3 ms with a range of 23–33 ms and MG H-reflex latency 28 ± 4 ms, a range of 23–35 ms, which are in line with previous reports (Mazzocchio et al., 2001).

Regression slope between SRT and stretch velocity

Figure 2 shows the individual and group mean or median slopes for the original and latency corrected methods. In Sol, the mean regression slope between the original (0.014 ± 0.012) and latency corrected (0.010 ± 0.012) methods were statistically different [t (11) = −19.3, $p < 0.001$, 95%CI = −0.03 to -0.02; hedge's g = 2.0 (1.0–3.0)]. Similarly, in MG the median regression slope in the original method [−0.021 (0.01)] was statistically different from the latency corrected method [0.001 (0.01), $p < 0.001$].

Effects of stretch velocity on SRT

In the original method, SRTs occurred at statistically different joint angles for both Sol ($p = 0.008$) and MG ($p < 0.001$). Bonferroni post-hoc analysis revealed that SRTs in the two slowest stretch velocities occurred significantly earlier than SRTs at the fastest (291°/s) velocity for both Sol (55°/s: $p = 0.04$; 110°/s: $p = 0.009$) and MG (55 and 110°/s: $p < 0.001$). With latency correction, no statistically significant differences across the stretch velocities were found for Sol ($p = 0.552$) or MG ($p = 0.315$). Table 2 shows the SRT results for the four stretch velocities.

Coefficient of determination (R2)

Figure 3 shows the individual R^2 results for the SRT-velocity linear regression and group medians for both methods. In Sol the R^2 between the original [0.53 (0.93)] and latency corrected [0.27 (0.34)] methods were not statistically different ($p = 0.301$). In MG, R^2 in the original method [0.91 (0.68)] was statistically higher than in the latency corrected method [0.08 (0.15), $p = 0.01$].

TSRT

No statistically significant difference between Sol TSRT [16° (11)] and Sol $TSRT_{corrected}$ [16° (11), $p = 0.910$] was found. Likewise, MG TSRT [18° (2)] and MG $TSRT_{corrected}$ [19° (3), $p = 0.102$] were not statistically different.

Discussion

The present work sought to verify the effects of the SR latency correction on the SRT and TSRT methods of stretch hyperreflexia assessment. The main findings were that latency correction significantly changed the SRT-velocity slopes and rendered the group-level SRT for both Sol and MG velocity independent. Thus, the lack of linear relationship between SRT and stretch velocity invalidates the use of a linear regression to find the TSRT.

Regression slopes

To group individual SRT-velocity slopes, we defined a 'near zero slope' as having a modulus value smaller than 0.01, which

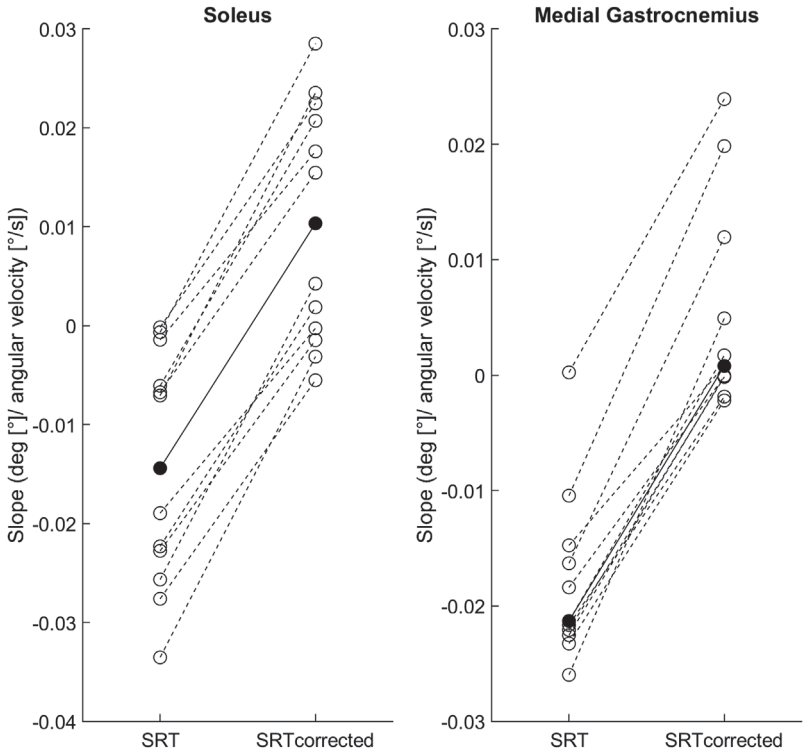

FIGURE 2
Individual SRT-velocity linear regression slopes (open circles) and soleus group mean/medial gastrocnemius median (filled circles) for both methods: original (SRT) and latency corrected ($SRT_{corrected}$).

TABLE 2 Effects of stretch velocity on Sol and MG SRT with and without latency correction.

Variables	Median (IQR) for stretch velocities			
	55°/s	110°/s	210°/s	291°/s
Sol SRT (°)	14 (13)*	16 (9)*	13 (5)	11 (4)
Sol $SRT_{corrected}$ (°)	15 (13)	19 (9)	18 (5)	18 (3)
MG SRT (°)	18 (2)†	16 (1)†	14 (1)	11 (1)
MG $SRT_{corrected}$ (°)	19 (2)	19 (1)	19 (1)	19 (1)

Sol, soleus; MG, medial gastrocnemius; SR, stretch reflex; SRT, stretch reflex threshold; IQR, interquartile range. Stretch velocities 55°/s and 110°/s are significantly different from 291°/s: *$p < 0.05$/†$p < 0.01$.

would result in a maximum 2.5° difference between the slowest and fastest stretch velocities used in this study. Since within participant and velocity SRT median range was 1.5° (min–max range: 0.1–16°), a slope smaller than 0.01 would cause changes in SRT that are indistinguishable from the subject's natural variability. At the individual level, with SR latency correction, six participants had near zero slopes in Sol (i.e., velocity independent) while the other six had positive slopes (i.e., earlier SRTs at higher velocities). In MG, nine participants had near zero slopes and two had positive slopes. Interestingly, all participants that showed velocity independency, had an early SRT within the first 2° of the stretch, whereas positive slopes were present in participants with SRTs later in the range of motion. These individual differences suggest that stretch velocity has a negligible effect on participants with early SRTs. A possible explanation may be that the muscle-tendon unit is already under tension and/or the IA arc is highly excitable (Nielsen et al., 2005).

The changes in the regression slopes caused by the SR latency correction towards positive values were expected since the correction shifts the SRTs of higher velocities to earlier angles. This means that when stretch velocity is increased, SRTs without the correction occurred progressively later in the range of motion, whereas the corrected SRTs occurred progressively earlier. Only the latter is an expected phenomenon of the velocity-dependent nature of hyperreflexia, and is also expected due to the viscoelastic behavior of the muscle-tendon unit [i.e., increased stretch resistance at higher velocities; (Taylor et al., 1990; Wu et al., 2010)].

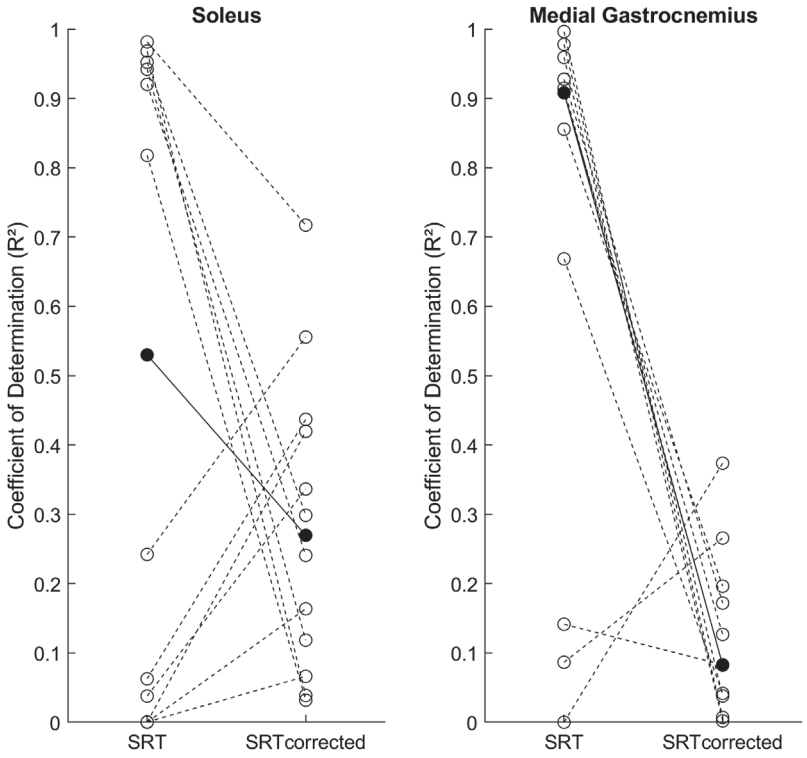

FIGURE 3
Individual SRT-velocity linear regression coefficients of determination (R^2, open circles) and group medians (filled circles) for both methods: original (SRT) and latency corrected ($SRT_{corrected}$).

Coefficient of determination

Although the regression slope changes with SR latency correction were unidirectional (i.e., towards positive slope values), its effect on R^2 was bidirectional among our participants: 1) negative slopes shifting towards near zero slopes reduced R^2 (50% of participants in Sol and 82% in MG); 2) near zero slopes shifting towards positive slopes increased R^2 (50% of participants in Sol and 18% in MG). This explains why the statistically significant effect of SR-latency correction on R^2 was observed only in MG. The lower SRT variability in MG was probably due to the extended knee testing position, which placed the biarticular MG under considerably more tension than Sol. Overall, the low median latency corrected R^2 values for both muscles (Sol = 0.27, MG = 0.08) and high variability among participants, argues against the utilization of the linear regression to calculate the TSRT, at least in our sample. Nevertheless, previous studies have reported positive slopes without the SR latency correction for the same muscles that we studied (Blanchette et al. 2016 ; Germanotta et al. 2017). These studies would have had steeper slopes with the latency correction, and if a high R^2 was found, the TSRT method would be justified. Even though latency correction increased the slopes significantly in the current study, as expected, no differences in TSRT (i.e., y-intercept) were found between methods in both examined muscles. Since latency correction had a minimal effect on the SRTs at slow velocities (e.g., 28 ms * 55°/s = 1.5° correction), even considerable changes in the regression slope had small effect on the TSRT.

Methodological remarks

Several important aspects of this study require further clarification: 1) a powerful motor-driven ankle-joint movement actuator was used to induce the stretches, whereas most of the aforementioned studies applied manual stretches. It took only 20–40 ms to achieve the target velocity in our actuator, which seems unlikely in manual stretches or even in the mechatronic device utilized by Germanotta et al. (2017), which had a maximum torque output of 7.1 Nm. Thus, it is possible that although mean joint velocities are comparable

between studies, the joint acceleration profiles were very different (Sloot et al., 2021). Notably, during clinically applied manual tests such as the Modified Tardieu Scale, the stretch velocity is unknown making it impossible to perform the latency correction to the catch angle; 2) the stretch range of motion in the present study was shorter than other studies assessing the same joint (Blanchette et al., 2016; Germanotta et al., 2017). This was due to the extended knee testing position and the use of a motor driven dynamometer with end stop limits set for safety reasons. Fortunately, even in the slowest stretch velocity consistent SRs were evoked within this range of motion; 3) the H-reflex bypasses the muscle spindle and is evoked at the popliteal fossa, thus a small systematic error underestimating the SR latency by a few milliseconds is unavoidable, causing a small error in the SRT calculation at high stretch velocities. Nevertheless, the distance from the SR onset location in the tested muscles to the popliteal fossa could not be more than 15% of the entire IA arc pathway, thus for the current dataset it would represent a maximum of 4.2 ms (i.e., 15% of the mean latency) or an error of 1.2° in the fastest stretch velocity, still inferior to the within-subject and velocity SRT variability. Since using electrical nerve stimulation to assess SR latency is not feasible in most clinical setups, it would be very helpful to create easy measures using for example height and limb length that could estimate the SR latency of different muscles. Furthermore, since there is already considerable amount of data published on the subject, an effort to re-analyze it correcting for reflex latency would be of immense help for the scientific community; 4) the chosen EMG onset method performed very well in the fast stretches since the signal-to-noise ratio was very high. However, in the two slowest stretch velocities, many false positives and negatives were identified by visual inspection, and manual onset correction was extremely time-consuming. All onset corrections were logged, and the information will be available at the project's repository. The lower SRT variability and better automatic EMG onset detection at higher stretch velocities strongly suggests designing SRT testing protocols with higher minimum stretch velocities.

The present study demonstrated that it is vital to consider SR latency when assessing the SRT and consequently the TSRT. To the best of our knowledge, most if not all current research utilizing the SRT as a measure of hyperreflexia has incurred in this error, thus a careful re-examination of data is important to update our understanding of this promising assessment method. Future research should assess the SRT and concomitantly measure muscle fascicle velocity (e.g., using ultrasonography) and the joint angular acceleration profile of the stretch. This information would allow better comparison between studies and perhaps elucidate why some participants exhibit velocity dependent SRT while others do not.

Data availability statement

Analysis scripts are available at https://github.com/Pedro-Valadao/EXECP_Neuromechanics. Raw data will be available upon request once EXECP's sub-project I-SENS finishes data acquisition since it is not currently possible to anonymize the data.

Ethics statement

Ethical approval was granted by the ethics committee of the Central Finland Healthcare District (U8/2017). Written informed consent to participate in this study was provided by participants or their legal guardians.

Author contributions

PV, HP, JA, and TF defined the study protocol. PV performed the data acquisition and together with FC performed the data analysis and statistics. All authors were involved in data interpretation and preparing the manuscript for publication. All authors read and approved the final manuscript.

Funding

This work was supported by the Olvi Foundation, Research Foundation of Cerebral Palsy Alliance (PHD00321), Finnish Cultural Foundation, University of Jyväskylä, Academy of Finland (grants #296240, #307250, #327288, #311877, #326988), including "Brain changes across the life-span" profiling funding to University of Jyväskylä, Jane and Aatos Erkko Foundation (#602.274), and the Netherlands Organization for Scientific Research (NWO-016.186.144). Ministry of Education and Culture (OKM/28/626/2022).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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III

NEUROMECHANICAL AND NEUROPHYSIOLOGICAL ANALYSIS OF HYPER-RESISTANCE AND HYPERREFLEXIA IN CHILDREN AND YOUNG ADULTS WITH CEREBRAL PALSY

by

Valadão, P., Cenni, F., Bar-On, L., Piitulainen, H., Avela, J & Finni, T.

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IV

EFFECTS OF THE EXECP INTERVENTION ON MOTOR FUNCTION, MUSCLE STRENGTH AND JOINT FLEXIBILITY IN INDIVIDUALS WITH CEREBRAL PALSY

by

Valadão, P., Cenni, F., Piitulainen, H., Avela, J & Finni, T. (2023).

Medicine & Science in Sports & Exercise, 10.

https://doi.org/10.3389/fbioe.2022.897852

Effects of the EXECP Intervention on Motor Function, Muscle Strength, and Joint Flexibility in Individuals with Cerebral Palsy

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ABSTRACT

VALADÃO, P., F. CENNI, H. PIITULAINEN, J. AVELA, and T. FINNI. Effects of the EXECP Intervention on Motor Function, Muscle Strength, and Joint Flexibility in Individuals with Cerebral Palsy. *Med. Sci. Sports Exerc.*, Vol. 56, No. 1, pp. 1–12, 2024. **Purpose:** Numerous exercise interventions to enhance motor function in cerebral palsy (CP) have been proposed, with varying degrees of effectiveness. Because motor function requires a combination of muscle strength, joint flexibility, and motor coordination, we designed a supervised multicomponent exercise intervention (EXErcise for Cerebral Palsy, or EXECP) for individuals with CP. Our aim was to evaluate the effects of the EXECP intervention and its retention after it ceased. **Methods:** The EXECP intervention combined strength training for the lower limbs and trunk muscles, passive stretching for the lower limb muscles, and inclined treadmill gait training. Eighteen participants with CP (mean age, 14 yr; 13 were male) were tested twice before the 3-month intervention and twice after the intervention, each test separated by 3 months. Seventeen typically developing age- and sex-matched controls were tested twice. Motor function was assessed with the 6-min walking test (6MWT) and the gross motor function measure dimensions D and E. Passive joint flexibility was measured with goniometry. Isometric and concentric muscle strength were assessed at the knee, ankle, and trunk joints. **Results:** The EXECP intervention successfully increased 6MWT ($P < 0.001$), gross motor function measure ($P = 0.004$), and muscle strength for knee and trunk muscles ($P < 0.05$), although no changes were observed for ankle joint muscles. Hip and knee joint flexibility also increased ($P < 0.05$). After the retention period, all tested variables except the 6MWT and knee joint flexibility regressed and were not different from the pretests. **Conclusions:** The improvements in strength, flexibility, and possibly motor coordination brought by the EXECP intervention were transferred to significant functional gains. The regression toward baseline after the intervention highlights that training must be a lifelong decision for individuals with CP. **Key Words:** CHILDREN, YOUNG ADULTS, TRAINING, REHABILITATION, WALKING

Cerebral palsy (CP) is a neurodevelopmental condition caused by a lesion in the developing fetal or infant brain. During development, the lesion leads to secondary symptoms such as muscle weakness, reduced joint flexibility, and incoordination (1–4), which hinder motor function for everyday activities such as walking and climbing stairs (5–7). Furthermore, these secondary symptoms may induce lower physical activity level (8,9) and cardiometabolic performance (5), increasing the risk of chronic diseases such as cardiovascular problems (10,11). Thus, there is an urgent need for therapeutic interventions capable of breaking this downward spiral of inactivity and loss of function.

Strength training is among the most studied interventions in CP, and it is effective if the established training guidelines (11–13) are followed (14–21). Although successful in increasing muscle strength, some interventions failed (15,17–20), whereas others succeeded in improving motor function (14,17). The diverging results in motor function gains induced by exercise interventions seem to arise from three main reasons. First, the neural and morphological adaptations are highly specific to the strength training methods (17,22), which varied in these studies. Second, functional tasks involve a

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Submitted for publication April 2023.
Accepted for publication July 2023.

0195-9131/23/5601-0001/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/MSS.0000000000003273

complex interaction between muscle strength, joint flexibility (i.e., maximum passive joint range of motion (ROM)), and motor coordination. Lastly, functional tasks involve activation of several muscle groups spanning across multiple joints. Thus, an adequate intervention should train all relevant muscles with naturalistic patterns of neural activation.

Despite the broad use of stretching to prevent or alleviate loss of joint flexibility in CP, its efficacy is unclear (4,23,24). Longitudinal studies applying manual passive stretching therapies for 6–9 wk have typically reported an increase in joint ROM with no mechanical changes (e.g., resting fascicle length), suggesting that increased stretch tolerance played a major role in the ROM increase (25–28). Little is known about the effects of more prolonged stretching interventions (28), and it is possible that structural changes (e.g., sarcomerogenesis) require more time. It may also be that combined training is more effective. Kalkman et al. (26) demonstrated that combined ankle plantarflexors strength training and passive static stretching for children with CP was more effective in increasing resting fascicle length than solely stretching. The mechanistic explanation is that strength training increased tendon stiffness, decreasing the relative muscle stiffness, and thus increasing the stretch taken by the muscle and decreasing the stretch taken by the tendon.

Gait training has been shown to be safe, feasible, and able to improve walking ability in children and young adults with CP (29). The inclined treadmill setup seems particularly effective, as it requires a greater ankle dorsiflexion (DF) in the swing phase and serves as a stretch for the ankle plantarflexors in the stance phase (30,31). Daily gait training with an inclined treadmill has been demonstrated to increase walking speed, DF strength, and active ROM, and reduce ankle stiffness in only 4–6 wk (32,33). Walking ability and overall gross motor function are usually measured both in research and clinical evaluation using the 6-min walking test (6MWT) and the gross motor function measure (GMFM; (34)), and both have been shown reliable in people with CP (35–37).

Our EXErcise for Cerebral Palsy (EXECP; Valadão et al. (38)) intervention was conceived to address some of the aforementioned limitations, combining three training modes: 1) strength training for the main lower limb and trunk muscles, using both mono and multiarticular exercises; 2) flexibility training for diagnosed short lower limb muscles; and 3) inclined treadmill gait training. The first aim of the present study was to examine the effects of the EXECP intervention on motor function, muscle strength, and joint flexibility. We hypothesized that the EXECP intervention would successfully increase performance in the 6MWT (main outcome), GMFM score, muscle strength, and lower limb joint flexibility. The second aim was to examine whether the adaptations induced by the EXECP intervention were still present 3 months after intervention. We hypothesized that all variables would regress toward baseline values after the 3-month retention period, not differing from control values. Finally, we hypothesized that CP participants would have significantly lower values for all studied variables compared with their typically developing (TD) controls.

METHODS

Study Design

Detailed information about the EXECP intervention can be found in our protocol paper (38). A nonconcurrent multiple-baseline design (39,40) was used (Fig. 1). The experimental CP group performed the EXECP intervention and had four testing sessions every 3 months: 1) first pretest (Pre1) at the beginning of the study, 2) second pretest (Pre2) after the 3-month control period, 3) first posttest (Post1) after the 3-month EXECP intervention, and 4) second posttest (Post2) after the 3-month retention period. During the control and retention periods, normal physical activity and physiotherapy were permitted; however, no structured physical training program was allowed. The intervention effects were assessed by comparing both pretests (Pre1, Pre2) with Post1. Because changes during the control period (between Pre1 and Pre2) represent the effects of normal development and activities (e.g., maturation, physiotherapy, sports), only Post1 and Post2 values that were statistically different from both pretests were deemed to have a significant intervention effect. Finally, Post2 was compared with Post1 and the pretests to verify the retention of the adaptations induced by the intervention.

The TD group performed only the two pretests interspaced by 3 months (Pre1, Pre2) and did not take part in the intervention. The Pre2 results were used in TD versus CP group comparisons, because Pre2 had fewer missing data compared with Pre1. Figure 1 depicts the experimental design with all tests.

Participants

A total of 20 children and young adults diagnosed with spastic CP and 17 age- and sex-matched TD controls volunteered for this study. Two participants with CP dropped out during the control period; thus, the final sample was 18 CP and 17 TD participants. The gross motor function classification system (GMFCS; [41]) diagnostic was provided by the participant's neurologist and confirmed by the first author. None of the participants with CP had lower limb surgery, serial casting, pharmacological treatments (except epilepsy medication, $n = 3$; baclofen, $n = 1$) or had participated in a resistance-training program for the lower limbs in the past 6 months. A participant who had a selective dorsal rhizotomy surgery 1 yr ago was included in the sample because his scores for muscle strength, joint flexibility, and gait performance were within the range of the other participants with CP. All participants were able to stand with both heels touching the floor (i.e., ankle in anatomical position). None of the TD participants had any musculoskeletal or neurological impairments. A positive ethical statement was granted by the ethics committee of the Central Finland Healthcare District (U8/2017, amended 7/2021). *A priori* sample size calculation was performed for the main outcome of meters walked in the 6MWT. Using a pre–post parallel-group randomized control trial model as an upper-bound reference, with a power of 0.8 and an α of 0.05, a sample size of 24 participants per group was required. Written informed consent to participate in this study was provided by participants and legal guardians of the underaged (Table 1).

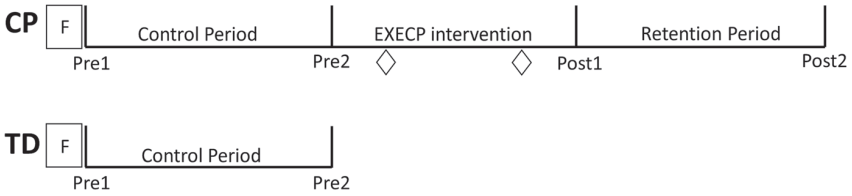

FIGURE 1—Experimental design. Within-group longitudinal analysis in the CP group had four time points for most variables (ankle and knee muscle strength and joint flexibility). The exceptions were as follows: 1) GMFM was measured only at Pre2 and Post1, and (2) trunk muscle strength was measured at the first and last intervention months (diamonds). For the TD group, GMFM and trunk strength were not assessed; thus, all variables were measured at the two time points (Pre1, Pre2). Both groups had a familiarization session (F) before Pre1. Between-group cross-sectional comparisons were done at Pre2.

EXECP Intervention

The EXECP intervention consisted of 2–3 training sessions per week, depending on each participant's physical activity levels: those engaged in regular physical activity chose to perform two or three sessions weekly, whereas sedentary participants were encouraged to train three times per week. A minimum of 24 and a maximum of 36 sessions were enforced for all participants. Each session had the following components and order: treadmill walking (5–10 min), strength training (60–75 min), and flexibility training (0–20 min; only for those with restricted flexibility). All sessions were individually supervised by a physiotherapist or strength and condition coach trained by P. V. Furthermore, gait training at home was performed in addition to the participant's ongoing physiotherapy and other possible sport hobbies.

Strength training. Five single-joint and five multijoint strength exercises were divided into two training protocols performed weekly (i.e., AB or ABA/BAB) with a range of 5–7 exercises per session. The exercises were as follows: seated and standing calf raise, seated DF, knee extension (KE) and flexion (KF) weight machine, leg press, squat, hip flexion while laying down, trunk extension (roman chair), and isometric hollow rocks.

The training load was adjusted monthly. In the first month, three sets of eight repetitions maximum (i.e., the ninth repetition could not be attained), with a movement duration of 6 s (3 s concentric and 3 s eccentric) and 60 s of rest were performed. In the second month, the training volume was maintained while the intensity was increased by reducing the concentric movement duration to 1 s and increasing the rest to 90 s. In the third month, training volume and rest were maintained, but sets were increased to four while repetitions were decreased to six, and concentric muscle actions were done as fast as possible while eccentric movement duration was decreased to 2 s. Exceptionally, the squat exercise followed a different progression: 1 to 4 sets of 10 repetitions with the largest attainable ROM were performed. Movement duration was similar to the other exercises, whereas rest started at 90 s and was decreased, when possible, to 60 s. After the entire volume could be executed with 60 s of rest, balance disks (Casall, Vantaa, Finland) were placed under the participant's feet to cause instability and increase exercise difficulty; also, unilateral squats were used to further increase the exercise intensity. Squats were always performed with the participant holding a support for safety. An assisted training procedure was adopted, the exercise resistance was selected based on the participant's strength on the optimal joint angles, and the instructor assisted in the concentric phase of the movement in the positions where the participant was not able to perform by himself. The eccentric phase was performed unassisted, and constant feedback about movement velocity was given. Whenever necessary, a dense foam ball was used to prevent hip adduction during the lower-limb strength exercises.

TABLE 1. Participant characteristics in the CP and TD groups.

Participant Characteristics	CP (n = 18), Mean ± SD (Min–Max)	TD (n = 17), Mean ± SD (Min–Max)
Male/female	13/5	12/5
Age, yr	14 ± 4 (9–22)	15 ± 4 (9–22)
Height, cm	158 ± 14 (131–180)	161 ± 16 (133–188)
Weight, kg	51 ± 16 (29–81)	53 ± 17 (28–90)
Level of involvement, bilateral/Unilateral	6/12	n/a
GMFCS, I/III	14/4	n/a

n/a, not applicable.

Flexibility training. Four sets of 45-s manual passive-static stretching at the pain threshold (i.e., position where the participant acknowledges an initial stretch pain sensation) were performed for each muscle group diagnosed short in the pretests (38). The possible trained muscles were one- and two-joint hip flexors, hip adductors, and knee flexors. One- and two-joint hip flexors were stretched in the modified Thomas test position (42). The participant laid supine on a table holding one of the lower limbs in full hip flexion (assistance was provided when needed), whereas the other leg hung outside the table (i.e., hip extension). The only difference between the stretches was that the trainer applied the hip extension torque at the distal thigh with the knee joint positioned either in full flexion or in a relaxed position. The hip adductors were stretched with the seated butterfly stretch. The hamstrings were stretched in supine position, and the trainer secured the untrained leg on the bench then flexed the participant's hip to approximately −90° (anatomical position, 0°; negative values, hip flexion) while applying a KE torque at the posterior aspect of the shank.

Gait training. A portable mechanical treadmill with an adjustable inclination of 6° or 7.3° (Vida XL, Venlo, the Netherlands) was used for training. Participants were instructed to walk at a comfortable speed, avoid toe walking, and try their best to attain heel strike. Verbal feedback was constantly given to improve gait quality, and the participant was allowed to rest at any time. In addition to 5–10 min of gait training in every session, all participants received a treadmill to take home and were

asked to walk a minimum of 10 min every day also at a comfortable speed, throughout the intervention duration. Weekly walking duration was logged on to a diary by the participants or their guardians, and total gait training duration was calculated. During the retention period, the participants chose if they wanted to keep using the treadmill at home and updating their diary or stop and return it.

Testing Protocol and Data Analysis

Muscle strength: ankle joint. Maximum isometric and concentric ankle plantarflexion (PF) and DF were assessed using a custom-build motor-driven dynamometer (Neuromuscular Research Center, University of Jyväskylä, Jyväskylä, Finland). Participants were seated with the knee joint fully extended (0°), hip joint flexed at −60°, and the ankle joint at an initial position of 0° (i.e., anatomical position) or 28° into PF. The foot was firmly attached to a footplate mounted on the rotation platform so that the rotation axes of the ankle joint and the motor-driven platform coincided. Participants were securely stabilized by an assembly of straps that fastened both shoulders and connected to a waist belt. An additional strap with a foam support prevented the tested leg knee joint from flexing. The torque around the rotational axis of the motor was measured by a piezoelectric crystal transducer (Kistler Holding, Winterthur, Switzerland), and the ankle joint angle was monitored by a linear potentiometer. Torque and joint angle were sampled at 1 kHz with a 16-bit A/D converter (CED power 1401; Cambridge Electronic Design, Cambridge, UK) using Spike2 software (v4, Cambridge Electronic Design).

The PF test started with a 2-s maximum isometric muscle action at 0°, followed by an isokinetic (14 deg·s^{-1}) concentric effort until 28°. The DF test started with a 2-s maximum isometric muscle action at 28°, followed by an isokinetic (14 deg·s^{-1}) concentric effort until 0°. Three to five trials with 1–2 min of rest in between were performed for each test, and the highest value among all trials was used for the following variables: peak isometric torque, rate of force development, concentric angular impulse, and curve width. Rate of force development was calculated from the onset of the muscle action (i.e., torque >1 N·m) to 200 ms as delta torque divided by delta time. Concentric angular impulse was calculated as the torque–time integral. Curve width was defined as the concentric ROM where the participant was able to exert continuously at least 50% of the peak isometric torque (43). Figure 2 depicts the four studied variables. No gravity correction was performed on torque data for two reasons. Firstly, the passive delta torque between the two end positions (0° and 28°) was very small (0.5–2 N·m), because of the foot's small moment arm and weight. Secondly, the CP participants had high variability in background muscle activity.

Muscle strength: knee joint. Maximum isometric and concentric KF and KE were assessed using a custom-build motor-driven dynamometer (Neuromuscular Research Center, University of Jyväskylä). Participants were seated with the hip joint flexed at −80° and the knee joint fully extended (0°) or at 105°. The distal part of the shank was secured with a Velcro strap to a strain gauge capable of measuring both tensile and compressive forces. The distance from the dynamometer axis of rotation, aligned with knee joint axis of rotation, to the strain gauge (i.e., moment arm) was measured in each test. Participants were securely stabilized by an assembly of straps fastening both shoulders and connected to a waist belt. The KF test started with a 2-s maximum isometric muscle action at 0°, followed by an isokinetic (15 deg·s^{-1}) concentric effort until 75°. An examiner kept strong downward pressure at the distal part of the thigh to prevent hip flexion during the trial. The KE test started with a 2-s maximum isometric muscle action at 75°, followed by an isokinetic (15 deg·s^{-1}) concentric effort until 0°. Torque was calculated by multiplying the moment arm by the force, and joint angle was monitored by a linear potentiometer. Both signals were sampled at 1 kHz with the same hardware and software used in the ankle joint tests. Passive trials (i.e., passive joint movement) were used to measure torque caused by the weight of the leg, which was subtracted from the active torque curves.

The same test procedures (number of trials and rest) and test variables described for the ankle joint tests were used for the knee joint tests. For all strength tests, a preparatory activity consisting of 10 progressively stronger efforts from 20% to 90% of the perceived maximum voluntary effort was performed before each strength test. Both ankle and knee dynamometers performed the target movement velocities with less than 2% variation throughout all trials in this study. All strength measurements were performed on the most affected leg for the CP group and on the corresponding leg of the matching control participant. Participants were instructed to produce maximum isometric torque as fast as possible and maintain maximum effort throughout the concentric movement in each trial. Furthermore, visual feedback of the torque signal was provided in real time, and participants received strong verbal encouragement during every trial.

Muscle strength: trunk muscles. Because both the trunk extension on the roman chair and hollow rocks exercises were trained isometrically and both time and weight recorded in every training session, the best performance in the first training month (to avoid learning effects) and in the last month was used to evaluate strength gains in these muscle groups. Best performance was measured in time, as the duration of the isometric muscle action (maximum 60 s), and intensity, as the maximum weight held during the isometric muscle action. Once the participant was able to hold a given weight for the maximum duration, the intensity was increased.

Joint flexibility. Hip flexor flexibility was tested using the modified Thomas test position described in the flexibility training section. An assistant kept a goniometer with its fulcrum at the greater trochanter of the femur and held the stationary arm perpendicular to the bench. The examiner placed the goniometer's moving arm aligned to the lateral femoral condyle and slowly pushed the distal aspect of the thigh toward hip extension and, once the participant acknowledged an initial stretch pain sensation, read the resulting joint angle in the goniometer. Participants with less than 20° of hip extension were diagnosed with short hip flexors. Hip adductor flexibility and the differentiation between one-joint and two-joint

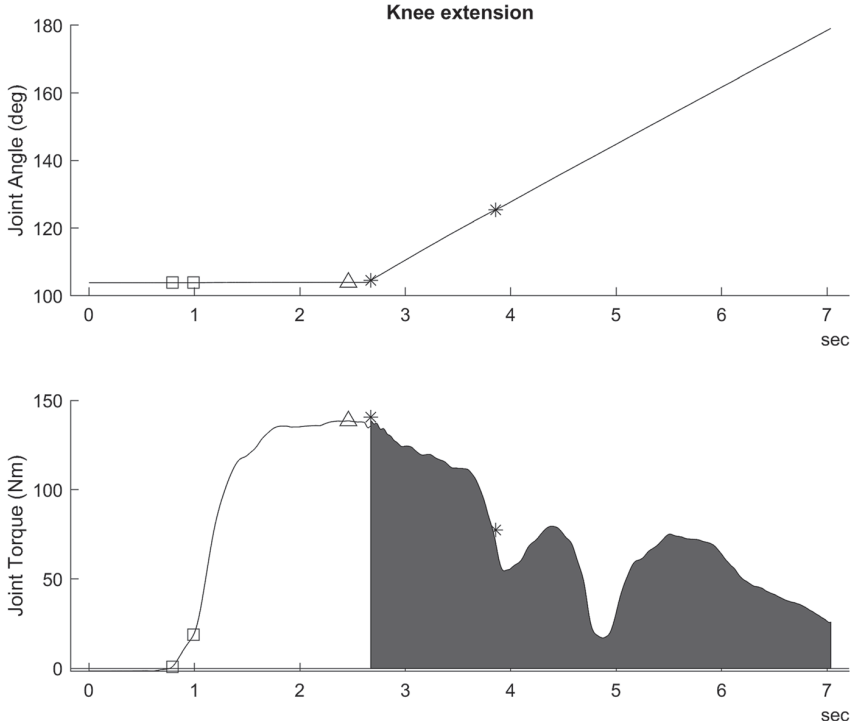

FIGURE 2—Example of the muscle strength variables analyzed in the KE test. From left to right: 1) rate of force development was calculated as the slope between torque onset (>1 N·m) and torque onset plus 200 ms (two squares), 2) peak isometric torque (triangle), 3) concentric angular impulse (shaded dark area), and 4) curve width is the concentric ROM that is greater than 50% of the peak isometric torque (range between the two asterisks). Concentric movement onset is marked by the first asterisk.

hip flexor flexibility were assessed based on the modified Thomas test position (i.e., hip and knee angles); details can be found at the protocol paper (38).

Passive knee extension (PKE) test was performed in the same position as the hamstring stretch, with the participant lying supine with the hip and knee flexed at right angles. The examiner slowly applied torque at the posterior aspect of the shank causing KE, whereas an assistant maintained strong downward pressure on the untested thigh to stabilize the pelvis. The test stopped when the participant acknowledged an initial stretch pain sensation, and the examiner read the goniometer angle (fulcrum: lateral femoral condyle, reference lines: lateral malleolus and greater trochanter of femur). Three measures per leg for each test were performed, and the mean value was used for statistical analysis. Participants with more than 40° of KF in the passive KE test were diagnosed with short knee flexors.

Motor function. Motor function was assessed with two tests, the 6MWT and the GMFM. The 6MWT was performed on an indoor 30-m rubber track, and its result was the distance walked in 6 min. GMFM was assessed in the CP group at Pre2 and Post1 using the absolute scores of the 66-item version of the GMFM (34) dimensions D (standing) and E (walking, running and jumping).

Maturation. Because of the 9-month study duration and a sample composed mainly of children, the growth spurt could be a confounding factor in our experimental design. The estimated time from peak height velocity (i.e., maturity offset, or MO) was computed for all participants at each testing point using sex-specific equations provided by Moore et al. (44), described in our protocol paper (38). For all available data points (i.e., participants multiplied by tests minus data loss: 100 of 106), the participants had an MO smaller than 6 months in only two tests, participants had MOs of 6–12 months in eight tests, and MOs larger than 1 yr in all remaining 90 tests. Thus, for our sample, the growth spurt was not an issue and MO was not included as a covariate in our statistical model.

Test–retest reliability. Before the first test time point (i.e., Pre1), all participants performed a 60-min familiarization session to experience all strength and flexibility tests, and the 6MWT (not necessarily the whole test). Whenever feasible, the familiarization session was used to duplicate tests to allow comparison with Pre1 for test–retest reliability assessment. However, because of limited time in the familiarization session, not many tests could be performed, and only variables with a minimum of five participants were used for this analysis. The two-way mixed-effects absolute agreement intraclass correlation coefficient (ICC; 3.1/3.k; [45]) was calculated between tests (i.e., familiarization vs Pre1) for each group, and flexibility data from both legs were pooled.

Statistical Analysis

Generalized linear mixed models (GLMM) were used to compare the four time points (i.e., Pre1, Pre2, Post1, Post2)

in the CP group. Time was set as a fixed factor and the subject intercept as a random factor; thus, participants had different *y*-intercepts but similar regression slopes. The GLMM model used the unstructured correlation structure and the identity link function, and two distributions were tested: normal and gamma. The best distribution for each dependent variable based on the Akaike information criterion and the residuals Q–Q plot was used. Theoretically, higher motor function should be associated with higher 6MWT and muscle strength performance. Thus, for these variables, the absolute Pre2 GMFM score ($GMFM_{co}$) was used as a covariate, centered and conditioned to the mean ± 1 SD. For the statistical analyses, three participant subgroups based on $GMFM_{co}$ were created: high (>mean + SD), mid (within mean ± SD), and low (<mean − SD) scores. The effects of time, $GMFM_{co}$, and their interaction were tested. For the flexibility tests, in addition to time as a fixed factor, the muscle training status (i.e., trained or not trained during the intervention) was also included, and the interaction between time and training status was verified. The amount of stretching performed during the intervention was not used as a covariate because the participants were allowed to maintain their stretch routines throughout the project duration, and keeping track of it through the 9 months was not feasible.

The GLMM model ICC (ICC_{GLMM}; i.e., subject variance divided by subject plus residual variance) was calculated for each dependent variable to verify the percentage of the total variance that was explained by the participants (i.e., random effect, different β_0 for each participant). The total number of gait training minutes and the total number of strength and stretch exercises were tested as covariates to improve the model, because more training volume could translate into better adaptation of the dependent variables. However, none of these variables were successful in improving the models and therefore were removed. Bonferroni *post hoc* and simple effect tests were used to find the specific differences between tests. Because we hypothesized that most changes would occur between pretests and Post1, although most likely nothing would happen between pretests and between posttests, simple effect tests for the interaction term were performed regardless of the omnibus main effect result. Only pre–post differences in which the posttests were significantly different from both pretests were reported and discussed. The rationale for this decision was that only effects that were statistically different from the control period (i.e., Pre1–Pre2), which has its inherent variability, are statistically meaningful. GLMM results are presented as parameter estimates (β), 95% confidence intervals (95% CI), SE, and the *P* value. The paired *t*-test or the nonparametric analog Wilcoxon signed rank was used to compare 1) the two time points (Pre1 and Pre2) in the TD group, 2) the CP GMFM values between Pre2 and Post1, and 3) the trunk strength parameters between the first and last intervention months. Independent *t*-tests or the nonparametric analog Mann–Whitney *U* test was used to compare Pre2 between groups. All statistical analyses were performed using jamovi 2.3 software (The jamovi project, https://www.jamovi.org). α was set to 0.05.

RESULTS

Training Compliance and Side Effects

The participants with CP performed 24–36 (mean ± SD, 29 ± 4) training sessions, which contained 360–1984 min (683 ± 352 min) of gait training (i.e., supervised sessions plus home training) and 32–96 min (67 ± 16 min) of stretching in the training sessions. Most participants also stretched the same muscles at the physiotherapy or at home. Only five participants chose to continue gait training in the retention period, reporting 600 min of training, 10 min per day as instructed. In a total of 529 training sessions, there were 12 complaints of acute knee pain (2%) by four participants, which subsided in the same day. Furthermore, two participants reported moderate muscle soreness three times, and one subject reported high muscle soreness once and had to recover for a week before restarting training. Finally, one participant reported knee pain during the first week of inclined treadmill training at home, which subsided after online consulting was done to correct the gait movement pattern.

Data Loss

Two CP 6MWT were excluded from the analysis because of noncompliance with test execution guidelines (1 Pre2/1 Post1). Because of the COVID-19 lockdowns, six participants with CP were unable to perform Post2. Furthermore, three participants started the intervention 3 months after Pre2, and one started 5 months after Pre2. Thus, it is conceivable that this lower physical activity period lowered some study variables below the pretests values.

Test–Retest Reliability

Figure 3 presents ICC values and 95% CI for all repeated measures between the familiarization and Pre1 testing sessions. The CP group had the following sample sizes: PF peak torque and rate of force development (n = 8), other PF and all DF variables (n = 5), and PKE (n = 16). The TD group had the following sample sizes: all PF and DF variables (n = 11), 6MWT and all KE/KF variables (n = 7), and PKE (n = 27). Because of the slower familiarization process in the CP group (e.g., mobility and positioning), the 6MWT and KE/KF tests could not be performed in the familiarization session because of time constraints. Most KE and PF variables, PKE and 6MWT had excellent to moderate reliability, whereas DF and KF had a wider 95% CI ranging from excellent to poor reliability (45). Curve width reliability for all four muscle actions was very poor.

Within-Group Comparisons in the Control Period (Pre1 vs Pre2)

In the CP group, no statistically significant *post hoc* test differences for time were found between Pre1 and Pre2 in any of the studied variables. However, a few significant simple effects were statistically significant in the control period: 1) KE peak isometric torque was higher in Pre2 compared with Pre1 in the low $GMFM_{co}$ subgroup (β = 11; SE = 3; 95% CI, 4–17; $P < 0.001$) and the mid $GMFM_{co}$ subgroup (β = 5;

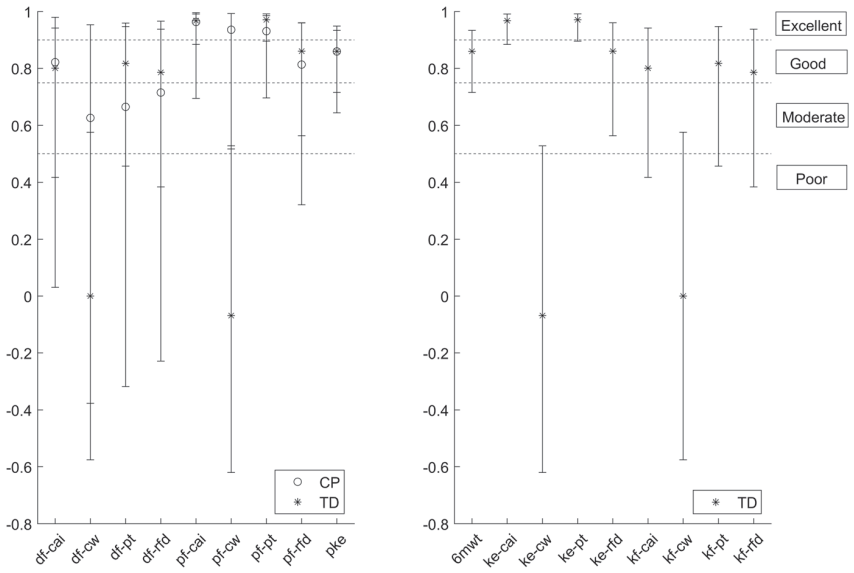

FIGURE 3—ICC and 95% CI for successfully replicated study variables in both groups (*left panel*) and only in TD (*right panel*). The *top right* corner displays reliability thresholds (45). 6mwt, 6-min walking test; cai, concentric angular impulse; cw, curve width; df, dorsiflexion; ke, knee extension; kf, knee flexion; pf, plantarflexion; pke, passive knee extension; pt, peak torque; rfd, rate of force development.

SE = 2; 95% CI, 0–10; $P = 0.033$), 2) KE rate of force development was higher in Pre2 compared with Pre1 for the high $GMFM_{co}$ subgroup (β = 52; SE = 18; 95% CI, 15–88; $P = 0.005$), and 3) KF rate of force development was higher in Pre1 compared with Pre2 for the mid $GMFM_{co}$ subgroup (β = 16; SE = 7; 95% CI, 2–30; $P = 0.027$). Thus, although no differences between pretests were found for the entire CP group, a few subgroups changed significantly during the control period. In the TD group, no statistically significant differences were found between Pre1 and Pre2 in any of the studied variables, with one exception. PF rate of force development was significantly higher in Pre2 compared with Pre1 (mean difference, 65 $(N \cdot m) \cdot s^{-1}$; $t = -2.686$, $P = 0.017$).

Within-Group Comparisons for Intervention Effects (Pre vs Post)

Six-minute walking test. Significant main effects of time (i.e., time points) and $GMFM_{co}$ (i.e., GMFM as a covariate) were found ($P < 0.001$), but no time–$GMFM_{co}$ interaction ($P = 0.126$). *Post hoc* analysis showed that Post1 had statistically higher scores than pretests (β = 33; 95% CI, 12–53; SE = 11; $P = 0.024$). Likewise, Post2 had statistically higher scores than pretests (β = 43; 95% CI, 20–67; SE = 12; $P = 0.006$). The simple effects test showed that the three $GMFM_{co}$ subgroups were statistically different in all four time points ($P < 0.001$) and that the pre–post differences occurred only in the two lower score $GMFM_{co}$ subgroups ($P < 0.001$).

GMFM (Pre2 vs Post1). Four participants with CP had the maximum GMFM score at Pre2 (i.e., highly functional) and were excluded from this analysis because of the test insensitivity to measure changes induced by the intervention. The Wilcoxon signed rank test showed a statistically significant difference ($n = 14$, $P = 0.004$) between Pre2 (median, 101; interquartile range (IQR), 46) and Post1 (median, 104; IQR, 41) GMFM scores. Eleven participants improved a median of 3 points with a range of 1–11, and three participants had the same score on both tests.

Trunk isometric strength (first vs last intervention month). A statistically significant difference in trunk extension weight between the first (median, 0.5; IQR, 5; $P = 0.001$) and last (median, 5; IQR, 7) months was found. There was no difference between the duration of the trunk extension exercise in the first (median, 60; IQR, 7) and the last (median, 60; IQR, 0) months. For trunk flexion, the opposite happened: no difference in trunk flexion strength between the first (median, 0; IQR, 0) and last (median, 0; IQR, 0) months was found, whereas the duration of the trunk flexion exercise was statistically lower in the first (median, 45; IQR, 30; $P = 0.013$) compared with the last (median, 60; IQR, 15) month.

Peak isometric torque. For the PF test, a significant main effect of $GMFM_{co}$ ($P < 0.001$) was found, whereas there were no main effects of time or their interaction. $GMFM_{co}$ subgroups were statistically different in all four time points ($P \leq 0.001$). No significant main effects were found for the DF test. In the KE test, a significant main effect of time ($P < 0.001$) and its interaction with $GMFM_{co}$ ($P = 0.014$) were found. *Post hoc* analysis showed that Post1 torque was higher than pretests (β = 23; SE = 3; 95% CI, 17–29; $P < 0.001$). The simple effects test showed that the *post hoc* differences occurred for all $GMFM_{co}$ subgroups ($P < 0.001$). In the KF test, only a significant main effect for time was found ($P < 0.001$). *Post hoc* analysis showed that Post1 torque was higher than the pretests (β = 16; SE = 3; 95% CI, 9–22; $P < 0.001$).

Rate of force development. For the PF test, a significant main effect of $GMFM_{co}$ ($P = 0.011$) was found, with

statistically significant differences between the three $GMFM_{co}$ subgroups in all time points ($P < 0.05$), except Post2. For the DF test, a significant main effect of time ($P = 0.034$) was found. However, *post hoc* analysis showed that Post1 was only statistically different from the lower value pretest and thus was disregarded. For the KE test, significant effects of time ($P < 0.001$) and its interaction with $GMFM_{co}$ ($P = 0.005$) were found. *Post hoc* analysis showed that Post1 had higher values than the pretests ($\beta = 68$; SE = 17; 95% CI, 37–101; $P < 0.001$). The simple effects test showed that pre–post effects of time were significant for all three $GMFM_{co}$ subgroups ($P < 0.001$). For the KF test, significant effects of time ($P = 0.12$) and $GMFM_{co}$ ($P = 0.010$) were found; however, *post hoc* analysis and simple effects test revealed no significant pre–post differences.

Concentric angular impulse. For the PF test, a significant main effect for $GMFM_{co}$ ($P = 0.001$) was found. Simple effects test showed that $GMFM_{co}$ subgroups were statistically different in all four time points ($P \leq 0.011$). No significant main effects were found for DF concentric angular impulse. In the KE test, significant effects of time ($P < 0.001$) and its interaction with $GMFM_{co}$ ($P = 0.009$) were found. *Post hoc* analysis showed that Post1 concentric angular impulse was statistically higher than the pretests ($\beta = 46$; 95% CI, 22–70; SE = 12; $P = 0.001$). The simple effects test showed that pre–post differences occurred only in the two lower score $GMFM_{co}$ subgroups (<0.001), and that $GMFM_{co}$ subgroups were statistically different only in the pretests ($P < 0.05$). For the KF test, significant effects for time ($P = 0.005$) and $GMFM_{co}$ ($P = 0.033$) were found. *Post hoc* analysis showed that Post1 concentric angular impulse was statistically higher than the pretests ($\beta = 45$, 95% CI, 19–71; SE = 13; $P = 0.01$). Simple effects test showed that the pre–post differences were only present in the two higher score $GMFM_{co}$ subgroups ($P \leq 0.005$), and that $GMFM_{co}$ subgroups were statistically different in Pre1 and Post1 ($P < 0.05$).

Curve width. No significant effects in PF, DF, and KF curve width were found. In the KE test, a significant effect of $GMFM_{co}$ ($P < 0.001$) was found, while no effects of time or their interaction. Simple effects analysis showed that only in Pre1, the $GMFM_{co}$ subgroups had statistically different curve width values ($P < 0.001$), with higher $GMFM_{co}$ scores having higher curve width.

PKE flexibility. For the PKE test, significant main effects of time ($P = 0.001$) and training status ($P < 0.001$) were found. *Post hoc* analysis showed that the legs chosen for flexibility training during the intervention had statistically lower flexibility compared with the untrained legs ($\beta = 14$; SE = 4; $P < 0.001$), and the simple effects test further revealed that the differences were present in all four time points ($P \leq 0.004$). The second *post hoc* analysis (i.e., merged training status) showed that both posttests had statistically higher joint flexibility compared with the pretests ($P < 0.05$). Furthermore, the simple effects test revealed that pre–post differences were present only in the trained legs ($P = 0.003$). Post1 had significantly higher joint flexibility than both pretests ($\beta = 5$; SE = 2; 95% CI, 1–8; $P = 0.008$), and so did Post2 ($\beta = 4$; SE = 2; 95% CI, 0–8; $P = 0.037$).

Hip extension flexibility. No main effects of time, training status, and their interaction were found for the hip extension test. In addition, *post hoc* tests showed no differences. Oppositely, the simple effects test found a significant effect for time only in the trained legs ($P = 0.019$), and pairwise comparisons showed that Post1 flexibility was statistically higher than the pretests ($\beta = 5$; SE = 2; 95% CI, 1–9; $P = 0.018$).

Within-Group Comparisons for the Retention Period (Post1 vs Post2)

No significant differences between posttests were found in the present study. In all dependent variables with a significant difference between pretests and Post1, Post2 values were in between pretests and Post1 and thus not different from any time points. The only exceptions were the 6MWT and PKE, in which Post2 remained statistically higher than the pretests. Table 2 shows the estimated marginal means for all variables at the best pretest, Post1, and Post2 time points.

Between-Group Comparisons

Table 3 shows the Pre2 comparisons between CP and TD groups and the absolute difference in means between pretests for both groups. The TD group had statistically higher scores for all studied variables compared with CP, except for hip flexion flexibility.

DISCUSSION

The main finding of the present study was that the EXECP intervention successfully enhanced gait performance and overall gross motor function. Furthermore, knee and trunk muscle strength, and hip and knee joint flexibility improved. However, at the ankle joint, no significant effects of the intervention were found. The multicomponent intervention design has the disadvantage of not allowing the determination of the importance of each specific adaptation to the gains in motor function. However, it is reasonable to assume that the combination of the induced adaptations was beneficial. Importantly, the multicomponent training program was safe, feasible, and effective in increasing motor function in children and young adults with CP.

Motor function. We found a group mean increase in 6MWT distance of 33 and 44 m comparing pretests with Post1 and Post2, respectively. Maher et al. (36) performed the 6MWT with a similar sample (CP, same mean age and GMFCS levels; $n = 41$) and found a mean test–retest group difference of 1 m and a 95% CI for individual test–retest variability of ±43 m. Thus, our intervention effect seems clinically significant as it is very unlikely that the group mean would shift toward the upper test–retest variability bound per chance. Interestingly, Gillet et al. (14) induced similar changes in the 6MWT (mean difference, 48 m) with a combined strength training for ankle muscles and anaerobic training program for slightly older participants with CP (mean age, 20 yr). Oppositely, Kirk et al. (15) performed a strength training

TABLE 2. EMM, SE, and ICC_{GLMM} for all study variables at the best pretest and both posttests for CP participants.

Variable	ICC_{GLMM}	Pretest, EMM (SE)	Post1, EMM (SE)	Post2, EMM (SE)
6MWT, m	0.87	502 (21)	535 (21)*	546 (21)*
PKE trained, °	0.73	39 (5)	35 (5)*	35 (5)*
PKE untrained, °		24 (4)	21 (4)	22 (4)
Hip trained, °	1.00	19 (3)	24 (3)*	18 (4)
Hip untrained, °		26 (3)	24 (3)	24 (4)
PF torque, N·m	0.74	74 (6)	81 (6)	81 (7)
PF RFD, $(N·m)·s^{-1}$	0.49	93 (15)	119 (15)	95 (17)
PF CAI, (N·m)·s	0.83	68 (10)	77 (9)	81 (10)
PF CW, °	0.99	17 (2)	16 (2)	17 (2)
DF torque, N·m	1.00	25 (4)	25 (4)	26 (4)
DF RFD, $(N·m)·s^{-1}$	1.00	56 (9)	60 (10)	61 (10)
DF CAI, (N·m)·s	0.84	15 (4)	18 (4)	19 (4)
DF CW, °	0.66	14 (2)	13 (2)	14 (2)
KE torque, N·m	1.00	95 (11)	113 (11)*	103 (11)
KE RFD, $(N·m)·s^{-1}$	1.00	218 (36)	286 (37)*	264 (39)
KE CAI, (N·m)·s	0.79	190 (21)	236 (21)*	233 (23)
KE CW, °	0.23	40 (4)	41 (3)	39 (4)
KF torque, N·m	1.00	52 (6)	68 (7)*	62 (7)
KF RFD, $(N·m)·s^{-1}$	1.00	86 (13)	79 (12)	102 (15)
KF CAI, (N·m)·s	0.64	49 (16)	94 (16)*	73 (18)
KF CW, °	0.55	18 (5)	20 (5)	18 (5)

*Different from pretests ($P < 0.05$).
CAI, concentric angular impulse; CW, curve width; EMM, estimated marginal means; RFD, rate of force development.

program for lower limbs and trunk muscles for adults with CP (mean age, 36 yr), and although their gait kinematics and muscle strength improved, no change in the 6MWT was found. In all the three studies mentioned previously, participants had similar GMFCS distributions and a baseline 6MWT performance of 480–502 m, the interventions had adequate strength training load distribution, and other than the age differences, the only major difference was in the chosen training protocol structure. Although Gillet et al. (14) utilized anaerobic exercises very relevant to motor function (e.g., stair climbing, changing direction), and we trained gait on the treadmill, the protocol from Kirk et al. (15) may have failed to increase 6MWT because of the lack of a more generalized motor function training to enhance coordination. Regarding the GMFM scores, merging GMFCS I–III and GMFM dimensions D and E, Oeffinger et al. (46) reported a minimum clinically important difference of 1.2–1.6 and 1.8–2.6 for medium (0.5) and large (0.8) effect sizes, respectively. In the present study, absolute GMFM score changes ($n = 11$; median, 3) were equal or higher than two points for 9 participants and one point for two participants. It is worth considering that a one-point increase may or may not be functionally relevant. For example, five participants were able to climb stairs with alternating feet after the intervention, instead of stepping twice on the same step, as they did before the intervention. Other one-point increases, for example, being able to keep balance with one foot up from 2 to 4 s may not be so functionally relevant.

TABLE 3. Group comparisons for all studied variables at Pre2 session.

Group	Mean ± SD/Median (IQR)			Δ Pretests
Variables	CP	TD	*P*	CP/TD
6MWT, m	550 (135)	710 (106)	<0.001	1/4
PKE, °	34 (28)	13 (20)	<0.001	
Hip, °	21 ± 8	24 ± 6	0.124	
PF torque, N·m	75 (53)	163 (75)	<0.001	3/4
PF RFD, $(N·m)·s^{-1}$	67 (68)	397 (274)	<0.001	15/43
PF CAI, (N·m)·s	58 (56)	208 (106)	<0.001	11/9
PF CW, °	13 (15)	25 (4)	0.004	1/0.5
DF torque, N·m	21 (15)	38 (26)	<0.001	2/3
DF RFD, $(N·m)·s^{-1}$	43 (22)	136 (48)	<0.001	9/4
DF CAI, (N·m)·s	14 (19)	58 (23)	<0.001	2/7
DF CW, °	12 (17)	28 (0)	<0.001	0/1
KE torque, N·m	92 (53)	125 (104)	0.018	2/3
KE RFD, $(N·m)·s^{-1}$	109 (237)	388 (291)	0.001	33/13
KE CAI, (N·m)·s	202 (145)	346 (356)	<0.001	0/24
KE CW, °	37 ± 19	52 ± 14	0.013	7/7
KF torque, N·m	46 (46)	63 (57)	0.030	5/6
KF RFD, $(N·m)·s^{-1}$	29 (64)	155 (232)	<0.001	6/41
KF CAI, (N·m)·s	40 (74)	147 (172)	<0.001	14/4
KF CW, °	9 (21)	39 (20)	<0.001	4/3

"Δ Pretests" indicates the absolute difference between pretests means for both groups (e.g., for the 6MWT the mean difference was 1 and 4 m for the CP and TD groups, respectively).
CAI, concentric angular impulse; CW, curve width; RFD, rate of force development.

Muscle strength. Thigh muscles responded well to the EXECP intervention. KE had an increase of 19% in peak torque, 31% in rate of force development, and 24% in concentric angular impulse, whereas KF had an increase of 31% in peak torque and 92% in concentric angular impulse. Acknowledging that previous studies had different training loads, testing procedures, and CP populations, we think that all studies discussed hereinafter had adequate training loads and testing procedures and thus will be cautiously compared with the present study. Previous studies reported similar gains for KE peak torque (12%–27%; [18,19]), concentric KE/KF peak torque (25%), and work (21%; [16]). Kirk et al. (15) reported a one-repetition maximum (1RM) increase of 82% for KF and 45% for KE, which is fairly comparable to our concentric angular impulse variable (i.e., assuming a similar repetition duration) and has similar magnitude strength gains. The increase in KF concentric angular impulse was exceptionally high, and it is probably not a result of only increased muscle strength but also enhanced motor control to maintain higher torque output throughout the concentric muscle action, even though curve width did not increase significantly after the intervention.

Our intervention did not cause significant changes in the strength of the shank muscles, which is in contrast with other studies that successfully induced adaptations in PF peak torque (25%; [14]), and in PF and DF 1RM (137% and 87%, respectively, [15]). Gillet et al. (14) had a similar training load to that used in our study; however, most of their exercises (four of five) trained PF, whereas one trained DF. Thus, it is reasonable that they successfully increased PF peak torque, whereas no changes were reported for the DF. Kirk et al. (15) also had a similar training load compared with the present study and reported a large increase in PF (137%) and DF (87%) 1RM test, in the same device that training occurred. However, no changes in DF peak torque and rate of force development were found when measured by a stationary dynamometer (increases in rate of force development in shorter time windows were found), suggesting that specificity between testing and training is an important factor. In the present study, the PF testing position had the knee and ankle at the anatomical position, which placed both hamstrings and gastrocnemius muscles in a lengthened and often slightly uncomfortable position (participants with CP consistently asked to flex their knee during rest). Because the weight used for exercising these muscles consistently increased for all participants during the

intervention, we hypothesize that the testing position may have blurred the strength gains for PF. Lastly, DF was the only muscle action trained with manual resistance and rubber bands, highly dependent on the ability of the trainer to keep the appropriate stimulus. Therefore, these aspects may reduce the efficiency of training these ankle muscles, and a higher training load may be required for adaptations to be verified.

The EXECP intervention was not able to increase curve width in any of the four muscle actions, not even in the thigh muscles, which had a significant increase in concentric angular impulse, demonstrating an inability to maintain steady torque output during the movement. This result is reasonable, because only the first training month of the intervention had an optimum training load configuration (i.e., slow and controlled movement) for curve width enhancement. Increases in trunk muscles strength followed an expected pattern: a) for trunk extensors, maintaining the maximum exercise duration (60 s) was already possible in the first month of the intervention for 12 participants, and thus, increasing the weight (900%) was the main procedure for increasing the training load; and b) for trunk flexors, only five participants were able to achieve the maximum exercise duration (60 s) in the first month, and thus, increasing the hold duration (33%) was the main way to increase the training load. In addition, a slight increase of weight in the trunk flexion exercise (i.e., weight on the distal shank and held above the head) causes a large increase in difficulty due to the very long resistance arm. Finally, because the participants were not used to progressive resistance training, increases of muscle strength may be affected by psychological aspects (e.g., willingness to exert maximum efforts), as the 10-fold increase in trunk extension weight suggests.

Joint flexibility. The EXECP intervention caused a mean joint flexibility increase of 5° for both passive knee and hip extensions. Although statistically significant, the change magnitude seems functionally insignificant, although it was achieved with a low training volume (mean of 6 $min \cdot wk^{-1}$), and it is unclear if a higher training volume could have yielded a better outcome. Furthermore, it is also unclear if the enhanced flexibility was due to sensorimotor (i.e., increased tolerance to stretch) or structural adaptations (i.e., morphological changes in the muscle–tendon structure), as this topic is still under active investigation (28,47). It seems unlikely that flexibility training with a feasible training load can induce mechanical alterations in most participants with CP. We base this claim on the following arguments: 1) for clinical populations, there is an overall lack of evidence for stretching effectiveness (48) and specifically for CP (23,24); 2) morphological alterations in CP muscle, such as a lower number of satellite cells and ribosomes, should mechanistically hinder muscle growth response (1,49,50); and 3) sarcomerogenesis is hindered in CP, evidenced by the usual reduced number of lengthened sarcomeres found in CP muscle (1). Given the widespread use of stretch as a treatment for CP and the lack of evidence of its effectiveness, current therapeutic practices should focus on other interventions that have been shown successful, such as strength and gait training. Finally, our data show that the intervention had no negative effect on joint flexibility, which has been an anecdotal concern of many health practitioners.

Covariates. Overall, $GMFM_{co}$ was very useful in improving the GLMM models. $GMFM_{co}$ was expected to affect the 6MWT outcome because it includes many items related to gait, but $GMFM_{co}$ also worked very well for PF and KE (torque, rate of force development, and concentric angular impulse) and KF (rate of force development and concentric angular impulse). Interestingly, $GMFM_{co}$ was not useful to stratify participants in any of the DF variables, meaning that participants with high GMFM scores had similar performance than participants with low scores. Training load variables such as total number of training sessions and strength exercises, amount of gait training, and number of stretching exercises had no effect on the models. Participants with higher GMFM were able to perform more exercises during the training session because of improved training logistics (e.g., faster locomotion, easier setup attainment) and generally were more physically active. Furthermore, group heterogeneity was a much more prominent factor compared with the training load, which is evidenced by the high ICC_{GLMM} displayed in Table 2.

Control and retention periods. Maturation and normal daily activities did not affect the studied variables in both groups during the control period, except for PF rate of force development in the TD group. A few CP $GMFM_{co}$ subgroups had significant changes between pretests for KE (peak torque and rate of force development) and KF (rate of force development), although nonsignificant for the whole group. Although not significant, a clear declining trend toward pretest values was found in Post2, which was expected because the intervention stopped at Post1. This result suggests that a lifelong change in behavior is needed for people with CP: physical training must be continued throughout the person's life span.

Group comparisons. TD had superior performance in all dependent variables, except for hip extension flexibility. It is reasonable to infer that all these aspects of muscle strength and motor control should be targeted during an intervention. Regarding hip extension flexibility, only two participants had low flexibility (<20), and both group medians were very similar; thus, in our sample, hip extension flexibility was not a main problem for CP participants.

Study limitations. The main study limitation was the sample size; it was unfeasible to reach 24 participants because of constraints of time and resources. The ICC_{GLMM} displayed in Table 2 shows that the within-group variability was very large and that allowing the model to have a β_0 (i.e., y-intercept) for each participant was very helpful. A higher sample size would allow the use of the participants as a random effect, making it possible to find clusters of participants with different slopes (e.g., responders and nonresponders). Test–retest reliability (i.e., ICC) was adequate for PKE, 6MWT, and most KE and PF strength variables, whereas the lower 95% CI bounds of DF and KF strength variable reached the poor reliability threshold. This result suggests that similar intervention studies should perform longer familiarization sessions and at least two pretests before an intervention, not only to verify maturation effects but

also to evaluate the test–retest variability. Because Maher et al. (36) had reliability data for the 6MWT with a similar sample, we chose to use the available time to duplicate other measures. Overall, curve width for both groups had very poor reliability even with a familiarization session, clear instructions, and multiple trials per test, suggesting that this measurement is not reliable and should be modified in future studies. Another important inherent limitation of training studies is that not all training sessions were optimal: participants were sometimes tired, in a bad mood, or unmotivated. Thus, optimizing training for good execution and attitude, nutrition, and resting is a process that surely demands more than 3 months. Lastly, the criterion for diagnosing hip flexors shortness was 20° of hip extension, as it is evident that hip extension is necessary during the late stance phase of gait. Please note that, in our protocol paper, 0° was incorrectly mentioned as the criterion.

CONCLUSIONS

The EXECP intervention was effective in enhancing gait performance and overall gross motor function. Furthermore, the training program containing strength, flexibility, and gait training was found safe and feasible for children and young adults with CP. Hopefully, this intervention will inspire physiotherapists, trainers, and individuals with CP to pursue long-term training for a better, more functional life.

Analysis scripts can be found at https://github.com/Pedro-Valadao/EXECP. Raw data will be made available as soon as EXECP's subproject ISENS finishes its data analysis and all data can be properly anonymized.

This work was supported by the Olvi Foundation, the Research Foundation of Cerebral Palsy Alliance (PHD00321), Finnish Cultural Foundation, the University of Jyväskylä, the Academy of Finland (grant nos. 296240, 307250, 327288, 311877, and 326988), including "Brain changes across the lifespan" profiling funding to the University of Jyväskylä, Jane and Aatos Erkko Foundation (no. 602.274), and the Ministry of Education and Culture (OKM/28/626/2022).

All authors have read and approved the final manuscript. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The results are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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