Stretch hyperreflexia in children with cerebral palsy

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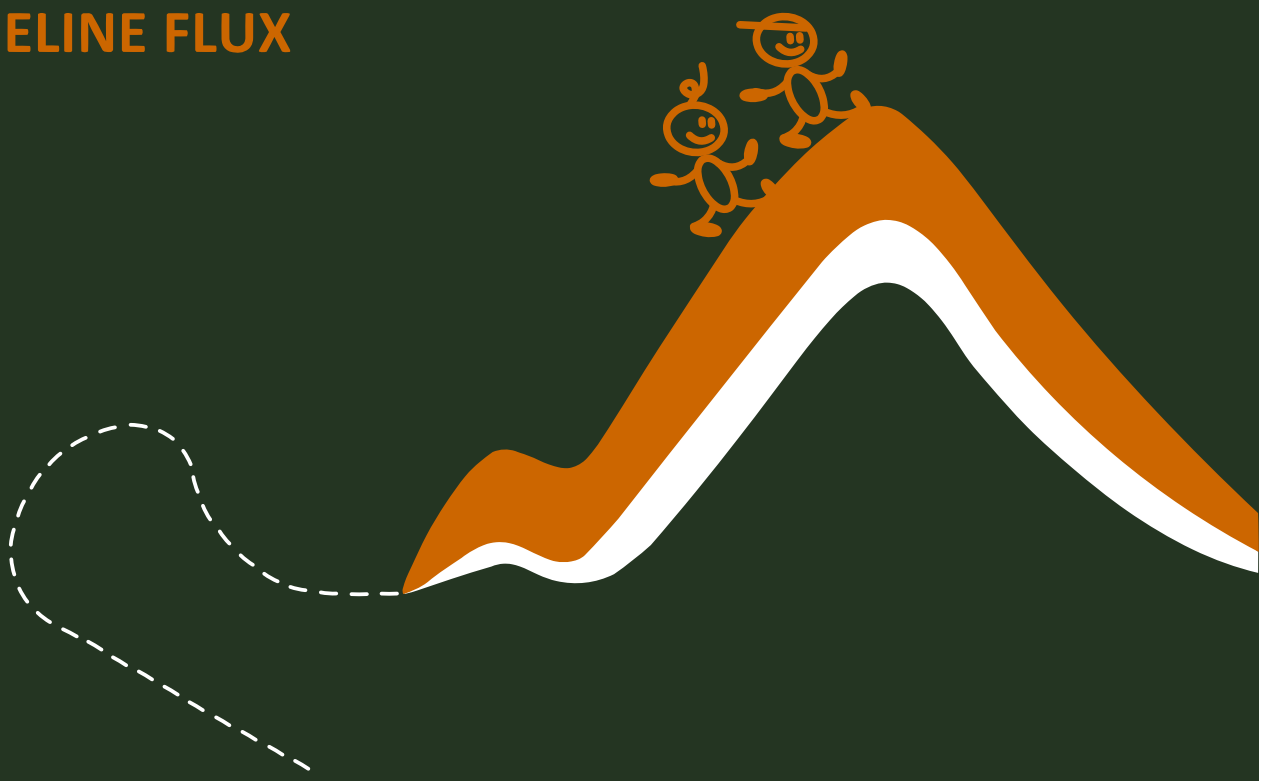
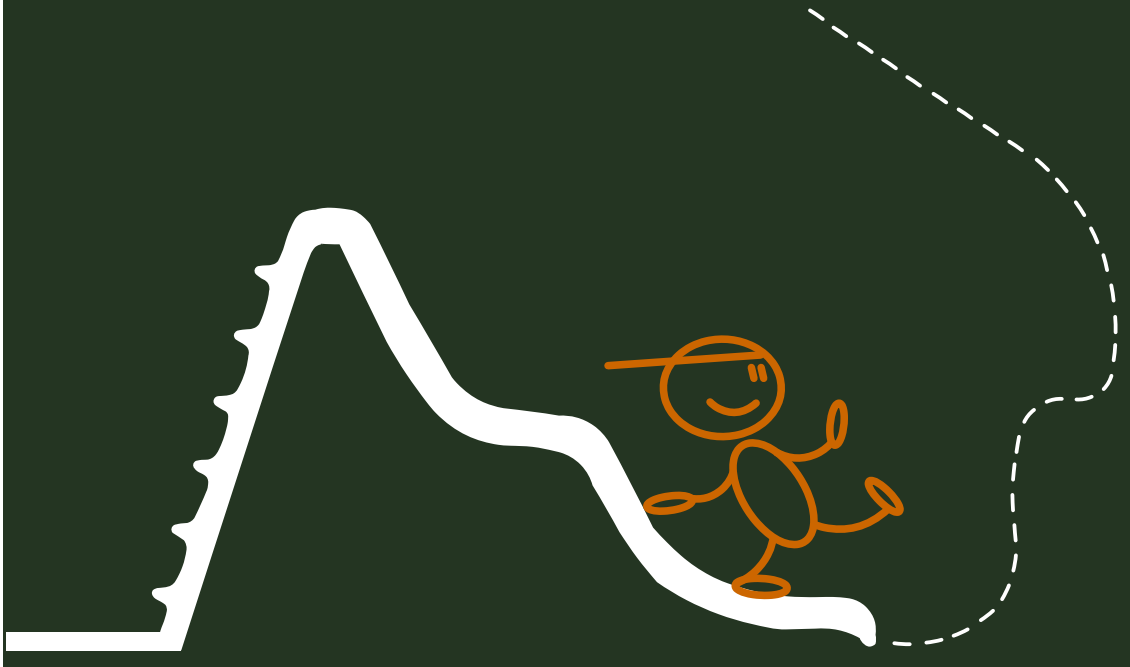


STRETCH HYPERREFLEXIA

IN CHILDREN WITH CEREBRAL PALSY

ASSESSMENT - CONTEXTUALIZATION - MODULATION



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Eline Flux

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door

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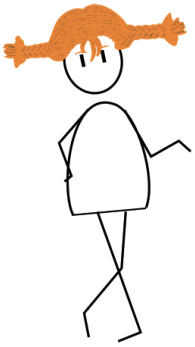
Het is nog nooit gedaan

dus ik denk dat het wel kan

—

~~Pippi Langkous~~

Eline Flux



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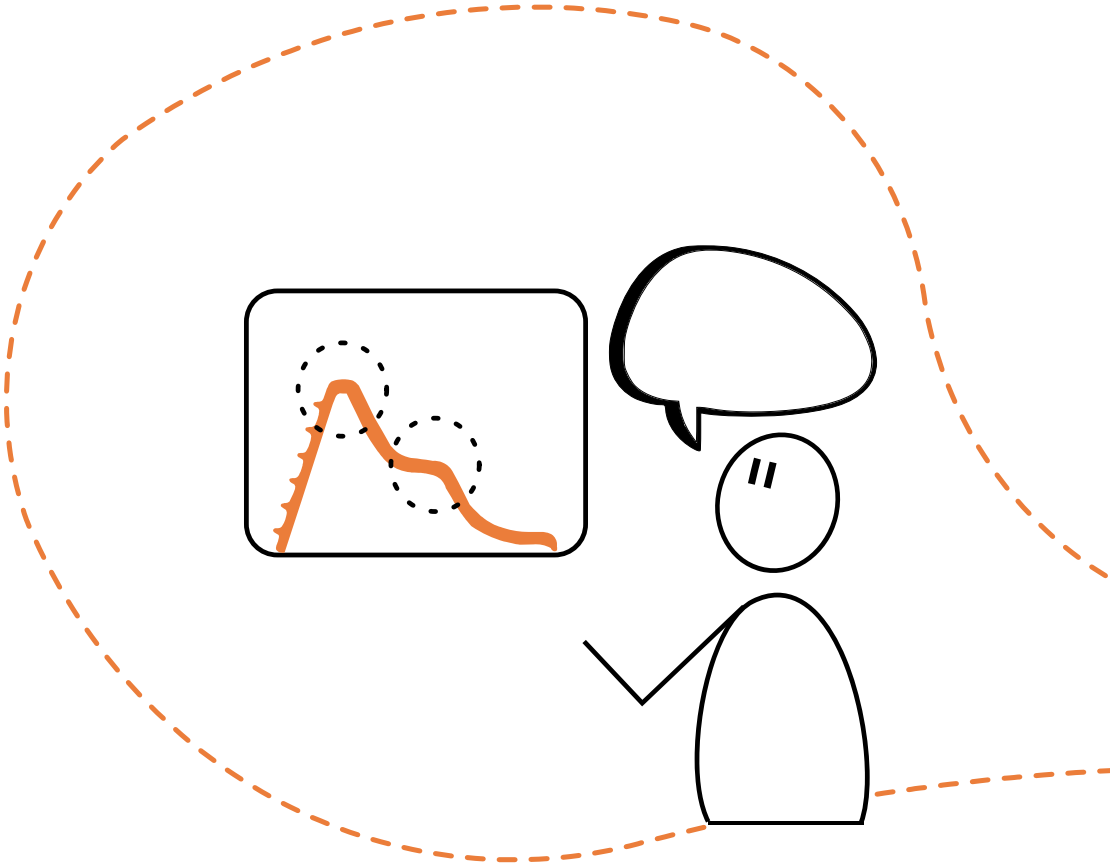
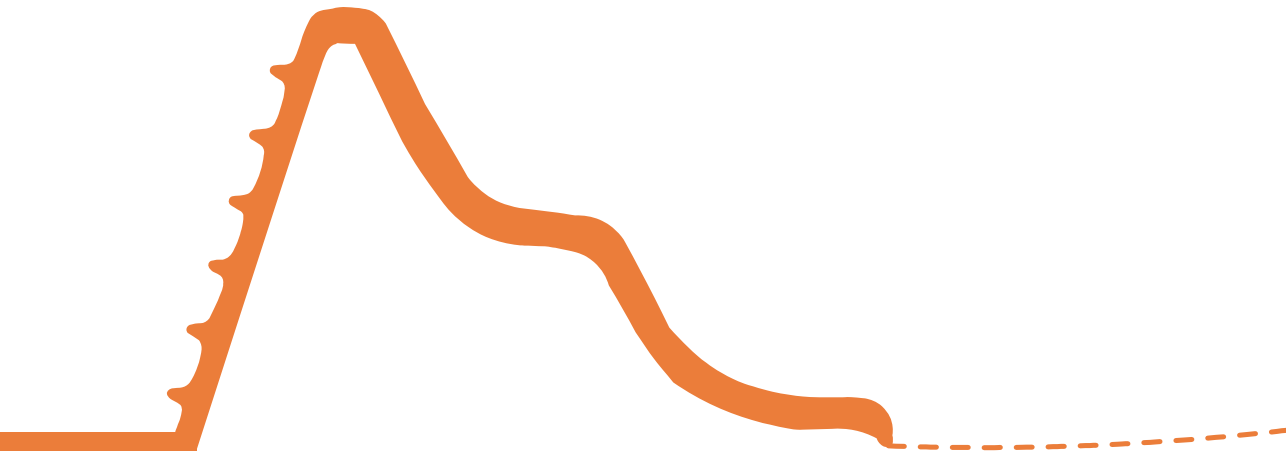
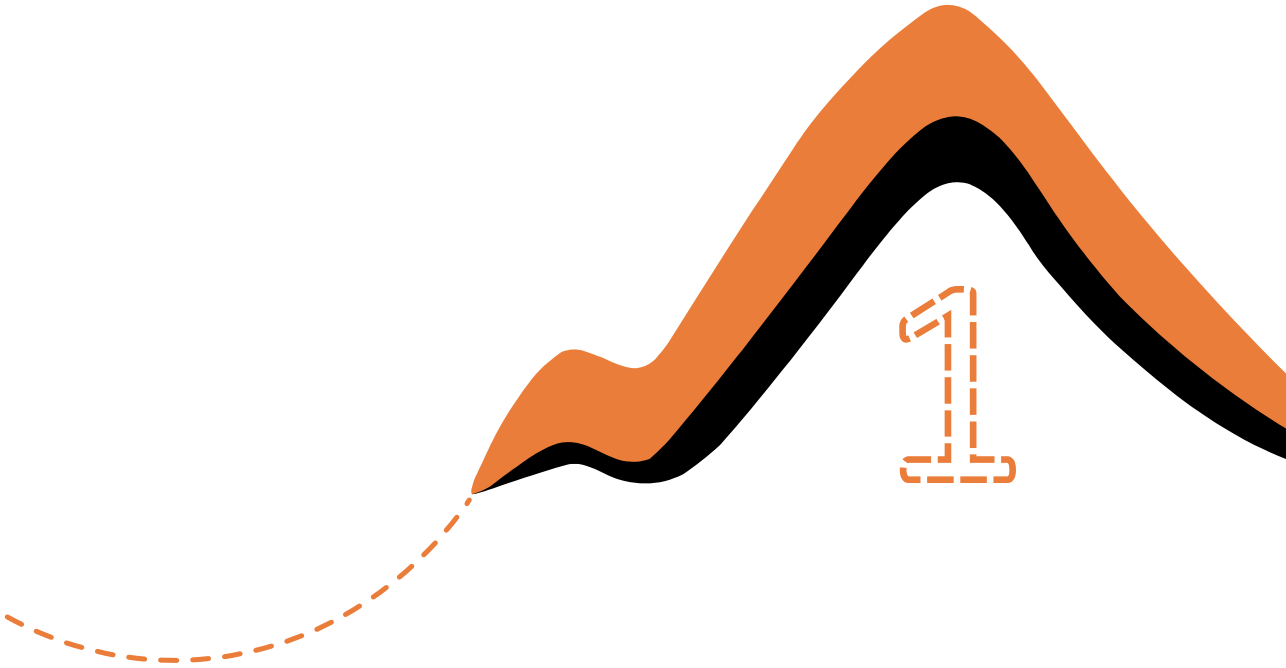






1

INTRODUCTION

1.0 Introduction

One of the biggest milestones in early life is learning to walk. Most parents can accurately recall the first steps of their child. Although the development of walking seems miraculous for most parents, children generally develop this skill without any assistance. It is often a process of falling and standing up, with a very unstable gait pattern at onset,[1] developing towards relatively mature gait around seven years of age.[2,3] However, this process is not self-evident in the case of pathologies. Children with neurological disorders, such as cerebral palsy, often experience difficulties with walking or never develop a customary gait pattern. A pathological gait pattern can seriously affect their mobility and therefore restrict them in daily-life activities. Therefore, interventions need to be considered to assist them toward improved mobility in daily life.

1.1 Cerebral palsy

Cerebral palsy (CP) is a neurological disorder and the most frequent cause of motor impairment in children, with a prevalence of around 2 per 1000 live births in Europe.[4,5] It is defined as a group of permanent disorders of posture and movement development resulting in activity restrictions. These disorders are caused by non-progressive disturbances in the developing fetal or infant brain,[6,7] such as perinatal lesions and oxygen deprivation. Premature[5] children and children with low birth weight[4,5] are at increased risk for encountering these disturbances. Furthermore, genetic pathways may play a role in the development of CP.[8] Genetic pathways can also cause hereditary spastic paresis (HSP), another group of childhood-onset disorders presenting with similar neurological impairments. Although this thesis mainly focuses on CP, most information can also be generalized toward HSP.

There is quite some heterogeneity among children with CP. Some have co-morbidities, for example, 20-30% of children experience severe intellectual deficits[4,5] and 12.5% have severe visual impairments.[5] All children with CP experience some level of impaired motor control. The overall severity of the gross motor impairments is often assessed using the Gross Motor Function Measure (GMFM).[9] Restrictions in mobility are commonly classified according to the Gross Motor Function Classification System (GMFCS),[10] based on the functional limitations and the need for assistive devices. GMFCS levels range from I, slightly affected children with the ability to walk and climb stairs, towards V, severely affected children who are wheelchair bound. This thesis focuses on ambulant children at GMFCS levels I and II (Fig. 1.1).

Depending on the specific brain lesion, the distribution and type of impairment differ among children. The distribution can be unilateral, with only one side of the body affected, or bilateral with both sides affected (Fig. 1.1). The most affected side is considered in the treatment plan,[11] since congnitive impairments can also differ between these classifications. For example, children with left unilateral CP more often experience difficulties with decreased working.[12] The type of impairment can be divided into three motor subtypes, the spastic, dyskinetic and ataxic subtype. All subtypes present with abnormal patterns of posture and/or movements. Around 85% of children are classified as having predominantly the spastic subtype.[4,5] Given the high prevalence of the spastic form, this thesis mainly focuses on spastic CP.

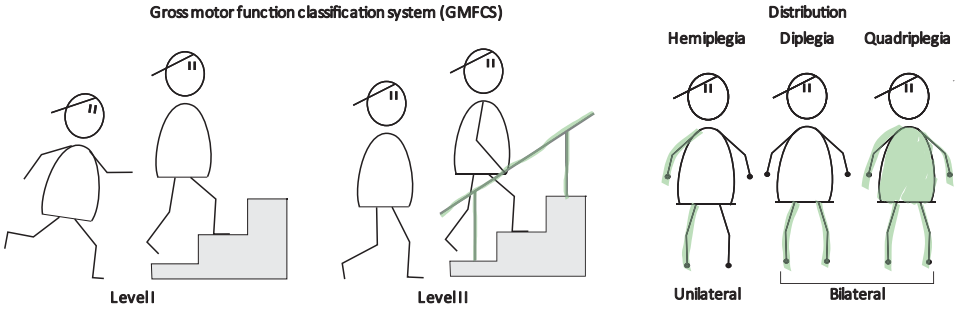


Figure 1.1. Gross motor function classification system (GMFCS) levels,[10] and distribution of cerebral palsy. GMFCS level I indicates lightly affected children with the ability to walk and climb chairs, and level II indicates slightly more affected children that are able to walk in most settings and climb stairs with the use of handrails. Distribution is sometimes classified as hemi-/di- or quadriplegia, but is classified as uni- and bilateral in this thesis, according to the general consensus on terminology.[6]

Children with spastic CP experience increased joint resistance, i.e. joint hyper-resistance. This can be caused by primary neural impairments, directly caused by the brain lesion, and secondary non-neural impairments that gradually develop over time (Fig. 1.2). The most common neural impairments in children with CP include decreased selective motor control, increased involuntary background muscle activity, decreased voluntary activity, and increased stretch reflexes, i.e. stretch hyperreflexia.[4,5,13,14]

Stretch hyperreflexia is often referred to as 'spasticity'[13] and describes an excessive muscle response to stretch. It is a common neural impairment in patients with central nervous disorders. Stretch reflexes are predominantly thought to be velocity-dependent,[13,15,16] although recent studies also present evidence for acceleration or force dependency.[17–20] The increased response to stretch is likely caused by both a reduced excitability threshold of the α-motor neurons and an exaggerated response intensity.[21] Several pathways might be involved in the increased excitability of the reflex loop, as presented in the box "stretch reflex neural pathways". The size of the muscle response can be influenced by multiple factors within these pathways, as presented in "stretch reflex magnitude". In addition to the generally enlarged muscle response, children with CP often experience increased stretch reflex durations. This can present itself through clonus, being a rhythmic oscillating activation, and decreased post-activation depression, being a reduction in activity after previous activation through inhibition of transmitter release from Ia afferents.[22]

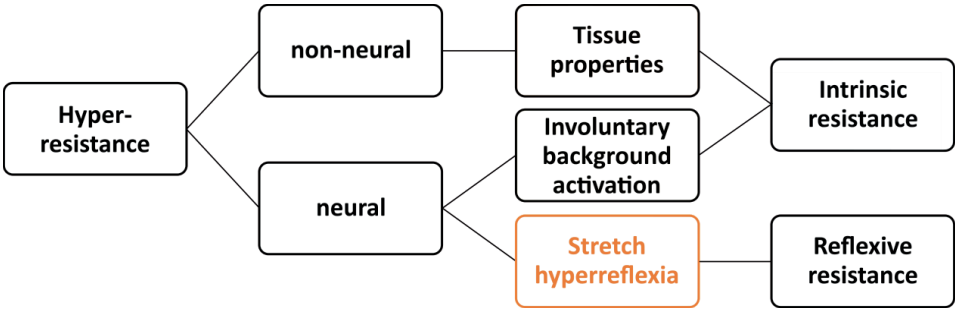


Figure 1.2. The terminology to describe joint hyper-resistance. The figure is adapted from van den Noort et al.[13]

The primary neural impairments can lead to secondary non-neural impairments, for example due to disuse and undesirable loading. Secondary impairments include skeletal impairments such as bony deformities, and muscle tissue-related impairments, such as muscle shortening and increased stiffness of muscle fibers, tendons, or connective tissue.[23] Subsequently, the non-neural impairments can lead to increased intrinsic joint stiffness and reduced range of motion (Fig. 1.2).

Together, the neural and non-neural impairments can lead to abnormalities during walking. While the pathological gait patterns differ largely between patients, several characteristic gait patterns can be recognized. These can be described through several classifications, for example crouch gait, stiff knee gait, and equinus gait.[24] This thesis mainly focuses on children with stretch hyperreflexia in the calf muscles, who most often present with equinus gait, i.e. a toe-walking pattern. They commonly have a typical muscle activation pattern with increased activity around early stance and decreased activity around push-off.[25,26] This results in impairments such as insufficient push-off power, decreased walking speed,[27] and increased energy costs.[25]

Stretch reflex neural pathways

There is no consensus on the exact mechanisms of stretch reflexes, but it is known that multiple pathways are involved. This box provides a schematic overview of the most likely involved pathways.

Short latency

Type IA: The fastest reflex circuit is the monosynaptic response, from muscle spindles, via type Ia-afferent nerves to the alpha motor neuron and then via efferent neurons to the muscle.

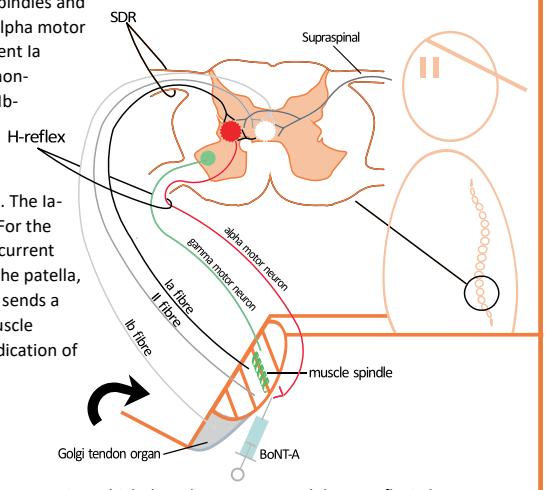
- Muscle spindles sense changes in muscle length and rate of change, but may also be sensitive to acceleration and/or intra-fusal force.[18]
- Gamma motor neurons influence the sensitivity of the muscle spindles (fusimotor activation).
- The Ia fibre also inhibits the antagonistic alpha motor neuron, a process referred to as reciprocal inhibition.

Long latency

- Type II: A slower reflex circuit is through the polysynaptic response. Muscle spindles provide signals via the type II afferent nerves through interneurons.
- Type Ib: Another reflex circuit is a polysynaptic response originating at the Golgi tendon organs, which sense force or tension in the muscle tendon. This is a non-reciprocal inhibitor response, intended to provide muscle relaxation.

Stretch hyperreflexia

Patients with stretch hyperreflexia have increased sensitivity of the stretch reflex loop, caused by increased excitation of the alpha motor neuron due to increased sensitivity of the muscle spindles and altered supraspinal input. Furthermore, inhibition of the alpha motor neuron is reduced, amongst others due to reduced or absent Ia reciprocal inhibition from antagonist activation, reduced non-reciprocal inhibition from the golgi tendon organs via the Ib-interneurons, and reduced post-activation depression.



H-reflex

The H-reflex is the electrical analogue of the stretch reflex. The Ia-afferent nerve is stimulated with an electrical stimulator. For the soleus muscles, this is performed by sending an electrical current from the popliteal fossa to a position slightly proximal to the patella, therefore triggering the tibial nerve. The Ia afferent nerve sends a signal to the alpha motor neurons, which in turn elicits muscle activity. The magnitude of the response can provide an indication of the sensitivity of this part of the stretch-reflex loop.

SDR surgery

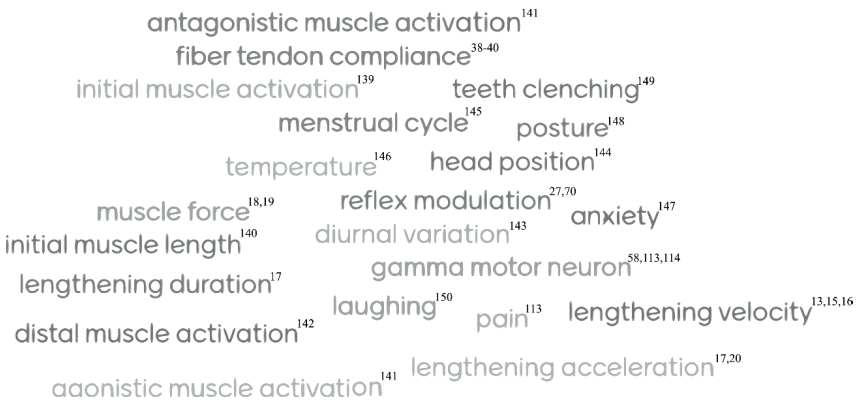
Selective Dorsal Rhizotomy is a permanent neurosurgical intervention which directly targets stretch hyperreflexia by intervening in the stretch reflex loop. Due to the sectioning of dorsal rootlets, alpha motor neuron excitation from the stretch reflex loop is decreased. Therefore, muscle activation in response to stretch will be highly reduced.

Botulinum toxin A

Children with stretch hyperreflexia often receive botulinum toxin A (BoNT-A) injections in the muscles with stretch hyperreflexia. This locally blocks the transmission of signals in the neuromuscular junctions, decreasing muscle activity and consequently the stretch reflex activity.

Stretch reflex magnitude

The sensitivity of the stretch reflex loop is enlarged in patients with stretch hyperreflexia. Multiple factors are known to further influence reflex magnitudes. They all work through different pathways involved in the reflex loops. Some of the most important factors are:



1.2 Assessments of gait and stretch hyperreflexia in CP

The impact of CP on a child's life is measured using the International Classification of Functioning, Disability and Health for Children and Youth ICF-CY[28] (Fig. 1.3). This is a model to assess human functional ability, by looking at the dynamics of functioning at three levels: body functions, activities, and participation.[29] At the level of body functions, impairments regard body function and structure, such as stretch hyperreflexia and increased muscle stiffness. These impairments often impair gait patterns. In activities, children often experience limitations such as decreased walking speed and distance and increased energy costs. At the level of participation, difficulties can arise in for example sports activities, and are referred to as restrictions. Environmental and personal factors may further influence these factors since they interact with the health condition. Improving the gait pattern is particularly interesting for most ambulant children, because it can reduce limitations and restrictions in daily life. Stretch hyperreflexia is hypothesized to be a major contributor to impaired gait patterns in children with CP. Therefore, this thesis mainly focuses on the impairment stretch hyperreflexia and how it affects the gait pattern.

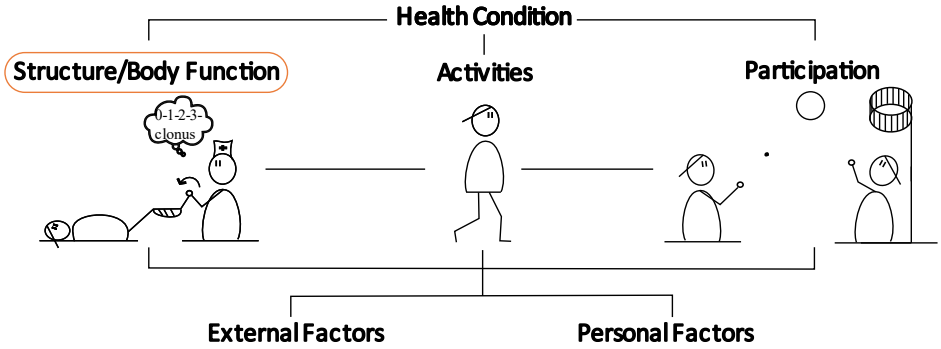


Figure 1.3. ICF-CY model: International Classification of Functioning, Disability and Health for Children and Youth.[28]

1.2.1 Assessment of gait

Abnormalities of walking are most often assessed using clinical gait analysis (Fig. 1.4).[30,31] Parameters of interest can be spatiotemporal parameters, such as walking speed and step length, and kinematic and kinetic parameters, such as ankle angle and push-off power. Clinical gait analysis to compute such kinematic and kinetic parameters can be performed using multiple marker models.[32–34] Recently, a new marker model, the Human Body Model, has been developed which has the advantage that it is optimized explicitly for real-time gait analysis.[35] Although all marker models compute kinematics and kinetics, outcomes can differ between models[36] due to differences in marker sets, assumptions, and definitions. The required method can depend on the application.

Additional measures, such as activation and stretch of the muscles, can complement kinematic and kinetic gait analysis (Fig. 1.4). Muscle activation patterns are commonly assessed using surface electromyography (EMG) and musculotendon stretch can be measured using musculoskeletal modeling, for example with the Gait2392 model.[34] A limitation of musculoskeletal models is that they do not incorporate the individual specific parameters of the muscle and tendon structures.[37] This can lead to incorrect findings, as tendon compliance is often altered in children with CP compared to typically developing children.[38–40] Dynamic ultrasound imaging can be used to measure the contribution of the muscle-tendon structures to musculoskeletal lengthening.[41–44]

Gait analysis data can be used to inform clinical decision-making. This requires identification of the primary cause of gait deviations. The interpretation of gait analysis and physical examination data can be facilitated by using a tool called GAIT.SCRIPT developed by Van der Krogt et al.[45] They created a core set of gait features that are linked to certain underlying impairments. For example, toe walking during stance is linked to gastrocnemius and soleus stretch hyperreflexia or contractures. Further examination is still required to disentangle stretch hyperreflexia from contractures[45] to further guide the appropriate treatment.[13] For example, stretch hyperreflexia can be targeted by suppressing muscle activity[46,47] or intervening in the stretch reflex loop,[47,48] while contractures are commonly treated by lengthening of the muscle-tendon complex through casting, physiotherapy or surgery.[47,49] Therefore, accurate assessment methods to quantify motor impairments, such as stretch hyperreflexia, are required.

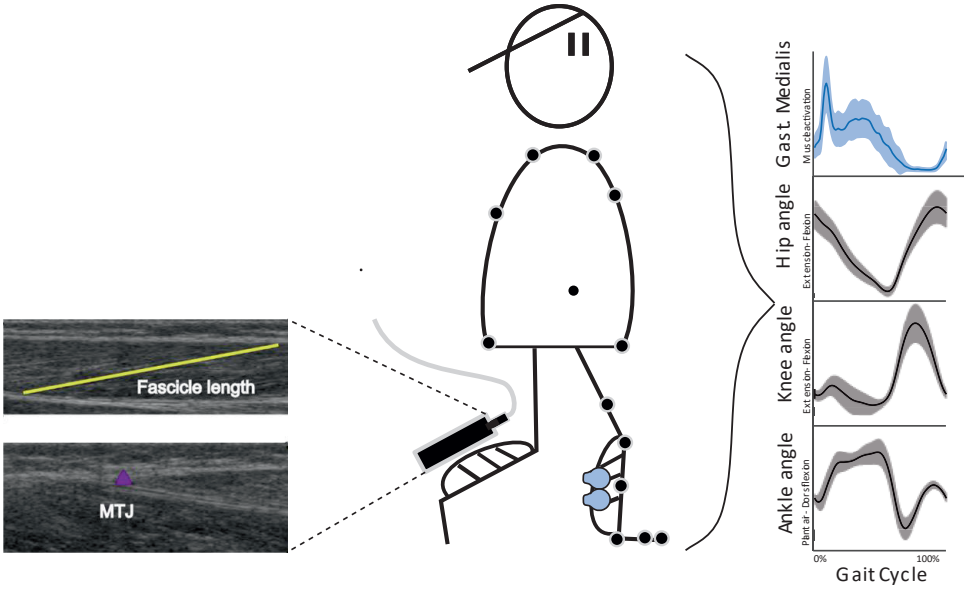


Figure 1.4. Clinical gait analysis: Marker data can be used to compute kinematics, such as hip, knee and ankle angles. Kinematic gait analysis can be complemented with analysis of muscle activation using electromyography (EMG), for example for the m. Gastrocnemius Medialis. Furthermore, ultrasound imaging can be added to provide information on muscle-tendon lengthening, by analyzing fascicle lengthening and displacement of the muscle-tendon-junction (MTJ).

1.2.2 Assessment of stretch hyperreflexia

Clinically, stretch hyperreflexia is most often assessed by quantifying the manually sensed joint resistance with one of many available subjective scales, such as the modified Ashworth Scale,[50] Tardieu Scale,[51] or the Spasticity Test (SPAT).[52] With most clinical scales, clinicians assess joint resistance by rotating the ankle joint at low and high velocities. Increased resistance at low velocities is attributed to intrinsic resistance, and resistance at high velocities is attributed to increased reflexive resistance (Fig. 1.2).[13] Unfortunately, these manual tests fall short in terms of objectivity, reliability, validity, sensitivity, and standardization,[13,53–55] and therefore might disturb clinical decision making.

Multiple other methods exists to capture (part of) the stretch reflex loop, as presented in the box "stretch reflex assessments". The assessments using electrical stimulation, such as H-reflexes and transcranial magnetic stimulation, specifically target parts of the stretch reflex loop. These tests generally have sufficient repeatability,[56] objectivity, and sensitivity. However, clinical applicability is low for children with CP given the electrical stimulus required, which is known to cause vasovagal syncope in some people. This is expressed as an uncomfortable feeling, including sweating hands and sometimes even fainting. Furthermore, H-reflexes can not be performed on most muscles, due to the location of the afferent nerves. Moreover, H-reflexes only capture part of the stretch reflex loop and exclude for example the sensitivity of the muscle spindles, which can be regulated through changes in gamma drive,[57] and is often impaired in patients with neurological disorders.

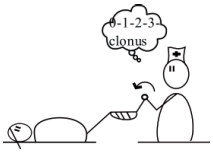
Several other stretch reflex assessment methods have been developed to improve objectivity, precision, repeatability, and standardization of stretch hyperreflexia assessments. For example, manual assessments can be instrumented by measuring muscle activity and/or torque, improving objectivity, reliability and sensitivity.[58–60] With instrumented assessments, the rotations around joints are still performed by the researcher. Differences in movement profiles can influence the reflex response, as shown in the "stretch reflex magnitude" box. Motorization of assessments will make them more controlled, further improving reliability. Motorization can be performed using an ankle dynamometer to apply fast, small dorsiflexion rotations to elicit stretch in the calf muscles. The resulting increase in muscle activity is attributed to stretch reflex activity.[61] This method is fast, simple to perform, and only requires a limited movement range. However, it remains difficult to separate the stretch reflexes from the intrinsic contribution of joint hyper-resistance using this method.

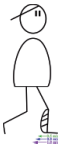
Other instrumented motorized methods have been developed, such as a motorized version of the SPAT.[60] This method is similar to the SPAT, but the slow and fast movements around the ankle joint are motorized and response activity can be assessed through EMG or torque analysis. Additionally, other methods requiring physics-based neuromechanical modeling have been developed.[62–65] Most methods entail multiple repeated movements over the entire passive ROM, but they include multiple model assumptions,[62,63,65] which might be violated in patient populations. To tackle this, a method requiring only limited assumptions has been developed. This model entails A parallel cascade system identification (SI) method requiring only limited assumptions has been developed. This method

Stretch reflex assessments

Multiple different tests are available that are developed to assess the increased sensitivity of (part of) the reflex loop. The main ones are presented here:

- **Manual subjective tests**
  - Modified Ashworth scale (MAS)[50]
  - Modified Tardieu scale[150]
  - The spasticity test (SPAT)[52]
- **EMG response to electrical stimuli**
  - H-reflex
  - F-wave
- **EMG response to externally applied movements**
  - Tendon reflex
  - Polysynaptic responses
  - EMG during passive movements
  - Wartenberg Pendulum test
  - Instrumented SPAT
- **EMG responses during active movements**
  - Treadmill perturbations
  - Orthotics perturbations
- **Evoked potentials**
  - Motor evoked potentials: transcranial magnetic stimulation
  - Sensory evoked potentials: lumbosacral potentials.
- **Motorized assessments**
  - Motorized SPAT
  - Parallel cascade system identification





entails repeated movements over a limited movement range,[64] which might be more favorable for children with anxiety. On the other side, the limited movement range might not translate to experienced resistance over the entire movement range. Finally, there is a trade-off between feasibility of clinical implementation and measurement accuracy. As all presented methods have their advantages, the required method can depend on the application. Therefore, it is important to study if results from different methods to assess stretch hyperreflexia are interchangeable.

1.2.3 Assessment of stretch hyperreflexia during gait

The meaningfulness of passive stretch hyperreflexia measures is criticized, i.e. whether or not they generalize to functional activities such as walking.[23,66] As presented in the box "stretch reflex magnitudes", the size of the stretch reflex is influenced by multiple factors, such as posture and muscle activation, and is known to be centrally regulated depending on the activity. For example, stretch reflexes are generally lower during sitting than standing and even lower during walking.[67,68] Scores on passive stretch hyperreflexia assessments might not reflect limitations encountered in daily life, which is an important factor in clinical decision-making. Therefore, it might be better to contextualize stretch hyperreflexia assessments.

To do so, several studies have assessed reflex magnitudes during gait by applying H-reflexes – the electrical analog of the stretch reflex – during different phases of the gait cycle.[27,67,69–72] These studies revealed that healthy adults generally have decreased reflex sensitivity during walking compared to rest. Furthermore, they modulate their reflex sensitivity throughout the gait cycle, with lower reflex magnitudes during swing than stance.[67] Likewise, children with CP show some modulation. While children with CP have increased reflex magnitudes compared to typically developing children in general, this increase is most pronounced during the stance phase of gait.[27] Despite these interesting findings, the clinical feasibility of trainings requiring H-reflexes is low, as described in paragraph 1.2.2.

Another way to explore the sensitivity of the entire stretch reflex loop is to apply external perturbations during gait. This was done using several methods, such as tapping the quadriceps tendon.[68] Tendon tapping reveals information about the stretch reflex throughout the gait cycle, but is uncomfortable and has limited applicability to other muscles. Another method to provide external perturbations is through an actuated ankle orthosis, which can be used to stretch and thus evoke calf muscle stretch reflexes during the stance phase of gait.[73] This method is less painful and can be translated to other joints, but it is unknown to what extent gait is altered by the mass and movement restrictions of such an orthosis. Recently, we proposed a new perturbation method to evoke stretch reflexes while maintaining a comfortable walking pattern. For this method, participants walk on an instrumented split-belt treadmill and acceleration impulses are applied on a single belt during the stance phase of gait. This causes a backward shift of the foot, resulting in increased dorsiflexion and hence stretch on the muscle-tendon complex, which evokes a stretch reflex. This method can be used to assess stretch reflex magnitudes during gait in healthy adults.[74] Still, this method's applicability has not yet been demonstrated for typically developing children and children with CP.

The role of stretch hyperreflexia during gait remains a topic of debate,[19,23,75–79] despite the evidence for increased reflexive responses to external stimuli such as H-reflexes or stretch reflexes through orthotic perturbations. The increased responses do not mean that unperturbed pathological gait is altered due to stretch hyper-reflexes.[80] Therefore, it is also important to assess the unperturbed gait

pattern. Multiple studies have shown increased muscle activity during late swing[26] and early stance[25,81] in children with spastic CP. Additionally, several studies attempted to quantify the relationship between muscle stretch and increased muscle activity during unperturbed gait.[82–87] Results from these studies are conflicting, with some studies finding evidence for stretch reflexes during the swing[88] or stance phase[89,90] or no evidence for a relation between muscle-tendon stretch and increased muscle activation.[87] While these studies used different assessment methods, they all relied on the modeled lengthening velocity of the entire musculotendon unit to represent muscle spindle stretch during gait. However, measuring the lengthening of the muscle tendon and muscle belly or muscle fascicles separately can increase insight into the relation between stretch and muscle activation. Dynamic ultrasound imaging can be used to estimate lengthening behavior of the fascicles, muscle belly and tendon during walking.[41] Additionally, recent studies have shown acceleration- and force-dependency of stretch reflexes.[19,20,91] Therefore, it would be relevant to extend analysis of the relation between increased muscle activity and stretch during unperturbed gait with the assessment of lengthening velocity and acceleration, and with the fascicles and muscle tendon separately.

1.3 Stretch hyperreflexia interventions for children with spastic CP

Given the impact of the impairment stretch hyperreflexia, multiple interventions have been developed that aim to reduce stretch hyperreflexia. The most common intervention is the administration of botulinum toxin A, which locally blocks the transmission of signals in the neuromuscular junctions, decreasing muscle activity and consequently the stretch reflex activity. However, this also reduces voluntary muscle activation and muscle strength.[92,93] Furthermore, botulinum toxin A only lasts around 3-6 months.[92,94,95]Baclofen is another medical intervention to minimize stretch hyperreflexia. Baclofen reduces the release of excitatory neurotransmitters by afferent neurons. Baclofen is most often provided orally, but when a high dose is needed, intrathecal delivery is considered.[47,96] Unfortunately, long-term use of baclofen can decrease muscle strength and motor unit relaxation.[92]

A permanent neurosurgical intervention is Selective Dorsal Rhizotomy (SDR), which directly targets stretch hyperreflexia by intervening in the stretch reflex loop, as visualized in the box "stretch reflex neural pathways". Due to the targeted sectioning of dorsal rootlets, alpha motor neuron excitation resulting from the stretch reflex loop is decreased, and therefore stretch hyper reflexes are decreased. Although reasonably effective,[48,97,98] it is only applicable for a small group of children with CP.[99,100] Because of the limitations of currently available medical interventions for stretch hyperreflexia, there is a need for non-invasive, specific therapies to decrease stretch reflexes.

1.3.1 Stretch hyperreflexia biofeedback

A potential method to decrease stretch hyperreflexia non-invasively is through biofeedback. Several researchers explored the efficacy of operant conditioning methods - a training technique using reinforcement and punishment to alter certain behavior - by providing biofeedback on the size of the reflexes, in order to modulate reflexes. These methods were successful in down-conditioning reflexes in rats,[101] monkeys,[102–104] healthy participants,[61,105–107] and participants with spinal cord injury.[108,109] Two studies aimed to down-condition stretch reflexes in children with CP. In the first study by Nash et al.[110], three children with CP received visual biofeedback on the magnitude of the calf muscle stretch

reflex during 40 sessions. The calf muscle stretch reflexes were evoked by manual, passive rotations of the ankle joint. As a result, two out of three children successfully reduced their stretch reflexes. In a follow-up study by O'Dwyer et al.[111], all eight included children successfully decreased stretch reflex magnitudes by around 50%.

Although biofeedback methods appear promising, they currently have several disadvantages. Firstly, most methods use H-reflexes as input for biofeedback,[102,106,108,112] which requires afferent nerve stimulation to evoke reflexes. As mentioned in paragraph 1.2.2, this method is not preferable for children with CP. Therefore, stretch reflexes might be more suitable as input for biofeedback. This has been explored by Mrachazc-Kersting et al.[61], Nash et al.[110] and O'Dwyer et al.[111] Within these studies, ankle dorsiflexion was applied by a motorized ankle perturbator[61] or manually by the researcher[110,111] and feedback was provided by showing the increased muscle activity, i.e. the stretch reflex. Even though most participants could decrease their stretch reflexes using these conditioning methods, several disadvantages remain that obstruct translation towards clinical practices. Another major limitation is the time-intensiveness of these protocols, consisting of 24[61] or even 40[110,111] conditioning sessions, requiring at least four sessions to achieve within-session results[61] and 12 sessions for the first between-session result.[106] Also, multiple baseline sessions are required to determine baseline values for the biofeedback threshold, as shown by the six[61] and 23[111] baseline sessions used in previous studies, probably caused by the high within-subject variability of stretch reflex magnitudes, as presented in the box "stretch reflex magnitudes".

Results might be obtained much faster when providing biofeedback on reflexive resistance as calculated using a parallel cascade system identification (SI) technique.[107] This method consists of two to three degrees ramp-and-hold ankle perturbations as applied by an ankle dynamometer. Ludvig et al.[64] performed a pilot study on healthy adults and revealed vast learning curve improvements, achieving within-session modulation of reflexive resistance within one to two sessions.[107] A potential explanation for the faster improvements might be that gamma motor neuron activity is eliminated in the H-reflex study, which is known to influence reflex size.[113,114] Therefore, evoking reflexes through muscle stretch, and thereby involving the gamma motor neuron circuitry, allows for additional modulation opportunities. Furthermore, most conditioning methods require relaxation periods between perturbations,[61,101,106,110,111] whereas the real-time biofeedback from the SI can be provided continuously, potentially yielding a shorter protocol. As a final advantage, the SI method is impedance-based, omitting the need for EMG measures and thus the need for accurate electrode placement. Electrical stimulation can be used to evaluate electrode placement, [61,106] but skipping this step can further improve time intensiveness. To our knowledge, the SI biofeedback method has only been studied over two biofeedback sessions. Therefore, the potential to decrease reflexes over multiple sessions is unknown.

1.3.2 Biofeedback during functional activities

All proposed conditioning methods in paragraph 1.3.1 regard training in static and mostly passive conditions. These measures allow for a controlled measure of reflexes, eliminating most factors that influence stretch reflex magnitudes (see box "stretch reflex magnitudes"). However, as presented in 1.2.3, the translation from passive to dynamic stretch hyperreflexia is criticized. In other words, it is unsure if improvements in passive stretch hyperreflexia translate toward improvements in functional activities. Thompson et al.[108] reported improvements in gait parameters after 24 conditioning sessions

in a passive, controlled session for adults with spinal cord injury. On the other hand, O'Dwyer et al.[111] found no improvements in passive ankle joint ROM in children with CP, despite the 50% decreased stretch reflexes. They conclude that reflex training should be complemented with other therapies. Similarly, an extensive review of the treatments for spasticity lead to the conclusion that "perhaps we should be exciting the spinal cord with functional, patterned activity … during natural movement to keep intrinsic, inhibitory mechanisms viable".[93p18] Therefore, feedback during functional, patterned activities like walking may outperform feedback during controlled, passive settings.

Indeed, feedback during functional activities was shown to induce alterations in the gait pattern of children with CP.[115–118] For example, repetitive gait training can cause changes in corticomotor pathways,[119,120] and is expected to achieve long-term effects. However, it is unknown whether this is through decreases in stretch hyperreflexia or by adjustments in supraspinal control. Functional training has induced decreases in stretch hyperreflexia in people with incomplete spinal cord injury,[121–125] and therefore also shows potential for reducing stretch reflexes in children with CP.

Functional training can directly aim to decrease the effect of stretch hyperreflexia during gait. Stretch hyperreflexia can cause deviating muscle activation patterns in children with spastic CP with a toe-walking pattern: they often experience stretch on the calf muscles and show a rapid increase in calf muscle activity during early stance,[25,26,81,116] which has been attributed to stretch hyperreflexia.[25,83] Colborne et al.[116] performed a functional training in which they provided visual feedback on these deviating calf muscle activation patterns. By down-conditioning the rapid increase in soleus activity after initial contact, it was hypothesized that stretch hyperreflexia would also be down-conditioned. Furthermore, soleus muscle activity during push-off was up-conditioned to improve power generation. While Reflex magnitudes were not assessed, results show that positive and negative work around the ankle improved. Although promising, the study presented with several limitations, such as an increases in walking speed for the pre and post-biofeedback assessments, which is known to be strongly related to peak push-off power.[126,127] Also, they only included children with relatively mild impairments.[116] Furthermore, Colborne et al.[116] they did not quantify actual changes in muscle activity as a result of the biofeedback. Therefore, the efficacy of biofeedback on the muscle activation pattern to alter gait in children with CP is still unknown and therefore should be studied further.

1.3.3 Methods to optimize compliance and treatment efficacy in biofeedback

Sufficient intrinsic motivation is important to optimize results of operant conditioning,[128] as the protocols are often lengthy, since they have to target the plasticity of the spinal cord. The amount of personal control and perceived competence highly influences intrinsic motivation.[129] Hence, it appears important that patients already have high success rates during the early stages of biofeedback training. Previous conditioning studies have attempted to heighten motivation by high percentages of positive feedback,[106,110] and monetary incentives.[106,130] One study assessed the efficacy of such monetary incentives in EMG biofeedback in five stroke patients and attributed larger training results to these incentives.[131] However, external motivators, such as monetary incentives, can also decrease intrinsic motivation,[132] and therefore intrinsic motivators are preferred in operant conditioning protocols.

A promising method to increase intrinsic motivation is through gamification.[133–135] Gamification entails introducing a gaming element to non-gaming settings like rehabilitation. It can boost fun and long-term engagement,[134–137] but also increase treatment effectiveness.[133–135] Nash et al.[110] and O'Dwyer et

al.[111] already pointed out the need for increased motivation in operant conditioning and introduced audio and video material as an incentive. After the pilot study, O'Dwyer et al.[111] even introduced simple gaming elements to provide biofeedback to the children. Technological advancements aid the introduction of gamification in biofeedback methods. In conclusion, it is worthwhile to incorporate gamification in biofeedback studies.

1.4 Aims and outline of this thesis

Children with spastic CP are generally thought to have impaired gait patterns due to stretch hyperreflexia. Stretch hyperreflexia is often present in the calf muscles, leading to deviating calf muscle activation patterns and inefficient gait patterns. Multiple methods have been developed to measure stretch hyperreflexia, but evidence supporting the validity and clinical feasibility of these methods is still insufficient, especially for children with CP. Furthermore, current interventions to decrease stretch reflexes are often invasive, non-specific, and temporary and might have negative side-effects. The **main goal** of this thesis is therefore to develop methods to measure, contextualize and modulate stretch hyperreflexia in children with CP.

During the first part of this thesis, we focus on the assessment of the gait pattern. In **chapter two** we analyze a marker model, the Human Body Model, designed to compute kinematics in real-time. Since the Human Body Model is only recently developed - and received several major updates since the first validation study[35] - we perform a study to assess the similarity with conventional gait models for gait analysis in children with cerebral palsy. **Chapter three** assesses if we can include dynamic ultrasound imaging in gait analysis to gain more insights into the lengthening of the structures within the muscle, without interfering with the gait pattern of children with cerebral palsy.

In the second part of this thesis, we assess methods to quantify stretch reflexes in a controlled setting, since validation of the methods is limited due to the absence of a gold standard. We aim to provide additional evidence for the validity by comparing different methods. We first analyze the relation between multiple methods to quantify stretch reflexes in patients with spinal cord injury in **chapter four**. Thereafter, **chapter five** compares the same assessment methods in children with cerebral palsy, due to population specific limitations that might obstruct accurate assessments.

In the third part, we assess if it is possible to evoke and quantify stretch reflexes during gait of children with cerebral palsy, as expression of stretch hyperreflexia in passive conditions might not always translate to the presence of stretch hyperreflexia during functional tasks. In **chapter six** we analyze the possibilities to quantify stretch reflexes using treadmill perturbations. In **chapter seven** we explore the relation between increased muscle activity and stretch on the different muscle-tendon structures during unperturbed gait. Furthermore, we analyze if children with cerebral palsy have increased reflexes compared to typically developing children.

Finally, in the fourth part we assess the potential of operant conditioning methods to decrease stretch hyperreflexia. in **chapter eight** we explore if we can provide biofeedback on stretch hyperreflexia in controlled conditions in healthy adults. In this chapter we also study if gamification can be used to increase motivation during biofeedback. In **chapter nine** we explore if we can provide biofeedback on

the inefficient muscle activation pattern of children with cerebral palsy during gait. The outcomes of all studies are discussed in **chapter ten.**

References

1. Breniere Y, Bril B. Why do children walk when falling down while adults fall down in walking? *C R Acad Sci III* 1988; 307: 617–22.
2. Cheron G, Bengoetxea A, Bouillot E, Lacquaniti F, Dan B. Early emergence of temporal co-ordination of lower limb segments elevation angles in human locomotion. *Neurosci Lett* 2001; 308: 123–7.
3. Ledebt A, Bril B, Brenière Y. The build-up of anticipatory behaviour. An analysis of the development of gait initiation in children. *Exp Brain Res* 1998; 120: 9–17.
4. Johnson A. Prevalence and characteristics of children with cerebral palsy in Europe. 2002. DOI:10.1017/S0012162201002675.
5. McManus V, Guillem P, Surman G, Cans C. SCPE work, standardization and definition--an overview of the activities of SCPE: a collaboration of European CP registers. *Zhongguo Dang Dai Er Ke Za Zhi* 2006; 8: 261–5.
6. Rosenbaum P, Paneth N, Leviton A, et al. A report: The definition and classification of cerebral palsy April 2006. *Dev Med Child Neurol* 2007; 49: 8–14.
7. Cans C. Surveillance of cerebral palsy in Europe: a collaboration of cerebral palsy surveys and registers. *Dev Med Child Neurol* 2007; 42: 816–24.
8. MacLennan AH, Thompson SC, Gecz J. Cerebral palsy: causes, pathways, and the role of genetic variants. *Am J Obstet Gynecol* 2015; 213: 779–88.
9. Russell DJ, Rosenbaum P, Wright M, Avery LM. Gross motor function measure (GMFM-66 & GMFM-88) users manual. Mac keith press, 2002.
10. Palisano R, Rosenbaum P, Bartlett D, et al. Gross Motor Function Classification System. *Dev Med Child Neurol* 1997; 39: 214–23.
11. Van Der Kamp J, Steenbergen B, Masters RSW. Explicit and implicit motor learning in children with unilateral cerebral palsy Explicit and implicit motor learning in children with unilateral cerebral palsy. *Disabil Rehabil* 2017. DOI:10.1080/09638288.2017.1360403.
12. Buszard T, Farrow D, Zhu FF, Masters RSW. The relationship between working memory capacity and cortical activity during performance of a novel motor task. *Psychol Sport Exerc* 2016; 22: 247–54.
13. van den Noort JC, Bar-On L, Aertbeliën E, et al. European consensus on the concepts and measurement of the pathophysiological neuromuscular responses to passive muscle stretch. *Eur J Neurol* 2017; 24: 981-e38.
14. Fowler EG, Staudt L a, Greenberg MB. Lower-extremity selective voluntary motor control in patients with spastic cerebral palsy: increased distal motor impairment. *Dev Med Child Neurol* 2010; 52: 264–9.
15. Grey MJ, Larsen B, Sinkjær T. A task dependent change in the medium latency component of the soleus stretch reflex. *Exp Brain Res* 2002; 145: 316–22.
16. Schuurmans J, de Vlugt E, Schouten AC, Meskers CGM, de Groot JH, van der Helm FCT. The monosynaptic Ia afferent pathway can largely explain the stretch duration effect of the long latency M2 response. *Exp brain Res* 2009; 193: 491–500.
17. Van 't Veld RC, Van Asseldonk EHF, Kooij H Van Der, Schouten AC. Disentangling acceleration-, velocity-, and duration-dependency of the shortand medium-latency stretch reflexes in the ankle plantarflexors. *J Neurophysiol* 2021; 126: 1015–29.
18. Blum KP, Lamotte D'Incamps B, Zytnicki D, Ting LH. Force encoding in muscle spindles during stretch of passive muscle. *PLOS Comput Biol* 2017; 13: e1005767.
19. Falisse A, Bar-On L, Desloovere K, Jonkers I, De Groote F. A spasticity model based on feedback from muscle force explains muscle activity during passive stretches and gait in children with cerebral palsy. *PLoS One* 2018; 13: 1–20.
20. Sloot LH, Weide G, van der Krogt MM, et al. Applying Stretch to Evoke Hyperreflexia in Spasticity Testing: Velocity vs. Acceleration. *Front Bioeng Biotechnol* 2021; 8: 1–10.
21. Sheean G. Neurophysiology of spasticity. *Up Mot neurone Syndr spasticity Clin Manag Neurophysiol* 2001; : 12–78.
22. Grey MJ, Klinge K, Crone C, et al. Post-activation depression of Soleus stretch reflexes in healthy and spastic humans. *Exp Brain Res* 2008; 185: 189–97.
23. Dietz V, Sinkjaer T. Spastic movement disorder: impaired reflex function and altered muscle mechanics. *Lancet Neurol* 2007; 6: 725–33.
24. Wren TAL, Rethlefsen S, Kay RM. Prevalence of specific gait abnormalities in children with cerebral palsy: influence of cerebral palsy subtype, age, and previous surgery. *J Pediatr Orthop* 2005; 25: 79–83.
25. Olney SJ, MacPhail HA, Hedden DM, Boyce WF. Work and Power in Hemiplegic Cerebral Palsy Gait. *Phys*

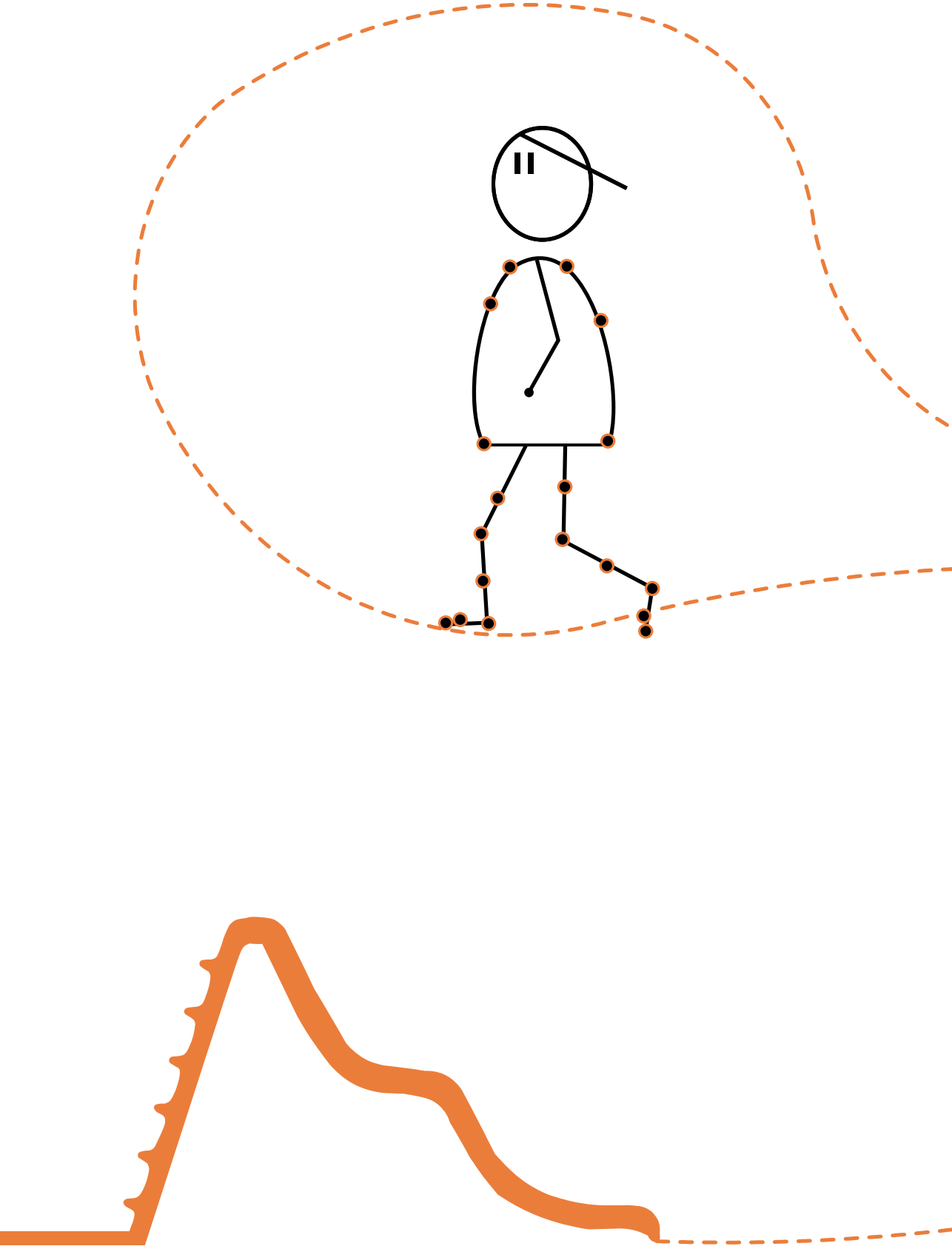
Ther 1990; 70: 431–9.
26. Van der Krogt MM, Doorenbosch CAM, Becher JG, Harlaar J. Dynamic spasticity of plantar flexor muscles in cerebral palsy gait. *J Rehabil Med J Rehabil Med J Rehabil Med* 2010; 42: 656–63.
27. Hodapp M, Klisch C, Mall V, Vry J, Berger W, Faist M. Modulation of Soleus H-Reflexes During Gait in Children With Cerebral Palsy. *J Neurophysiol* 2007; 98: 3263–8.
28. Rosenbaum P, Stewart D. The world health organization international classification of functioning, disability, and health: a model to guide clinical thinking, practice and research in the field of cerebral palsy. *Semin Pediatr Neurol* 2004; 11: 5–10.
29. Rauch A, Cieza A, Stucki G. How to apply the International Classification of Functioning, Disability and Health (ICF) for rehabilitation management in clinical practice. *Eur J Phys Rehabil Med* 2008; 44: 329–42.
30. Kirtley C. Clinical gait analysis: theory and practice. Elsevier Health Sciences, 2006.
31. Wren T a. L, Gorton GE, Õunpuu S, Tucker C a. Efficacy of clinical gait analysis: A systematic review. *Gait Posture* 2011; 34: 149–53.
32. Davis RB, Ounpuu S, Tyburski D, Gage JR. A gait analysis data collection and reduction technique. *Hum Mov Sci* 1991; 10: 575–87.
33. Cappozzo A, Catani F, Della Croce U, Leardini A. Position and orientation in space of bones during movement: anatomical frame definition and determination. *Clin Biomech* 1995; 10: 171–8.
34. Delp SL, Anderson FC, Arnold AS, et al. OpenSim: open-source software to create and analyze dynamic simulations of movement. *IEEE Trans Biomed Eng* 2007; 54: 1940–50.
35. Van Den Bogert AJ, Geijtenbeek T, Even-Zohar O, Steenbrink F, Hardin EC. A real-time system for biomechanical analysis of human movement and muscle function. *Med Biol Eng Comput* 2013; 51: 1069–77.
36. Ferrari A, Benedetti MG, Pavan E, et al. Quantitative comparison of five current protocols in gait analysis. *Gait Posture* 2008; 28: 207–16.
37. Cronin NJ, Lichtwark G. The use of ultrasound to study muscle-tendon function in human posture and locomotion. Gait Posture. 2013; 37: 305–12.
38. Barber L, Carty C, Modenese L, Walsh J, Boyd R, Lichtwark G. Medial gastrocnemius and soleus muscle-tendon unit, fascicle, and tendon interaction during walking in children with cerebral palsy. *Dev Med Child Neurol* 2017; 59: 843–51.
39. Cronin NJ, Carty CP, Barrett RS, Lichtwark G. Automatic tracking of medial gastrocnemius fascicle length during human locomotion. *J Appl Physiol* 2011; 111: 1491–6.
40. Kalkman BM, Bar-On L, Cenni F, et al. Muscle and tendon lengthening behaviour of the medial gastrocnemius during ankle joint rotation in children with cerebral palsy. *Exp Physiol* 2018; 103: 1367–76.
41. van Hooren B, Teratsias P, Hodson-Tole EF. Ultrasound imaging to assess skeletal muscle architecture during movements: A systematic review of methods, reliability, and challenges. *J Appl Physiol* 2020; 128: 978–99.
42. Cronin NJ, Lichtwark G. The use of ultrasound to study muscle-tendon function in human posture and locomotion. *Gait Posture* 2013; 37: 305–12.
43. Kalsi G, Fry NR, Shortland AP. Gastrocnemius muscle–tendon interaction during walking in typically-developing adults and children, and in children with spastic cerebral palsy. *J Biomech* 2016; 49: 3194–9.
44. Barber L, Carty C, Modenese L, Walsh J, Boyd R, Lichtwark G. Medial gastrocnemius and soleus muscle-tendon unit, fascicle, and tendon interaction during walking in children with cerebral palsy. *Dev Med Child Neurol* 2017; 59: 843–51.
45. van der Krogt MM, Houdijk H, Wishaupt K, \van Hutten K, Dekker S, Buizer AI. Development of a core set of gait features and their potential underlying impairments to assist gait data interpretation in children with cerebral palsy. *Front Hum Neurosci* 2022; 714.
46. Rosales RL, Chua-Yap AS. Evidence-based systematic review on the efficacy and safety of botulinum toxin-A therapy in post-stroke spasticity. *J Neural Transm* 2008; 115: 617–23.
47. Novak I, Mcintyre S, Morgan C, et al. A systematic review of interventions for children with cerebral palsy: state of the evidence. *Dev Med Child Neurol* 2013; 55: 885–910.
48. Steinbok P. Outcomes after selective dorsal rhizotomy for spastic cerebral palsy. *Child's Nerv Syst* 2001; 17: 1–18.
49. Brouwer B, Wheeldon RK, Stradiotto-Parker N. Reflex excitability and isometric force production in cerebral palsy: the effect of serial casting. *Dev Med Child Neurol* 2008; 40: 168–75.
50. Bohannon RW, Smith MB. Interrater reliability of a modified Ashworth scale of muscle spasticity. *Phys*

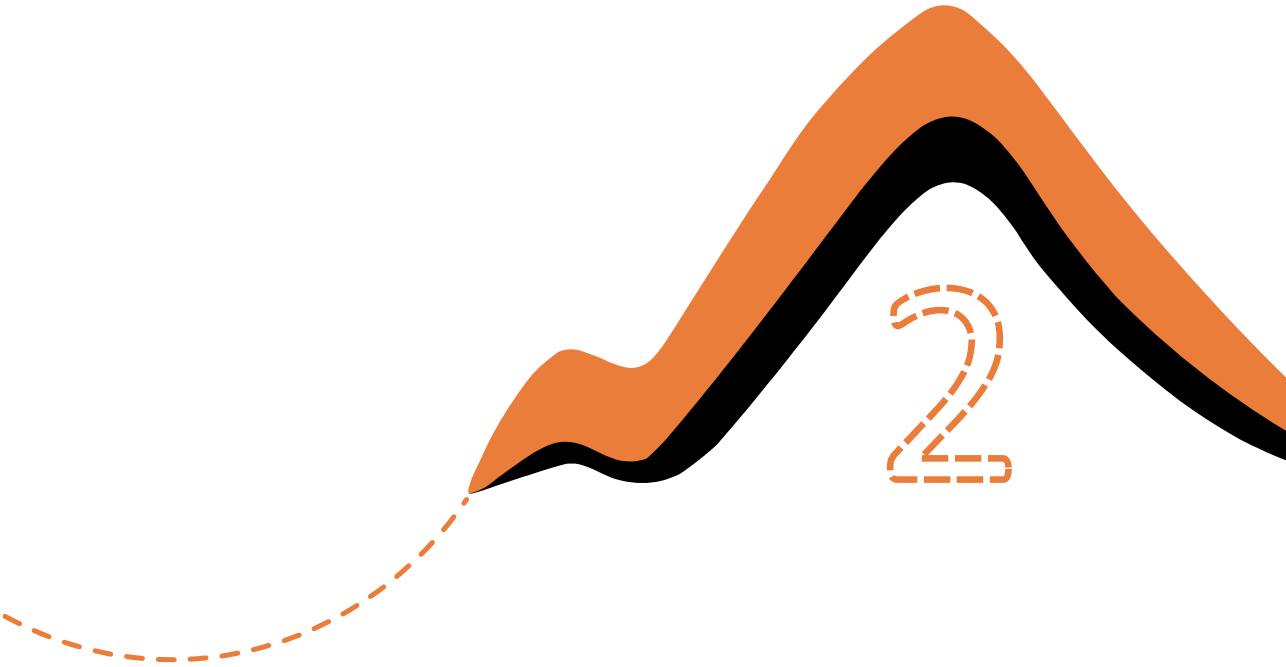
Ther 1987; 67: 206–7.
51. Tardieu G. A la recherche d'une technique de mesure de la spasticite. *Rev neurol* 1954; 91: 143–4.
52. Scholtes VAB, Dallmeijer AJ, Becher JG. The Spasticity Test: a clinical instrument to measure spasticity in children with cerebral palsy. In: The Effectiveness of Multilevel Botulinum Toxin Type A and Comprehensive Rehabilitation in Children with Cerebral Palsy. Citeseer, 2007: 29–64.
53. Fleuren JFM, Voerman GE, Erren-Wolters C V, et al. Stop using the Ashworth Scale for the assessment of spasticity. *J Neurol* 2009; 81: 46.
54. Haugh AB, Pandyan AD, Johnson GR. A systematic review of the Tardieu Scale for the measurement of spasticity. *Disabil Rehabil* 2006; 28: 899–907.
55. Bar-On L, Molenaers G, Aertbeliën E, et al. Spasticity and its contribution to hypertonia in cerebral palsy. Biomed Res. Int. 2015; 2015. DOI:10.1155/2015/317047.
56. Jaberzadeh S, Scutter S, Warden-Flood A, Nazeran H. Between-days reliability of H-reflexes in human flexor carpi radialis. *Arch Phys Med Rehabil* 2004; 85: 1168–73.
57. Matthews Pb. Evolving views on the internal operation and functional role of the muscle spindle. *J Physiol* 1981; 320: 1.
58. Yamaguchi T, Petersen TH, Kirk H, et al. Spasticity in adults with cerebral palsy and multiple sclerosis measured by objective clinically applicable technique. *Clin Neurophysiol* 2018; 129: 2010–21.
59. Lorentzen J, Grey MJ, Geertsen SS, et al. Assessment of a portable device for the quantitative measurement of ankle joint stiffness in spastic individuals. *Clin Neurophysiol* 2012; 123: 1371–82.
60. Sloot LH, Bar-On L, van der Krogt MM, et al. Motorized versus manual instrumented spasticity assessment in children with cerebral palsy. *Dev Med Child Neurol* 2017; 59: 145–51.
61. Mrachacz-Kersting N, Kersting UG, de Brito Silva P, et al. Acquisition of a simple motor skill: task-dependent adaptation and long-term changes in the human soleus stretch reflex. *J Neurophysiol* 2019; 122: 435–46.
62. Sloot LH, van der Krogt MM, Groep KL de G de, et al. The validity and reliability of modelled neural and tissue properties of the ankle muscles in children with cerebral palsy. *Gait Posture* 2015; 42: 7–15.
63. de Gooijer-van de Groep KL, de Vlugt E, de Groot JH, et al. Differentiation between non-neural and neural contributors to ankle joint stiffness in cerebral palsy. *J Neuroeng Rehabil* 2013; 10: 81.
64. Ludvig D, Kearney RE. Real-time estimation of intrinsic and reflex stiffness. *IEEE Trans Biomed Eng* 2007; 54: 1875–84.
65. de Vlugt E, de Groot JH, Schenkeveld KE, Arendzen JH, van der Helm FC, Meskers CG. The relation between neuromechanical parameters and Ashworth score in stroke patients. *J Neuroeng Rehabil* 2010; 7: 35.
66. Schindler-Ivens S, Brown DA, Lewis GN, Nielsen JB, Ondishko KL, Wieser J. Soleus H-reflex excitability during pedaling post-stroke. *Exp Brain Res* 2008; 188: 465–74.
67. Capaday C, Stein RB. Amplitude modulation of the soleus H-reflex in the human during walking and standing. *J Neurosci* 1986; 6: 1308–13.
68. Faist M, Ertel M, Berger W, Dietz V. Impaired modulation of quadriceps tendon jerk reflex during spastic gait: differences between spinal and cerebral lesions. *Brain* 1999; 122: 567–79.
69. Yang JF, Fung J, Edamura M, Blunt R, Stein RB, Barbeau H. H-Reflex Modulation During Walking In Spastic Paretic Subjects. *Can J Neurol Sci* 2017; 18: 443–52.
70. Dietz V, Faist M, Pierrot-Deseilligny E. Amplitude modulation of the quadriceps H-reflex in the human during the early stance phase of gait. *Exp Brain Res* 1990; 79: 221–4.
71. Fung J, Barbeau H. Effects of conditioning cutaneomuscular stimulation on the soleus H-reflex in normal and spastic paretic subjects during walking and standing. *J Neurophysiol* 1994; 72: 2090–104.
72. Mummidisetty CK, Smith AC, Knikou M. Modulation of reciprocal and presynaptic inhibition during robotic-assisted stepping in humans. DOI:10.1016/j.clinph.2012.09.007.
73. Andersen JBJ, Sinkjaer T. An Actuator System for Investigating Electrophysiological and Biomechanical Feature Around the Human Ankle Joint During Gait. *IEEE Trans Rehabil Eng* 1995; 3: 299–306.
74. Sloot LH, Van Den Noort JJC, van der Krogt MMM, Bruijn SMS, Harlaar J. Can treadmill perturbations evoke stretch reflexes in the calf muscles? *PLoS One* 2015; 10. DOI:10.1371/journal.pone.0144815.
75. Ada L, Vattanasilp W, O'dwyer NJ, Crosbie J. Does spasticity contribute to walking dysfunction after stroke? *J Neurol Neurosurg Psychiatry* 1998; 64: 628–35.
76. Marsden J, Ramdharry G, Stevenson V, Thompson A. Muscle paresis and passive stiffness: Key determinants in limiting function in Hereditary and Sporadic Spastic Paraparesis. *Gait Posture* 2012; 35: 266–71.
77. Willerslev-Olsen M, Andersen J, Sinkjaer T, Nielsen J. Sensory feedback to ankle plantar flexors is not

exaggerated during gait in spastic hemiplegic children with cerebral palsy. *J Neurophysiol* 2014; 111: 746–54.
78. Damiano DL, Laws E, Carmines D V., Abel MF. Relationship of spasticity to knee angular velocity and motion during gait in cerebral palsy. *Gait Posture* 2006; 23: 1–8.
79. Tuzson AE, Granata KP, Abel MF. Spastic velocity threshold constrains functional performance in cerebral palsy. *Arch Phys Med Rehabil* 2003; 84: 1363–8.
80. Nielsen JB. Motoneuronal drive during human walking. *Brain Res Rev* 2002; 40: 192–201.
81. Van der Krogt MM, Doorenbosch CAM, Becher JG, Harlaar J. Walking speed modifies spasticity effects in gastrocnemius and soleus in cerebral palsy gait. *Clin Biomech* 2009; 24: 422–8.
82. Bar-On L, Molenaers G, Aertbeliën E, Monari D, Feys H, Desloovere K. The relation between spasticity and muscle behavior during the swing phase of gait in children with cerebral palsy. *Res Dev Disabil* 2014; 35: 3354–64.
83. Crenna P. Spasticity and 'Spastic' Gait in Children with Cerebral Palsy. *Neurosci Biobehav Rev* 1998; 22: 571–8.
84. Lamontagne A, Malouin F, Richards CL. Locomotor-Specific measure of spasticity of plantarflexor muscles after stroke. *Arch Phys Med Rehabil* 2001; 82: 1696–704.
85. Crenna P, Inverno M, Frigo C, Palmieri R, Fedrizzi E. Pathological profile of gait in children with cerebral palsy. *Med Sport Sci* 1992; 36: 186–98.
86. Chow JW, Yablon SA, Stokic DS. Electromyogram-lengthening velocity relation in plantar flexors during stance phase of gait in patients with hypertonia after acquired brain injury. *Arch Phys Med Rehabil* 2012; 93: 2287–94.
87. De Niet M, Latour H, Hendricks H, Geurts AC, Weerdesteyn V. Short-Latency Stretch Reflexes Do Not Contribute to Premature Calf Muscle Activity During the Stance Phase of Gait in Spastic Patients. *Arch Phys Med Rehabil* 2011; 92: 1833–9.
88. Van der Krogt MM, Doorenbosch CAM, Becher JG, Harlaar J. Dynamic spasticity of plantar flexor muscles in cerebral palsy gait. *J Rehabil Med* 2010; 42: 656–63.
89. Crenna P. Spasticity and 'Spastic' Gait in Children with Cerebral Palsy. *Neurosci Biobehav Rev* 1998; 22: 571–8.
90. Lamontagne A, Malouin F, Richards CL. Locomotor-Specific measure of spasticity of plantarflexor muscles after stroke. *Arch Phys Med Rehabil* 2001; 82: 1696–704.
91. Van 't Veld RC, Van Asseldonk EHF, Van Der Kooij H, Schouten AC. Disentangling acceleration-, velocity-, and duration-dependency of the short-and medium-latency stretch reflexes in the ankle plantarflexors. 2021. DOI:10.1152/jn.00704.2020.
92. Thomas CK, Hager-Ross CK, Klein CS. Effects of baclofen on motor units paralysed by chronic cervical spinal cord injury. *Brain* 2010; 133: 117–25.
93. D'Amico JM, Condliffe EG, Martins KJB, Bennett DJ, Gorassini MA. Recovery of neuronal and network excitability after spinal cord injury and implications for spasticity. *Front Integr Neurosci* 2014; 8: 1–24.
94. Williams PE, Goldspink G. The effect of immobilization on the longitudinal growth of striated muscle fibres. *J Anat* 1973; 116: 45–55.
95. Williams PE, Goldspink G. Longitudinal Growth of Striated Muscle Fibres. *J Cell Sci* 1971; 9.
96. Chow JW, Yablon SA, Stokic DS. Effect of intrathecal baclofen bolus injection on ankle muscle activation during gait in patients with acquired brain injury. *Neurorehabil Neural Repair* 2015; 29: 163–73.
97. McLaughlin J, Bjornson K, Temkin N, et al. Selective dorsal rhizotomy: meta-analysis of three randomized controlled trials. *Dev Med Child Neurol* 2002; 44: 17.
98. Steinbok P. Selective dorsal rhizotomy for spastic cerebral palsy: a review. *Child's Nerv Syst* 2007; 23: 981–90.
99. McLaughlin JF, Bjornson KF, Astley SJ, et al. Selective dorsal rhizotomy: efficacy and safety in an investigator-masked randomized clinical trial. *Dev Med Child Neurol* 1998; 40: 220–32.
100. Macwilliams BA, Johnson BA, Shuckra AL, D'Astous JL. Functional decline in children undergoing selective dorsal rhizotomy after age 10. *Dev Med Child Neurol* 2011; 53: 717–23.
101. Chen XY, Wolpaw JR. Operant Conditioning of H-reflex in Freely Moving Rats. *J Neurophysiol* 1995; 73. http://jn.physiology.org/content/jn/73/1/411.full.pdf (accessed 14 July 2017).
102. Wolpaw JR, Kieffer VA, Seegal RF, Braitman DJ, Sanders MG. Adaptive plasticity in the spinal stretch reflex. *Brain Res* 1983; 267: 196–200.
103. Wolpaw JR. Adaptive plasticity in the primate spinal stretch reflex: reversal and re-development. *Brain Res* 1983; 278: 299–304.
104. Wolpaw JR, Seegal RF, O'Keefe JA. Adaptive plasticity in primate spinal stretch reflex: behavior of

synergist and antagonist muscles. *J Neurophysiol* 1983; 50. http://jn.physiology.org/content/50/6/1312.long (accessed 14 July 2017).
105. Makihara Y, Segal RL, Wolpaw JR, Thompson AK. Operant conditioning of the soleus H-reflex does not induce long-term changes in the gastrocnemius H-reflexes and does not disturb normal locomotion in humans. *J Neurophysiol* 2014; 112. http://jn.physiology.org/content/112/6/1439 (accessed 19 July 2017).
106. Thompson AK, Chen XY, Wolpaw JR. Acquisition of a Simple Motor Skill: Task-Dependent Adaptation Plus Long-Term Change in the Human Soleus H-Reflex. *J Neurosci* 2009; 29. DOI:10.1523/JNEUROSCI.4326-08.2009.
107. Ludvig D, Cathers I, Kearney RE. Voluntary modulation of human stretch reflexes. *Exp Brain Res* 2007; 183: 201–13.
108. Thompson AK, Pomerantz FR, Wolpaw JR. Operant Conditioning of a Spinal Reflex Can Improve Locomotion after Spinal Cord Injury in Humans. *J Neurosci* 2013; 33: 2365–75.
109. Thompson AK, Wolpaw JR, Taylor J, Mrachacz-Kersting N, Thompson AK, Wolpaw JR. H-reflex conditioning during locomotion in people with spinal cord injury. *J Physiol C 2019 Authors J Physiol* 2021; 599: 2453–69.
110. Nash J, Neilson PD, O'Dwyer NJ. Reduing spasticity to control muscle contracture of children with cerebral palsy. *Dev Med Child Neurol* 1989; 31: 471–80.
111. O'Dwyer N, Neilson P, Nash J. Reduction of spasticity in cerebral palsy using feedback of the tonic stretch reflex: a controlled study. *Dev Med Child Neurol* 1994; 35: 770–86.
112. Chen Y, Chen XY, Jakeman LB, Schalk G, Stokes BT, Wolpaw JR. The Interaction of a New Motor Skill and an Old One: H-Reflex Conditioning and Locomotion in Rats. *J Neurosci* 2005; 25. http://www.jneurosci.org/content/25/29/6898.long (accessed 19 July 2017).
113. Matre DA, Sinkjær T, Svensson P, Arendt-Nielsen L. Experimental muscle pain increases the human stretch reflex. *Pain* 1998; 75: 331–9.
114. Ditunno J, Little J, Tessler A, Burns A. Spinal shock revisited: a four-phase model. *Spinal Cord* 2004; 42: 383–95.
115. Bolek JE. A Preliminary Study of Modification of Gait in Real-Time Using Surface Electromyography. *Appl Psychophysiol Biofeedback* 2003; 28.
116. Colborne GR, Wright F V, Naumann S, Anonymous. Feedback of triceps surae EMG in gait of children with cerebral palsy: a controlled study. *Arch Phys Med Rehabil* 1994; 75: 40–5.
117. Booth AT, Buizer AI, Harlaar J, Steenbrink F, van der Krogt MM. Immediate Effects of Immersive Biofeedback on Gait in Children With Cerebral Palsy. *Arch Phys Med Rehabil* 2019; 100: 598–605.
118. van Gelder L, Booth ATC, van de Port I, Buizer AI, Harlaar J, van der Krogt MM. Real-time feedback to improve gait in children with cerebral palsy. *Gait Posture* 2017; 52: 76–82.
119. Barbeau H. Locomotor training in neurorehabilitation: emerging rehabilitation concepts. *Neurorehabil Neural Repair* 2003; 17: 3–11.
120. Yen C-L, Wang R-Y, Liao K-K, Huang C-C, Yang Y-R. Gait training—induced change in corticomotor excitability in patients with chronic stroke. *Neurorehabil Neural Repair* 2008; 22: 22–30.
121. Trimble MH, Kukulka CG, Behrman AL. The effect of treadmill gait training on low-frequency depression of the soleus H-reflex: comparison of a spinal cord injured man to normal subjects. *Neurosci Lett* 1998; 246: 186–8.
122. Gorassini MA, Norton JA, Nevett-Duchcherer J, Roy FD, Yang JF, Gorassini M. Changes in Locomotor Muscle Activity After Treadmill Training in Subjects with Incomplete Spinal Cord Injury. *Artic Press J Neurophysiol* 2008. DOI:10.1152/jn.91131.2008.
123. Adams MM, Hicks AL. Comparison of the effects of body-weight-supported treadmill training and tilt-table standing on spasticity in individuals with chronic spinal cord injury. *https://doi.org/101179/2045772311Y0000000028* 2013; 34: 488–94.
124. Manella KJ, Field-Fote EC. Modulatory effects of locomotor training on extensor spasticity in individuals with motor-incomplete spinal cord injury Spinal Cord Injury Model Systems Centers View project Modulatory effects of locomotor training on extensor spasticity in individuals with motor-incomplete spinal cord injury. *Artic Restor Neurol Neurosci* 2013; 31: 633–46.
125. Knikou M, Mummidisetty CK. Locomotor training improves premotoneuronal control after chronic spinal cord injury. *J Neurophysiol* 2014; 111: 2264–75.
126. Booij MJ, Meinders E, Sierevelt IN, Nolte PA, Harlaar J, van den Noort JC. Matching walking speed of controls affects identification of gait deviations in patients with a total knee replacement. *Clin Biomech* 2021; 82: 105278.

127. Oudenhoven LM, Booth ATC, Buizer AI, Harlaar J, van der Krogt MM. How normal is normal: Consequences of stride to stride variability, treadmill walking and age when using normative paediatric gait data. *Gait Posture* 2019; 70: 289–97.
128. Lewthwaite R, Wulf G. Optimizing motivation and attention for motor performance and learning. *Curr Opin Psychol* 2017; 16: 38–42.
129. Fisher CD, Pritchard RD. Effects of Personal Control, Extrinsic Rewards, and Competence on Intrinsic Motivation. 1978. http://www.dtic.mil/docs/citations/ADA058417 (accessed 18 July 2017).
130. Mrachacz-Kersting N, Kersting UG, de Brito Silva P, et al. Acquisition of a simple motor skill: Task-dependent adaptation and long-term changes in the human soleus stretch reflex. *J Neurophysiol* 2019; 122: 435–46.
131. Santee JL, Keister ME, Kleinman KM. Incentives to enhance the effects of electromyographic Feedback Training in stroke patients. *Biofeedback Self Regul* 1980; 5: 51–6.
132. Ma Q, Jin J, Meng L, Shen Q. The dark side of monetary incentive: how does extrinsic reward crowd out intrinsic motivation. *Neuroreport* 2014; 25: 194–8.
133. Proença JP, Quaresma C, Vieira P. Serious games for upper limb rehabilitation: a systematic review. *Disabil Rehabil Assist Technol* 2018; 13: 95–100.
134. Bonnechère B, Jansen B, Omelina L, Van Sint Jan S. The use of commercial video games in rehabilitation: a systematic review. *Int J Rehabil Res* 2016; 39: 277–90.
135. Lopes S, Magalhães P, Pereira A, et al. Games used with serious purposes: a systematic review of interventions in patients with cerebral palsy. *Front Psychol* 2018; 9: 1712.
136. Deterding S, Sicart M, Nacke L, O'Hara K, Dixon D. Gamification. using game-design elements in non-gaming contexts. In: CHI'11 extended abstracts on human factors in computing systems. 2011: 2425–8.
137. Turan Z, Avinc Z, Kara K, Goktas Y. Gamification and education: Achievements, cognitive loads, and views of students. *Int J Emerg Technol Learn* 2016; 11.
138. De Groote F, Blum KP, Horslen BC, Ting LH. Interaction between muscle tone, short-range stiffness and increased sensory feedback gains explains key kinematic features of the pendulum test in spastic cerebral palsy: A simulation study. *PLoS One* 2018; 13: e0205763.
139. Meinders M, Price R, Lehmann JF, Questad KA. The stretch reflex response in the normal and spastic ankle: Effect of ankle position. *Arch Phys Med Rehabil* 1996; 77: 487–92.
140. Edamura M, Yang JF, Stein RB. Factors that Determine the Magnitude and Time Course of Human H-Reflexes in Locomotion. *J Neurosci* 1991; 17: 420–7.
141. Delwaide PJ, Figiel C, Richelle AC. Effects of postural changes of the upper limb on reflex transmission in the lower limb Cervicolumbar reflex interactions in man. *J ofNeurology, Neurosurgery, Psychiatry* 1977; 40: 616–21.
142. Wolpaw JR, Seegal RF. Diurnal rhythm in the spinal stretch reflex. *Brain Res* 1982; 244: 365–9.
143. Hayes K, Sullivan J. Tonic neck reflex influence on tendon and Hoffman reflexes in man. *Electromyogr Clin Neurophysiol* 1976; 16: 251–61.
144. Murata M, Yamaguchi H, Seki K, Takahara T, Saito T, Onodera S. Day-to-Day Variation in the Hoffmann Reflex in Females. *Kawasaki J Med Welf* 2014; 20: 1–7.
145. Racinais S, Cresswell AG. Temperature affects maximum H-reflex amplitude but not homosynaptic postactivation depression. *Physiol Rep* 2013; 1.
146. Sibley KM, Carpenter MG, Perry JC, Frank JS. Effects of postural anxiety on the soleus H-reflex. *Hum Mov Sci* 2007; 26: 103–12.
147. Mynark RG, Koceja DM. Down training of the elderly soleus H reflex with the use of a spinally induced balance perturbation. *J Appl Physiol* 2002; 93: 127–33.
148. Dowman R, Wolpaw JR. Jendrassik maneuver facilitates soleus H-reflex without change in average soleus motoneuron pool membrane potential. *Exp Neurol* 1988; 101: 288–302.
149. Overeem S, Lammers GJ, Van Dijk JG. Weak with laughter. *Lancet* 1999; 354: 838.
150. Boyd RN, Kerr Graham H. Objective measurement of clinical findings in the use of botulinum toxin type A for the management of children with cerebral palsy. 1999.





2

THE HUMAN BODY MODEL VERSUS CONVENTIONAL GAIT MODELS FOR KINEMATIC GAIT ANALYSIS IN CHILDREN WITH CEREBRAL PALSY

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Abstract

With the rise of biofeedback in gait training in cerebral palsy there is a need for real-time measurements of gait kinematics. The Human Body Model (HBM) is a recently developed model, optimized for the real-time computing of kinematics. This study evaluated differences between HBM and two commonly used models for clinical gait analysis: the Newington Model, also known as Plug-in-Gait (PiG), and the calibrated anatomical system technique (CAST). Twenty-five children with cerebral palsy participated. 3D instrumented gait analyses were performed in three laboratories across Europe, using a comprehensive retroreflective marker set comprising three models: HBM, PiG and CAST. Gait kinematics from the three models were compared using statistical parametric mapping, and RMSE values were used to quantify differences. The minimal clinically significant difference was set at 5°. Sagittal plane differences were mostly less than 5°. For frontal and transverse planes, differences between all three models for almost all segment and joint angles exceeded the value of minimal clinical significance. Which model holds the most accurate information remains undecided since none of the three models represents a ground truth. Meanwhile, it can be concluded that all three models are equivalent in representing sagittal plane gait kinematics in clinical gait analysis.

2.1 Introduction

Cerebral palsy (CP) is a common motor disorder affecting 2 in 1000 births in Europe,[1] often leading to an aberrant gait pattern.[2] The gait pattern in CP is often analyzed using three-dimensional joint kinematics to assist clinical decision-making and to evaluate treatment outcomes. Furthermore, gait kinematics can be used as parameters in real-time gait specific biofeedback.[3–5] Such biofeedback can be used for functional gait training. A recent literature review has shown that functional gait training outperforms standard physical therapy on several parameters[6] including walking speed, walking endurance and gait-related gross motor function. The study further addressed that virtual reality and biofeedback seem promising tools to improve engagement and rehabilitation outcomes in children with CP.[6] This has also been found for other patient populations.[7,8] Tate and Milner[9] specifically reviewed biofeedback on kinematics and found this an effective method to improve functional outcomes like gait speed and symmetry, at least on the short-term. With the rise of biofeedback training, the need for accurate real-time measurements of kinematics is rising.

Multiple models exist which can be used to measure kinematic gait features, including Plug-in-Gait (PiG)[10], the calibrated anatomical system technique (CAST)[11] and the Gait2392 model as implemented in OpenSim.[12] The human body model (HBM) is a recently developed model optimized for computing real-time kinematics.[13] Within HBM, several features are implemented to enhance the possibility to calculate kinematics in real-time. Technical markers are added to an anatomical marker set to provide redundancy of markers and thereby robustness against marker dropouts. Global optimization is used to further minimize the effects of marker dropout, as well as to limit effects of soft tissue artefacts.[14] The global optimization used in HBM is based on a weighted least squares problem to model marker positions: the distance between measured and modeled markers is minimized for the entire model at once, as is further described by Falisse et al.[15]

So far, only a few studies have assessed the outcomes of HBM. Van den Bogert et al.[13] first published healthy adult gait kinematics computed using an early version of HBM. Modifications have been made since to better match patient characteristics. The main modifications being the inclusion of anatomically defined knee and ankle axes, based on knee epicondyles and ankle malleoli respectively, an improved regression model to calculate hip joint centers,[16] and changes in shank and thorax definitions to better match ISB standards.[17] Falisse et al.[15] compared the modified HBM with the OpenSim gait2392 model, which is often used for musculoskeletal modeling purposes. Although several assumptions are similar between both models, kinematic outcomes deviated substantially.[15] These deviations could largely be attributed to differences in pelvic orientation and hip and knee joint center estimation methods.

Thanks to its real-time performance, HBM is very well suited for gait training purposes, but it can also be used for other purposes such as clinical decision making and treatment evaluation. HBM complies with several recent insights, such as the Harrington hip joint center equation[18] and global optimization[14] and is therefore expected to present accurate results for gait analysis. Obviously, it is beneficial to use the same model in gait analysis as in gait training, to avoid possible confusion due to differences between models. For clinical implementation of HBM, it is important to see how outcomes in terms of gait kinematics compare to marker models currently used in clinical care. Constraints (i.e. a reduction in the six degrees of freedom of a segment) can be a cause of differences between models.

A commonly used constraint includes linking two segments together (i.e. limiting translation between segments), thereby constraining the joint to three degrees of freedom. Another common constraint is analyzing the knee as a hinge joint, thereby reducing joint movements to one degree of freedom. Constraints can decrease the effect of soft tissue artefacts and marker placement errors, hence improve reliability.[14,19] However, modeled joint constraints should match the anatomy of the patient of interest, and are typically based on nonpathological anatomy. Children with CP often experience bony and joint deformities, which might violate these assumptions. Therefore, it is important to evaluate kinematic models for the specific patient group of interest. While previous comparisons made with HBM are performed using healthy adults, comparison in children with CP adds more to the insight of the differences in eventual care. Therefore, this study aimed to compare gait kinematics from HBM in children with CP with those from two commonly used marker models for clinical gait analysis; PiG and CAST.

2.2 Method

Subjects

Twenty-five children with CP participated in this study. Participants were recruited by their doctors from the VU University medical center Amsterdam (VUmc, N=10), Children's Hospital Bambino Gesù (OPBG, N=6) and the University of Leuven (KUL, N=9). No significant differences existed in patient characteristics between participant groups from each measurement site (Table 2.1). Inclusion criteria were: diagnosed with spastic CP, GMFCS level I-II, aged between eight and fifteen years, no orthopedic lower limb surgery or selective dorsal rhizotomy in the last year, nor chemo-denervation in the last six months prior to the assessment, and the ability to follow simple instructions. Participants aged twelve and older and all parents provided written informed consent before participation. The study was approved by each of the three local ethics committees.

Protocol

Forty-two retroreflective markers were placed on each subject, covering HBM, PiG and CAST (Fig. 2.1, Supplementary Materials 2.1). A medial ankle marker was added in PiG to account for tibial torsion as often present in CP.[20] This is further addressed in the Supplementary Materials 2.1. As inter-observer reliability increases by marker placement training,[21] all examiners were trained by internal

Table 2.1.
Mean values and standard deviations for patient characteristics separately for each measurement site, including statistical outcomes.

	VUmc	**OPBG**	**KUL**	**P-values ANOVA**
GMFCS level	6 x II, 3 x I	6 x I	6 x II, 2 x I	
Age	11.4y ± 2.1	11.0y ± 2.8	9.2y ± 2.6	0.410
Height	1.5m ± 0.1	1.4m ± 0.1	1.3m ± 0.1	0.138
Weight	41.1kg ± 9.2	34.2kg ± 8.3	31.3kg ± 9.0	0.266
Leg length	74.6cm ± 9.2	76.8cm ± 9.5	69.0cm ± 7.8	0.269
Foot length	22.7cm ± 2.2	23.3cm ± 3.8	20.0cm ± 2.6	0.437
Foot off	65.4% ± 1.5	66.1% ± 2.0	65.1% ± 1.9	0.630
Walking speed	1.1m/s ± 0.3	0.9m/s ± 0.2	1.0m/s ± 0.1	0.196
Stride length	1.1m ± 0.2	0.9m ± 0.2	1.0m ± 0.2	0.143

experts and involved examiners came together several times to standardize protocols. At each measurement site, two or three experienced gait analysts were in charge of applying markers and performing the measurements.

After marker placement, a static trial for subject calibration was captured with participants standing upright. At VUmc, participants walked on an instrumented treadmill at a self-paced[22], comfortable walking speed wearing thin flexible gym shoes. One participant was not capable of performing self-paced walking, therefore a self-selected fixed speed was used. All children got several minutes to familiarize to treadmill walking. Participants at KUL and OPBG walked barefoot at comfortable walking speed on a ten meter walkway. At least three gait cycles were collected for each participant and three cycles per subject were used for analysis. Differences in kinematics between over-ground and self-paced treadmill walking are generally small for children with CP[22] and therefore neglected in this study. Although footwear might cause different marker movement artefacts, the effect on differences between models is thought to be limited since similar foot markers were used, with the exception of the MT1/2/5 markers. Marker capture was performed using MX camera's (Vicon Motion Systems, Oxford, UK) at all sites. Marker trajectories reconstructed from raw marker data (Vicon Nexus, version 2.3, Oxford, UK) were used for data analysis.

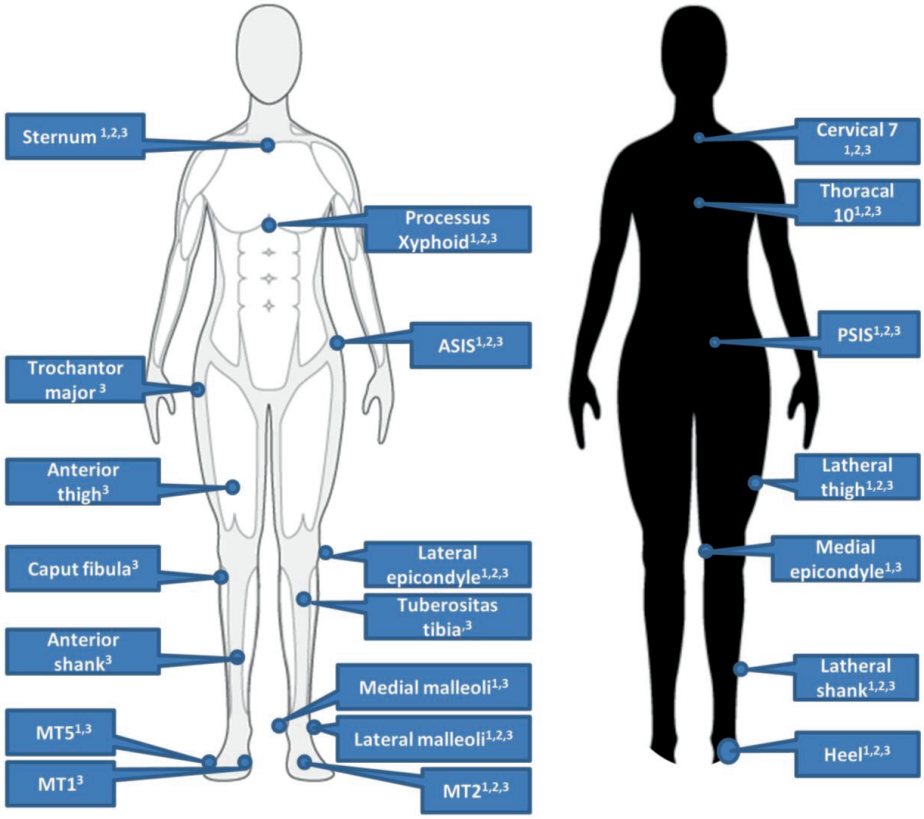

Figure 2.1. Overview of used marker set and the location of the markers. Numbers indicate which models include this marker, 1 being HBM, 2 PiG and 3 CAST. Abbreviations: MT, Metatarsal phalangeal joint; ASIS, anterior superior iliac spine; PSIS, posterior superior iliac spine.

Data analysis

For each participant, kinematics for hip, knee and ankle joint, as well as trunk, pelvis and foot segments were calculated using three models. First, HBM was used, as implemented in the Gait Offline Analysis Tool (GOAT; version 3.3; Motek BV, Amsterdam, The Netherlands). Multiple joint constraints and global optimization are used in HBM. Second, PiG was used as implemented in Vicon Nexus (version 2.3). PiG is based on the Newington model, using a minimum marker set mostly placed on bony landmarks. Constraints in PiG are implicit by shared markers and joint centers between adjacent segments, hence restricting segment movements to three degrees of freedom. Third, CAST was used, as implemented in custom-made software package BodyMech (www.bodymech.nl, Matlab 2014a, The Mathworks Inc., USA), based on clusters and virtual markers without constraints. Further biomechanical details of the three models are presented in the Supplementary Materials 2.1. Outcomes from each model were exported to Matlab for further analyses and statistics.

All joint and segment kinematics were filtered with a bi-directional 6 Hz 2nd order low-pass Butterworth filter, to harmonize bandwidth between methods. Gait cycles were defined from initial contact to next initial contact, with initial contact defined using the method of Zeni et al.[23] and gait cycles time-normalized to 0-100%. Three strides of the left leg were analyzed per subject. Next, eighteen clinically relevant outcome parameters, further referred to as kinematic parameters, were calculated (Table 2.2). The kinematic parameters included parameters as used previously,[24] as well as three parameters from frontal and transverse plane following Klejman et al.[25] and two parameters used in kinematic feedback training.[9]

Statistics

Differences between models were assessed using Statistical Parametric Mapping (SPM version M.0.4.5).[26] Differences between the two conventional models were also considered relevant, to assess whether differences of HBM with conventional models were similar to differences between conventional models. SPM was chosen as this method allows to detect specific differences in any part of the curve, reducing the necessity of a priori hypotheses. An SPM repeated measures analysis of variance (RM-ANOVA) was conducted over all kinematics with the three models as grouping variable, with post-hoc SPM paired t-tests. The Holm procedure[27] was used for all post-hoc tests to maintain probability of a type 2 error at 5%. Root mean square errors (RMSEs) were calculated as an effect size for all significantly different parts of the curves.

Kinematic parameters were tested for normality using Shapiro-Wilk tests and compared between models using RM-ANOVA. For post-hoc analysis, paired-samples t-test with Holm-correction were used. Furthermore, to quantify overall differences between models, RMSE values between the models were calculated over gait kinematic curves for each subject and averaged over all subjects. Additionally, differences in mean kinematics, representing offsets between signals, were calculated to evaluate systematic differences between models. Offset-corrected RMSEs were also calculated to assess differences attributed to the shape of the kinematic curves.[28] The measurement error associated with collecting 3D gait kinematics using the same model, e.g. inter session and inter therapist differences in kinematic outcomes, were generally below 5° in previous studies.[29–31] Systematic differences between models are especially of interest when they exceed those intra-model differences. McGinley et al.[31] recommended that differences above 5° were considered clinically

meaningful. Furthermore, improvements in kinematic parameters after feedback usually exceeded 5°.[5,9] Therefore, we considered the cut off of 5° to indicate relevant differences between models.

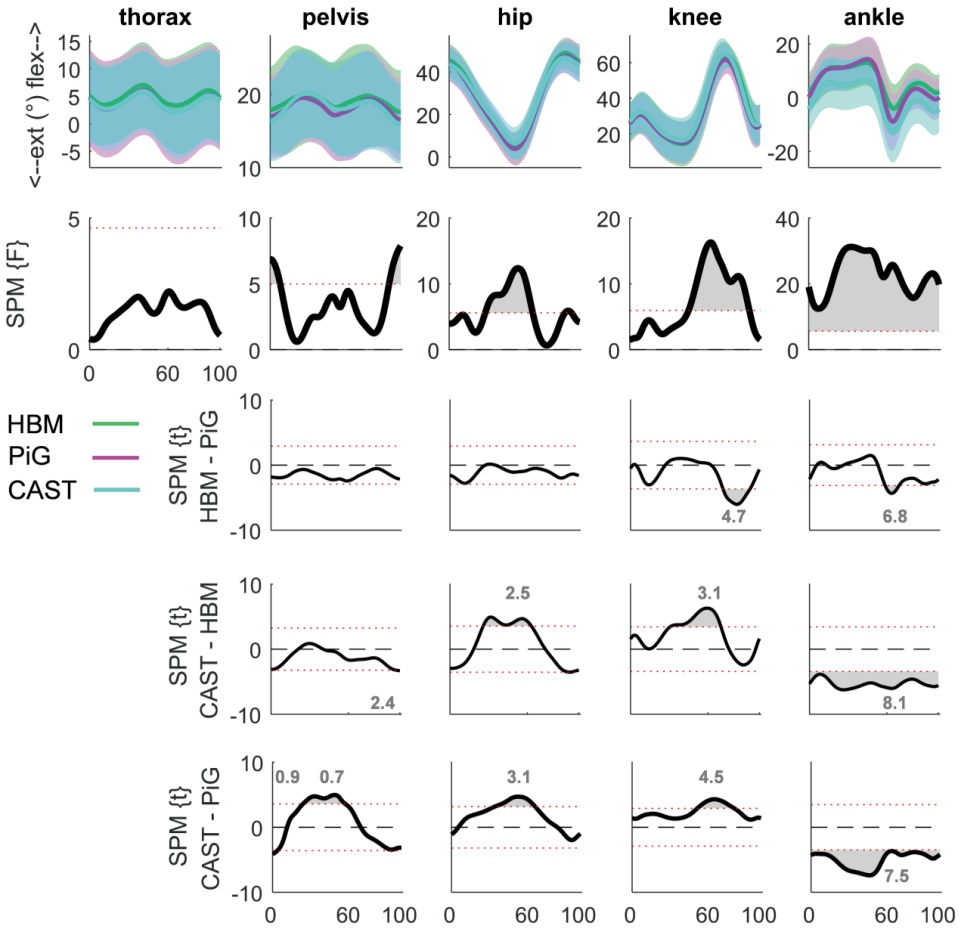

Figure 2.2. Sagittal plane angles. Mean and STD of joint angles over all subjects for the different marker models are presented in the top pane. SPM repeated-measures ANOVA F-values are depicted on the second pane. SPM post-hoc paired t-test t-values are represented on the bottom three panes. Red dotted lines indicate F/t-threshold values above which curves significantly differ. Grey shaded areas indicate significant differences, with bigger shaded areas indicating lower p-values. For each significant region in the t-tests, RMSE values are depicted in these graphs in black to quantify the size of a significant difference.

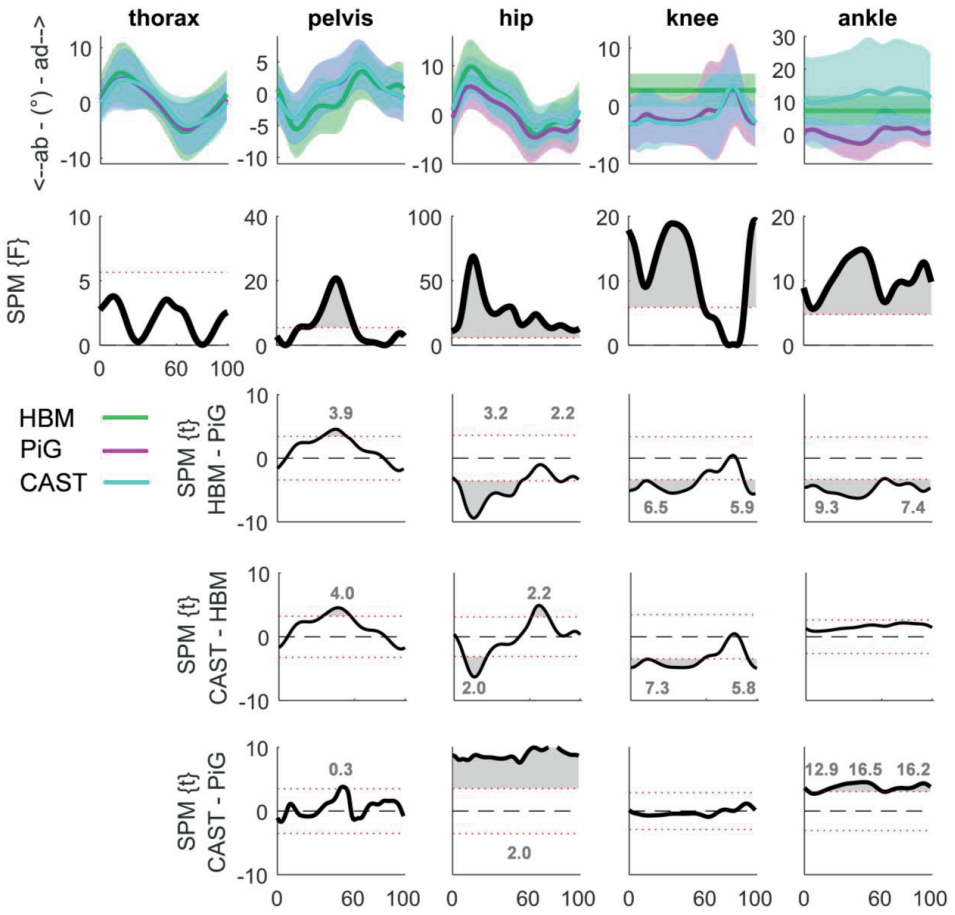

Figure 2.3. Frontal plane angles. Representation of the graphs is similar as described for Fig. 2.2. HBM knee and ankle angles are constraint to zero degrees movement in the frontal plane. The horizontal lines represent the static values for ab−/adduction.

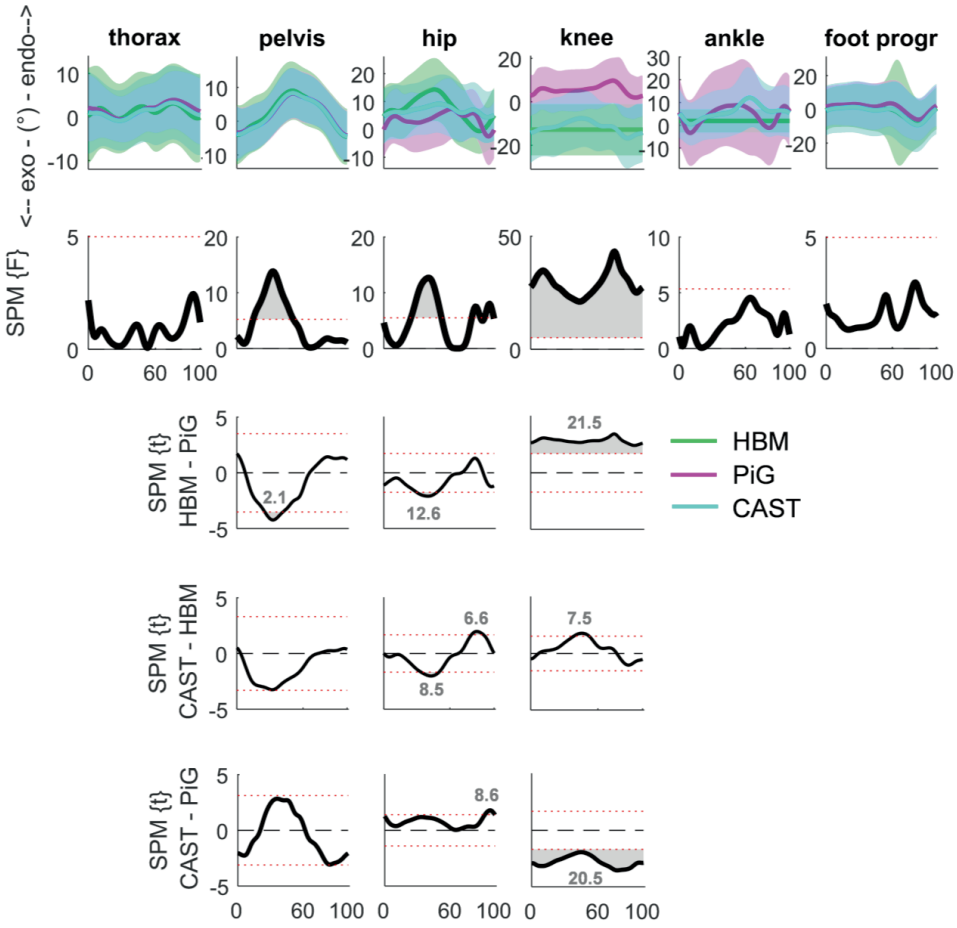

Figure 2.4. Transversal plane angles. Representation of the graphs is similar as described for Fig. 2.2. HBM knee and ankle angles are constraint to zero degrees movement in the transversal plane. The horizontal lines represent the static values for internal/external rotation.

Table 2.2.

Mean values and standard deviations for clinically relevant outcome parameters from the three models and the three laboratories, including statistical outcomes.

	Mean (°) ± STD			P-values ANOVA & post-hoc			
	PiG	**CAST**	**HBM**	**ANOVA**	**HBM-CAST**	**HBM-PiG**	**CAST-PiG**
Mean pelvic tilt	18.1 ± 6.0	18.1 ± 6.0	18.7 ± 6.1	0.126			
ROM[a] pelvic tilt	6.1 ± 2.9	6.1 ± 3.0	6.4 ± 3.1	0.699			
Mean pelvic rotation	2.5 ± 6.6	2.5 ± 6.6	2.9 ± 7.5	0.100			
ROM hip flexion	45.8 ± 8.6	42.4 ± 8.6	46.4 ± 9.0	**<0.001**	**<0.001**	0.214	**<0.001**
Minimal hip flexion	2.5 ± 7.8	5.1 ± 7.7	3.0 ± 7.0	**<0.001**	**<0.001**	0.434	**<0.001**
Peak hip abduction swing	-6.6 ± 3.9	-4.5 ± 3.5	-5.9 ± 3.6	**<0.001**	**<0.001**	**0.047**	**<0.001**
Peak hip exorotation	-4.2 ± 10.2	1.1 ± 9.7	-2.3 ± 8.4	**0.021**	**0.020**	0.369	**0.015**
Peak hip endorotation	11.7 ± 9.9	12.7 ± 9.9	16.3 ± 11.1	0.068			
ROM knee flexion	51.2 ± 12.0	53.4 ± 13.2	54.2 ± 12.6	**<0.001**	0.224	**<0.001**	**<0.001**
Peak knee flexion swing	63.8 ± 6.8	66.8 ± 7.0	66.2 ± 6.0	**<0.001**	0.418	**<0.001**	**0.003**
Peak knee extension stance	13.3 ± 12.1	14.1 ± 12.5	12.4 ± 12.6	0.052			
Knee flexion at IC[b]	25.7 ± 11.1	26.8 ± 11.0	26.0 ± 11.8	0.247			
Time to peak knee flexion	74.5[c] ± 4.2	74.2[c] ± 4.2	75.2[c] ± 4.6	**0.002**	**0.001**	0.035[d]	0.298
ROM knee adduction	13.6 ± 3.8	12.1 ± 4.8	0 ± 0[e]	**<0.001**	**<0.001[e]**	**<0.001[e]**	0.081
Peak ankle dorsal stance	16.3 ± 7.9	9.0 ± 8.5	14.6 ± 8.7	**<0.001**	**<0.001**	0.078	**<0.001**
Peak ankle dorsal swing	4.6 ± 7.4	-0.1 ± 8.5	6.4 ± 7.3	**<0.001**	**<0.001**	**0.048**	**0.001**
Peak plantar push-off	10.7 ± 10.5	14.4 ± 10.3	5.9 ± 9.0	**<0.001**	**<0.001**	**<0.001**	**0.005**
Mean foot progression	0.6 ± 13.0	-1.8 ± 15.5	-1.3 ± 15.8	0.204			

Note. Significant values are expressed in bold and differences exceeding 5° clinical significances are underlined. [a]ROM = range of motion. [b]IC = initial contact. [c]Percentage of gait cycle. [d]This value is not significant due to Holm-corrections. [e]ROM is constraint to 0° in HBM; therefore the other two models differ significantly by default.

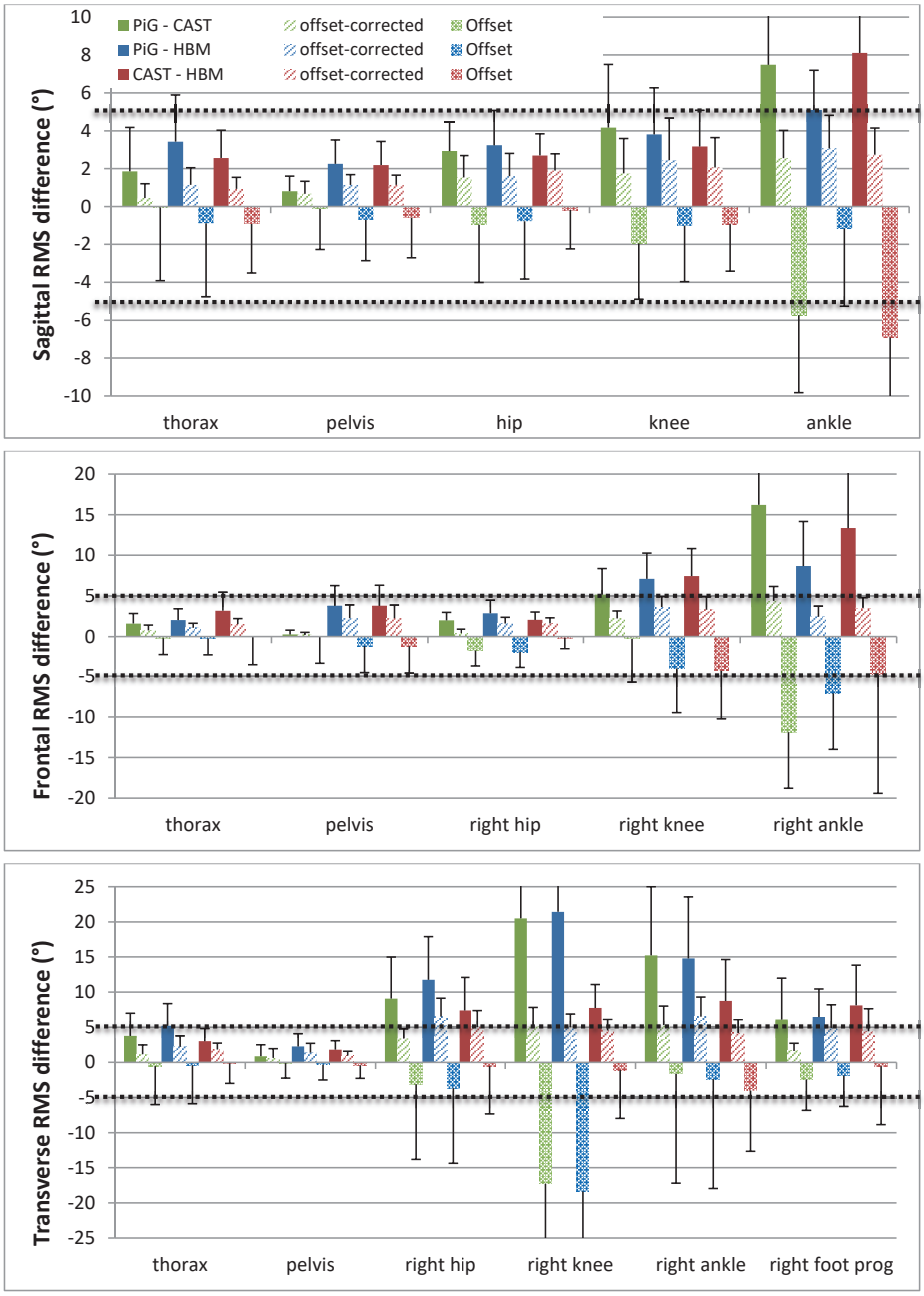

Figure 2.5. Differences between the three models, measured as root mean square error (RMSE), offset-corrected RMSE and offset values for the sagittal, frontal and transverse planes. Offset values are presented as negative values for visualization purposes. Dotted lines represent the 5° threshold for clinical decision making.

2.3 Results

Kinematic curves for the three models and outcomes from SPM can be found in Fig. 2.2, 2.3 and 2.4. Kinematic parameters are shown in Table 2.2 and RMSE values over the complete strides in Fig. 2.5.

For thorax, pelvis and hip kinematics, differences between models were not significant or stayed below 5° in all planes, except for transverse plane hip rotation. SPM showed significant differences in hip exorotation, reflected also by hip peak exorotation, showing 5.3° less exorotation for CAST compared with PiG. PiG showed the most amount of exorotation, followed by HBM. RMSE values over complete strides were 11.8° (PiG-HBM), 9.1° (PiG-CAST) and 7.4° (HBM-CAST), with an offset-corrected RMSE of 6.5° between PiG and HBM.

Knee sagittal kinematics were comparable between models, i.e. differences were below 5°. Since HBM knee angles are constraint to sagittal plane movements only, differences for the frontal and transversal plane were expected. In the frontal plane, knee adduction ROM of PiG and CAST were 13.6° and 12.1°, in contrast to the 0° assumption in HBM. In addition, all frontal and transverse knee RMSE values between the three models exceeded 5°, but offset-corrected RMSE values and offsets for knee adduction remained below 5°. Knee exorotation was over 15° less according to PiG than CAST and HBM over the entire gait cycle and offset-corrected RMSE values were around 5°.

Ankle kinematics differed significantly between models in sagittal and frontal plane over almost the entire gait cycle, as also reflected in the ankle kinematic parameters showing over 7.3° and 5.6 significantly less dorsiflexion during swing and 5.0° and 6.5° during stance for CAST than for PiG and HBM respectively. Additionally, peak plantarflexion during push-off was 8.5° larger for CAST than for HBM. Furthermore, all RMSE values for the ankle exceeded 5°, with offset values above 5° for comparisons of HBM with CAST in the sagittal plane and with PiG in the frontal plane. None of the offset-corrected values exceeded the 5° threshold.

2.4 Discussion

This study aimed to assess differences between HBM, a recent marker model using joint constraints, a redundant number of markers and global optimization versus two commonly used models in gait analysis: i.e. PiG, using a minimum marker set implying some implicit constraints, and CAST, based on cluster markers, calibrated with bony landmarks and no joint constraints, in children with cerebral palsy. Sagittal plane kinematics were found to be quite comparable for the three models, with differences generally below 5°. However, differences of up to 25° were found in the other planes for the hip, knee and ankle joints, between all three models.

HBM provided equivalent outcomes for sagittal plane gait kinematics, with only ankle kinematic differences with CAST exceeding 5° RMSE. Differences between CAST and HBM/PiG were visible as an offset towards plantar flexion, as was also found in a previous study by Ferrari et al.[29] between CAST and PiG. This is mainly caused by different markers that define the foot segment (see Supplementary Materials 2.1). MT1 and MT5 are used in CAST, which are placed lower on the foot than the MT2 marker used in HBM and PiG. This causes a difference in the line between the heel marker and the MT2 versus the heel marker and the MT1/MT5 plane of approximately 6.0°. This angular difference

can be corrected by replacing MT1 and MT5 markers in CAST with the MT2 marker used in the other models. A limitation of the study is that subjects walked on a treadmill in the VUmc and over ground in OPBG and KUL. The effect of treadmill walking versus over ground walking on the different models is not addressed in this study, although the effect is thought to be small based on previous studies.[32,33] Furthermore, children wore gymnastic shoes on the treadmill and not over ground, which may have affected the foot kinematics and the comparison between models. However, differences between ankle kinematics showed similar results for all three labs, and therefore treadmill walking and shoe movement artefacts are not thought to influence the findings. Overall, our findings suggest that sagittal plane kinematics are generally similar between models.

In contrast, important differences between all three models were found in the frontal and transverse plane. The differences found in this study in children with CP are comparable to differences previously found in healthy adults. For instance, Ferrari et al.[29] found very low correlation coefficients between PiG and CAST for hip and knee endo-/exorotation. The large differences in frontal and transverse planes are likely due to the differences in constraints applied by all models and partially due to different markers used. In HBM, the knee is modeled as a one degree of freedom hinge joint and the ankle is constrained to two degrees of freedom. Generally constraints will reduce the influence of soft tissue artefacts and marker placement errors.[14,19] On the other hand, actual rotations in the constrained angles will affect the modeled rotations in other planes and other joints, introducing kinematic errors. True knee or ankle exorotation will for example be measured as hip exorotation, which explains the larger hip exorotation ROM for HBM compared to CAST and PiG. Contrarily, average knee adduction ROM as found by CAST and PiG exceeded 10°, which exceeds the maximum for healthy knees.[34] This result is likely due to crosstalk from knee flexion as a consequence of malaligned coordinate system through misplacement of thigh markers.[34] This is supported by findings of Sangeux[35], who showed that PiG ab/adduction angles can be reduced when correcting the alignment by assuming only one or two degree of freedom movements in the knee. Such a misalignment error leads in turn to errors in calculated hip rotation angles.[35] Hence it is likely that all models show errors in hip exorotation angles to some extent, albeit of different origin.

Differences in hip kinematics can furthermore be caused by different methods to define the hip joint center. HBM uses the Harrington equation, suggested to be the most accurate regression method according to a recent validation study,[18] in contrast with the Davis equation used in PiG. Leboeuf et al.[36] showed that supplementing Davis equation with the Harrington equation improved PiG kinematics in the frontal plane, although not in other planes. Kainz et al.[18] advised the use of functional calibration over a regression method. However, experience during performing our measurements showed that most children with CP are not able to perform the star-arc movement with the required range of motion for functional calibration. Therefore, we did not use functional hip calibration in this study. An important note for HBM is that, in absence of medial knee and ankle markers, knee and ankle axes are defined parallel to the line between the ASIS markers in static calibration. Due to femoral and tibial torsion, this is unlikely to be valid for many children with CP and would probably result in errors in hip kinematics. It is clear from PiG outcomes that tibial torsion occurs in the patients in this study: Tibial torsion is accounted for in PiG in ankle angles, but not in knee angles, due to the use of a torsioned tibia. In HBM and CAST, torsions in the tibia are also reflected as an offset in knee exorotation. This results in large differences between PiG knee exorotation and HBM and CAST knee exorotation. Furthermore, the influence of tibial torsion on kinematic outcomes is presented in

Supplementary Materials 2.1. Due to the large influence of tibial torsion, it is recommended to apply medial markers when using HBM during static calibration when analyzing pathological gait.

Some differences between models may also be due to the use of global optimization, as applied in HBM, versus segment tracking, as used in PiG and CAST. Pelvic kinematics in HBM differed significantly from PiG and CAST, despite identical markers and anatomical frame definitions. Global optimization of the markers used in HBM distributes errors due to marker misplacement and soft tissue artefacts over all segments, but possibly also reduces soft tissue artefacts of the pelvic markers. This is reflected in the differences between models: small differences between HBM and the other two models occur in almost all joints. Despite these differences, overall RMSE differences between HBM and the two conventional models are smaller in magnitude than differences between the conventional models. This suggests that errors are distributed over multiple segments, thereby minimizing overall errors.

The measured differences give guidelines for use in clinical implementation. For all three models, differences exceeded 5°, implying that substituting a marker model with another model introduces a bias, that might not be accounted for in clinical decision making. These differences can present themselves as offsets between models, such as in ankle plantarflexion. Such a systematic error is relatively easy to account for. The largest difference in the sagittal plane was present as such an offset. Since those can be corrected for, it can be concluded that kinematic curves in the sagittal plane from different model can be compared for interpretation. This implies that HBM and the conventional models are functionally equivalent for sagittal plane kinematics. However, in general, given that small differences still exist between models, it remains important to collect data from the same subject before and after treatment using the same model. The same holds for a normative dataset used for comparison, which should be based on the same model. Lastly, sagittal plane kinetics can also be input for biofeedback,[3–5] hence validity of kinetics should be assessed before implementation.

Besides offsets, differences between the kinematic patterns are harder to correct for. Such differences in patterns were seen for the frontal and transverse plane kinematics, so for these planes the three models are not equivalent to each other. Constraints in knee and ankle angles in HBM limit the use of HBM in feedback for these angles. However, conventional models are probably also not suitable for providing accurate feedback on these angles, taking into account the large ROM found for knee adduction angles for example. Furthermore, the static angles as calculated by HBM can potentially be used in clinical decision making, for example to determine the amount of tibial rotation. The observed differences between models further emphasize the importance of using a normative database based on the same kinematic model, when comparing patients to healthy subjects. In HBM, global optimization influences all segments, which results in differences with conventional models for all segments, but since most differences remain below 5°, this is not expected to influence clinical decision making. Overall, differences between PiG and CAST were on average larger than between HBM and the two conventional models. Hence these findings do not suggest preferred use of conventional models over HBM. A ground truth would be required to decide on which of the three models is the most accurate. Considering the limited differences and their systematicity, HBM can be useful for providing rehabilitative feedback, and for clinical decision making at least regarding sagittal plane kinematics.

2.5 Conclusions

Overall, the differences in gait kinematics we found between marker models were not pointing to a specific outlier, i.e. based on agreement there is no preferred use of either PiG, CAST or HBM. For the sagittal plane angles the models were found to be equivalent, i.e. any non-systematic difference was below the minimal clinically significant difference of 5°. However, differences of up to 25° were found in frontal and transversal planes for hip, knee and ankle joints, between all models. For these planes the three models cannot be used interchangeably. A ground truth would be required to decide on which of the three models is the most accurate.

Acknowledgements

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References

1. Johnson A. Prevalence and characteristics of children with cerebral palsy in Europe. *Dev Med Child Neurol* 2002; 44: 633–40.
2. Davids JR, Rowan F, Davis RB. Indications for Orthoses to Improve Gait in Children With Cerebral Palsy. *JAAOS - J Am Acad Orthop Surg* 2007; 15.
3. Booth ATC, Steenbrink F, Buizer AI, Harlaar J, van der Krogt MM. Is avatar based real-time visual feedback a feasible method to alter gait parameters of interest? *Gait Posture* 2016; 49: 98.
4. van Gelder L, Booth ATC, van de Port I, Buizer AI, Harlaar J, van der Krogt MM. Real-time feedback to improve gait in children with cerebral palsy. *Gait Posture* 2017; 52: 76–82.
5. Booth AT, Buizer AI, Harlaar J, Steenbrink F, van der Krogt MM. Immediate Effects of Immersive Biofeedback on Gait in Children With Cerebral Palsy. *Arch Phys Med Rehabil* 2019; 100: 598–605.
6. Booth ATC, Buizer AI, Meyns P, Oude Lansink ILB, Steenbrink F, van der Krogt MM. The efficacy of functional gait training in children and young adults with cerebral palsy: a systematic review and meta-analysis. *Dev Med Child Neurol* 2018; 60: 866–83.
7. Giggins OM, Persson UM, Caulfield B, et al. Biofeedback in rehabilitation. *J Neuroeng Rehabil* 2013; 10: 1123–34.
8. Pfeufer D, Gililland J, Böcker W, et al. Training with biofeedback devices improves clinical outcome compared to usual care in patients with unilateral TKA: a systematic review. *J Pediatr Gastroenterol Nutr* 2018. DOI:10.1007/s00167-018-5217-7.
9. Tate JJ, Milner CE. Systematic Review Biofeedback During Gait Retraining in Patients: A Real-Time Kinematic, Temporospatial, and Kinetic Real-Time Kinematic, Temporospatial, and Kinetic Biofeedback During Gait Retraining in Patients: A Systematic Review. *PHYS THER* 2010; 90: 1123–34.
10. Davis RB, Ounpuu S, Tyburski D, Gage JR. A gait analysis data collection and reduction technique. *Hum Mov Sci* 1991; 10: 575–87.
11. Cappozzo A, Catani F, Della Croce U, Leardini A. Position and orientation in space of bones during movement: anatomical frame definition and determination. *Clin Biomech* 1995; 10: 171–8.
12. Delp SL, Anderson FC, Arnold AS, et al. OpenSim: open-source software to create and analyze dynamic simulations of movement. *IEEE Trans Biomed Eng* 2007; 54: 1940–50.
13. van den Bogert AJ, Geijtenbeek T, Even-Zohar O, Steenbrink F, Hardin EC. A real-time system for biomechanical analysis of human movement and muscle function. *Med Biol Eng Comput* 2013; 51: 1069–77.
14. Duprey S, Cheze L, Dumas R. Influence of joint constraints on lower limb kinematics estimation from skin markers using global optimization. *J Biomech* 2010; 43: 2858–62.
15. Falisse A, Rossom S Van, Gijsbers J, et al. OpenSim Versus Human Body Model: A Comparison Study for the Lower Limbs During Gait. *J Appl Biomech* 2018.
16. Harrington ME, Zavatsky AB, Lawson SEM, Yuan Z, Theologis TN. Prediction of the hip joint centre in adults, children, and patients with cerebral palsy based on magnetic resonance imaging. *J Biomech* 2007; 40: 595–602.
17. Wu G, Siegler S, Allard P, et al. ISB recommendation on definitions of joint coordinate system of various joints for the reporting of human joint motion—part I: ankle, hip, and spine. *J Biomech* 2002; 35: 543–8.
18. Kainz H, Modenese L, Lloyd DG, Maine S, Walsh HPJ, Carty CP. Joint kinematic calculation based on clinical direct kinematic versus inverse kinematic gait models. *J Biomech* 2016; 49: 1658–69.
19. Groen BE, Geurts M, Nienhuis B, Duysens J. Sensitivity of the OLGA and VCM models to erroneous marker placement: effects on 3D-gait kinematics. *Gait Posture* 2012; 35: 517–21.
20. Kerr Graham H, Selber P. Musculoskeletal aspects of cerebral palsy. *J Bone Jt Surg [Br]* 2003; 85: 157–66.
21. Gorton GE, Hebert DA, Gannotti ME. Assessment of the kinematic variability among 12 motion analysis laboratories. *Gait Posture* 2009; 29: 398–402.
22. Sloot LH, Van der Krogt MM, Harlaar J. Self-paced versus fixed speed treadmill walking. *Gait Posture* 2014; 39: 478–84.
23. Zeni JA, Richards JG, Higginson JS. Two simple methods for determining gait events during treadmill and overground walking using kinematic data. *Gait Posture* 2008; 27: 710–4.
24. Schutte LM, Narayanan U, Stout JL, Selber P, Gage JR, Schwartz MH. An index for quantifying deviations from normal gait. *Gait Posture* 2000; 11: 25–31.
25. Klejman S, Andrysek J, Dupuis A, Wright V. Test-retest reliability of discrete gait parameters in children with cerebral palsy. *Arch Phys Med Rehabil* 2010; 91: 781–7.

26. Friston K, Mattout J, Trujillo-Barreto N, Ashburner J, Penny W. Variational free energy and the Laplace approximation. *Neuroimage* 2007; 34: 220–34.
27. Holm S. A simple sequentially rejective multiple test procedure. *Scand J Stat* 1979; : 65–70.
28. Ancillao A, van der Krogt MM, Buizer AI, Witbreuk MM, Cappa P, Harlaar J. Analysis of gait patterns pre- and post- Single Event Multilevel Surgery in children with Cerebral Palsy by means of Offset-Wise Movement Analysis Profile and Linear Fit Method. *Hum Mov Sci* 2017; 55: 145–55.
29. Ferrari A, Benedetti MG, Pavan E, et al. Quantitative comparison of five current protocols in gait analysis. *Gait Posture* 2008; 28: 207–16.
30. Ferber R, McClay Davis I, Williams III DS, Laughton C. A comparison of within- and between-day reliability of discrete 3D lower extremity variables in runners. *J Orthop Res* 2002; 20: 1139–45.
31. McGinley JL, Baker R, Wolfe R, Morris ME. The reliability of three-dimensional kinematic gait measurements: A systematic review. *Gait Posture* 2009; 29: 360–9.
32. Van der Krogt MM, Sloot LH, Buizer AI, Harlaar J. Kinetic comparison of walking on a treadmill versus over ground in children with cerebral palsy. *J Biomech* 2015; 48: 3577–83.
33. van der Krogt MM, Sloot LH, Harlaar J. Overground versus self-paced treadmill walking in a virtual environment in children with cerebral palsy. *Gait Posture* 2014; 40: 587–93.
34. Lafortune MA, Cavanagh PR, Sommer HJ, Kalenak A. Three-dimensional kinematics of the human knee during walking. *J Biomech* 1992; 25: 347–57.
35. Sangeux M. Computation of hip rotation kinematics retrospectively using functional knee calibration during gait. *Gait Posture* 2018; 63: 171–6.
36. Leboeuf F, Reay J, Jones R, Sangeux M. The effect on conventional gait model kinematics and kinetics of hip joint centre equations in adult healthy gait. *J Biomech* 2019; 87: 167–71.

Supplementary Materials 2.1

Table S2.1

An overview of markers used during calibration and during gait (cluster) and equations used to determine segments and joints.

		HBM	PiG	CAST
Thorax	Markers	Jugular Notch (JN), Xiphoid (XIPH), Thoracal 10 (T10), Cervical 7 (C7)	JN, XIPH, C7	JN, XIPH, C7, T10
	Cluster	JN, XIPH, T10	JN, XIPH, C7	C7, T10, JN
	Origin	T10-T11 joint	0.5*marker width behind JN, in direction of x-axis.	JN
	Frontal axis	x=cross(y,z)	x=Midpoint(JN,XIPH)-midpoint(C7,T10)	y=0.5*(JN+C7)-0.5*(XIPH+T10);
	Transverse	y=cross(JN-C7, 0.5*(XIPH+T10))	y=cross(z,x)	xtemp=JN-0.5*(XIPH+T10); z=cross(xtemp,y);
	Longitudinal	z=0.5*(JN+C7) – 0.5*(XIPH+T10)	z=midpoint(XIPH,T10) - midpoint(JN,C7)	x=cross(y,z);
Pelvis	Markers	RASIS, LASIS, RPSIS, LPSIS	RASIS, LASIS, RPSIS, LPSIS. SACR if present, otherwise: sacrum = midpoint(RPSIS,LPSIS)	RASIS, LASIS, RPSIS, LPSIS sacrum = midpoint(RPSIS,LPSIS)
	Cluster	RASIS, LASIS, RPSIS, LPSIS	RASIS, LASIS, RPSIS, LPSIS	RASIS, LASIS, LPSIS
	Origin	0.5*(LASIS+RASIS)	0.5*(LASIS+RASIS);	0.5*(LASIS+RASIS);
	Frontal axis	x = cross(y,z)	ztemp= cross((RASIS – sacrum),y) x=cross(y,ztemp)	ztemp=cross(sacrum -origin,y); x=cross(y,ztemp);
	Transverse	y = LASIS-RASIS	y = LASIS-RASIS	y= RASIS-origin;
	Longitudinal	z = cross(y ,0.5(LASIS-RASIS) - 0.5(LPSIS-RPSIS))	z=cross(x,y)	z=cross(x,y)
Right Thigh	Markers	Right hip joint center (RHJC[1], right lateral epicondyle knee (RLEK), right knee joint center (RKJC)	RHJC[2], RLTHI, RLEK. Knee alignment device (KAD[C]) during static.	RHJC[3], RLEK, Right medial epicondyle knee (RMEK), right caput fibula (RCF)
	Cluster	RHJC, right lateral thigh (RLTHI), RLEK	RHJC, RLTHI, RLEK	Right anterior thigh (RATHI), RLTHI, RLEK
	Origin	RHJC	RHJC	0.5*(RLEK + RMEK)

	Frontal axis	x=cross((RKJC – RLEK[A]),Z)	x=cross(RKADAx – RKJC,z)	x=cross(y,z)
	Transverse	y=cross(Z,X)	y=cross(z,x) (not necessarily parallel to KAD axis)	QFP=cross(RCF-origin,RLEK-origin); y=cross(z,QFP);
	Longitudinal	z=RKJC-RHJC	z=RHJC – RKJC	z=RHJC-origin;
Right Tibia	Markers	Medial malleolus (RMM), lateral malleolus (RLM), RLEK, ankle joint center (RAJC),	RKJC, RLSHA, RLM	RLM, RMM, RCF, Tuberositas tibia (RTT).
	Cluster	Lateral shank (RLSHA), RLM, RKJC	RLSHA, RLM, RKJC	RTT, anterior shank (RASHA), RLM
	Origin	RKJC = 0.5*(MEK+LEK);	RKJC → medial to RLEK, perpendicular to HJC-KJC, and frontal axis direction of KAD-axis, if present.	0.5*(RMM+RLM);
	Frontal axis	x=cross(RAJC-RLM,Z)	x=cross(KADAx – RKJC, z) (Parallel to frontal knee axis).	x=cross(z,y);
	Transverse	y=cross(z,x)	y=cross(z,x)	y=cross(QFP,z);
	Longitudinal	z=RAJC-RKJC	z=RKJC – RAJC	QFP = cross(RCF[B]-origin,RLM-origin) QSP = cross(QFP,RTT-origin) ; z=cross(QSP,QFP);
Right Foot	Markers	RLM, RMM, calcaneus (RHEE), metatarsal (RMT) 2	RAJC, RLM, RMT2, RHEE	RLM, RHEE, RMT1, RMT2, RMT5
	Cluster	RLM, RHEE, RMT2	RAJC, RMT2, RHEE	RHEE, RMT1, RMT5
	Origin	Subtalar joint centre[4]	RAJC	RHEE
	Frontal axis	z=HEE-MT2	z= MT2 – RHEE	z=cross(y,QTP);
	Transverse	y=cross(z,x)	y=cross(z,x)	QTP=cross(MT5-RHEE,MT1-RHEE); QSP=cross(MT2-RHEE,QTP); y=cross(QTP,QSP);
	Longitudinal	x=cross(z,RMM-RLM)	x=cross(RLM– RAJC,z)	x=cross(y,z);

[A]In case of left segment, medial and lateral markers are switched to make sure that the vector points left.

[B]For some participants, RCF and RTT were not present. Midpoint of RLEK and RMEK were used instead.

[C]A knee alignment device (KAD) was used for the participants in the hospitals KUL and VUmc. For the participants at OPBG, the lateral thigh marker was placed in line with the HJC and the lateral epicondyle of the knee, following clinical practice. For the participants at KUL and VUmc, the medial ankle marker was included in the analysis (labeled in Vicon Nexus as LMED and RMED). If the medial ankle marker is not present in a data set, tibial torsion is neglected. However, children with CP often experience tibial torsion.[5] It is therefore important to add the value for tibial torsion, especially in children with CP. The influence of tibial torsion in children with CP is depicted in Fig. S2.1, where the ankle angle outcomes in the transversal plane are presented. Neglecting tibial torsion leads to significant different outcomes with RMSE values of 12.4 on average, ranging from 0.5 to 27.6 degrees over the patients. This further leads to differences in the frontal plane, with RMSE values of 2.5 (range: 0.2 – 5.0) as well as in the sagittal plane (RMSE 3.1, range 0.1 – 14.6).

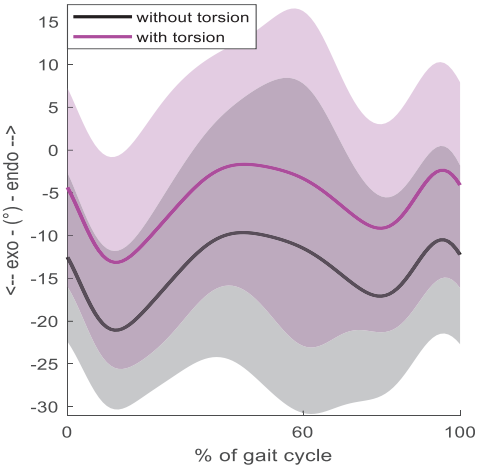
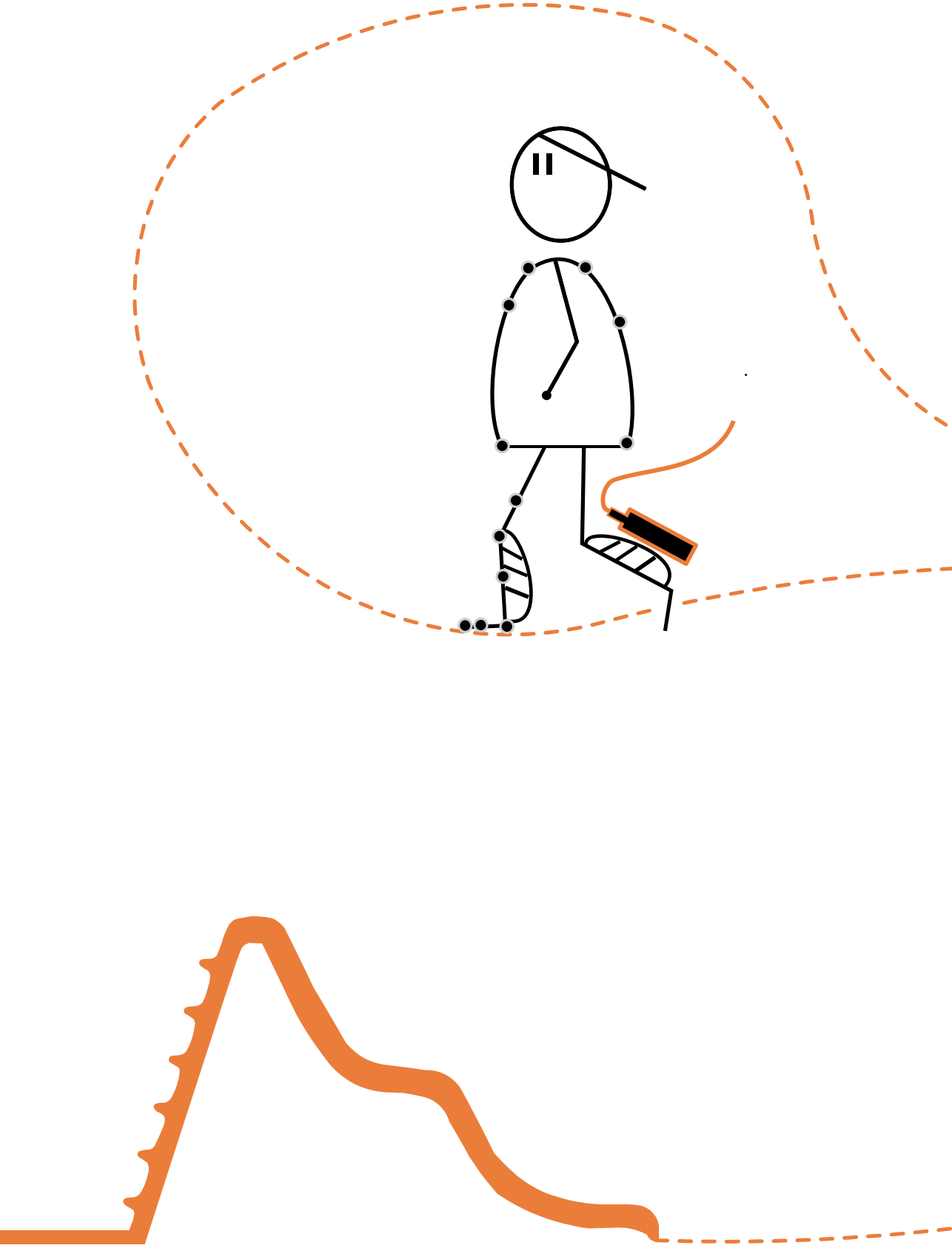


Figure S2.1. Ankle angles with and without tibial torsion in PiG

References Supplementary Materials

1. Harrington ME, Zavatsky AB, Lawson SEM, Yuan Z, Theologis TN. Prediction of the hip joint centre in adults, children, and patients with cerebral palsy based on magnetic resonance imaging. *J Biomech* 2007; 40: 595–602.
2. Davis RB, Ounpuu S, Tyburski D, Gage JR. A gait analysis data collection and reduction technique. *Hum Mov Sci* 1991; 10: 575–87.
3. Leardini A, Cappozzo A, Catani F, et al. Validation of a functional method for the estimation of hip joint centre location. *J Biomech* 1999; 32: 99–103.
4. van den Bogert AJ, Smith GD, Nigg BM. In vivo determination of the anatomical axes of the ankle joint complex: an optimization approach. *J Biomech* 1994; 27: 1477–88.
5. Kerr Graham H, Selber P. Musculoskeletal aspects of cerebral palsy. *J Bone Jt Surg [Br]* 2003; 85: 157–66.



3

THE INFLUENCE OF WEARING AN ULTRASOUND DEVICE ON GAIT IN CHILDREN WITH CEREBRAL PALSY AND TYPICALLY DEVELOPING CHILDREN

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Abstract

Background. Ultrasonography with motion analysis enables dynamic imaging of medial gastrocnemius (MG) muscles and tendons during gait. This revealed pathological muscle-tendon dynamics in children with spastic cerebral palsy (CP) compared to typically developing (TD) children. However, wearing an ultrasound probe on the lower leg could interfere with gait and bias muscle length changes observed with ultrasound.

Research question. Does wearing an ultrasound probe on the MG influence gait in children with CP and TD children?

Methods. Eighteen children with spastic CP and 16 age-matched TD children walked at comfortable walking speed on an instrumented treadmill. One baseline gait condition (BASE) and two conditions with an ultrasound probe and custom-made probe holder were measured: on the mid-muscle fascicles (FAS) and on the muscle-tendon junction (MTJ). The effect of condition and group on spatiotemporal parameters, hip, knee and ankle kinematics, ankle moment, ankle power, and modeled MG muscle-tendon unit (MTU) length was assessed using two-way repeated measures ANOVA's. Statistical non-parametric mapping was applied for time-series. Post-hoc paired-samples t-tests were conducted, and the root mean square difference was calculated for significant parts.

Results. Children took wider steps during FAS (CP,TD) and MTJ (TD) compared to BASE, and during FAS compared to MTJ (CP). Hip extension was lower (2.7°) during terminal stance for MTJ compared to FAS for TD only. There was less swing knee flexion (FAS 4.9°; MTJ 4.0°) and ankle plantarflexion around toe-off (FAS 3.0°; MTJ 2.4°) for both ultrasound placements, with no group effect. Power absorption during loading response was slightly increased for both ultrasound placements (0.12W/kg), with no group effect. MTU shortened less in swing for both ultrasound placements (FAS 3.6mm; MTJ 3.7mm), with no group effect.

Significance. Wearing an ultrasound probe causes minimal lower-limb gait alterations and MTU length changes that are mostly similar in CP and TD.

3.1 Introduction

Dynamic 2D B-mode ultrasonography (US) can be used to observe medial gastrocnemius (MG) length changes during gait.[1,2] This revealed an important decoupling mechanism between the contractile and elastic tissues of the MG muscle-tendon unit (MTU), which influences gait efficiency.[3] Additionally, it revealed pathological tendon and muscle dynamics during gait in children with spastic cerebral palsy (CP), that may underlie their gait pattern alterations.[4–7]

However, several methodological issues are associated with dynamic ultrasound imaging during gait that may compromise the study results. For example, probe tilt, due to the probe weight, and muscle compression can affect measured length changes.[8] These issues could be largely avoided with a custom probe holder.[9] However, probe holders are bulky, which could interfere with gait especially in children and this could bias muscle length changes observed with US. Furthermore, adaptation capabilities have shown to differ in CP compared to typically developing (TD) children.[10,11] Therefore children with CP might be affected differently by wearing the probe. Many dynamic US studies focus on comparing CP to TD reference data,[4–7] emphasizing the relevance of assessing both CP and TD. Therefore, we investigated whether the presence of an US probe and probe holder on the MG influences the gait pattern and modeled MG MTU length in children with CP and TD children. We expected increased step width and decreased knee flexion in mid-swing due to the location and weight of the probe. Coinciding with the decreased knee flexion, decreased shortening of the MG MTU in swing is expected.

3.2 Methods

Eighteen children with spastic CP and 16 age-matched TD children (Table 3.1) participated in this study after providing informed consent. The study was approved by the local medical ethics committee (registration number: NL65846.029.18). Participants walked at comfortable walking speed on an instrumented split-belt treadmill (GRAIL, Motek ForceLink BV, The Netherlands), while wearing gymnastic shoes and a safety harness. 3D kinematics were collected at a sampling frequency of 100 Hz with a 10-camera system (Vicon Motion Systems, Oxford, UK) using the human body model marker set.[12,13] Following six minutes of habituation to determine comfortable walking speed,[14] a one-minute baseline condition (BASE) of the children's typical gait pattern was measured.

Subsequently, a 59mm linear US probe (Telemed SmartUS, Lithuania) was attached to the non-preferred (TD) or most-affected (CP) lower leg using a custom probe holder. The probe holder was designed to minimally compress the muscle and optimally align the probe to the fascicles[8], allowing movements in 5 degrees-of-freedom, and was equipped with four clustered infrared-reflective markers to enable 3D motion tracking (Probefix Dynamic, USONO, The Netherlands; Fig. 3.1). The complete probe-holder combination including plastic probe holder (219g) and probe (111g) weighs a total of 330g. Two one-minute trials were collected in random order; one with mid- muscle probe position to image fascicles (FAS) and one on the MG muscle-tendon junction (MTJ). Children were instructed to walk normally. After quality control, eight representative strides per condition were randomly selected for further processing.

Table 3.1.
Participant characteristics.

	CP (n=18)	TD (n=16)
Age (years)	11.1 ± 3.3	10.6 ± 4.2
Weight (kg)	39.0 ± 14.6	42.9 ± 17.5
Height (m)	1.45 ± 0.18	1.51 ± 0.21
Level of involvement (uni/bi)	8/10	NA
GMFCS (I/II)	9/9	NA
SPAT score GM (0-3)	1:4, 2:1, 3:3, CL:10	NA
Comfortable walking speed (m/s)	0.72 ± 0.14	1.01 ± 0.13

Abbreviations: TD, typically developing children. CP, children with spastic cerebral palsy; GMFCS, Gross Motor Function Classification System; Uni, unilateral; Bi, bilateral; SPAT, clinical Spasticity Test[15]; GM, Gastrocnemius medialis; CL, Clonus; no score for the SPAT can be assigned; NA, not applicable.

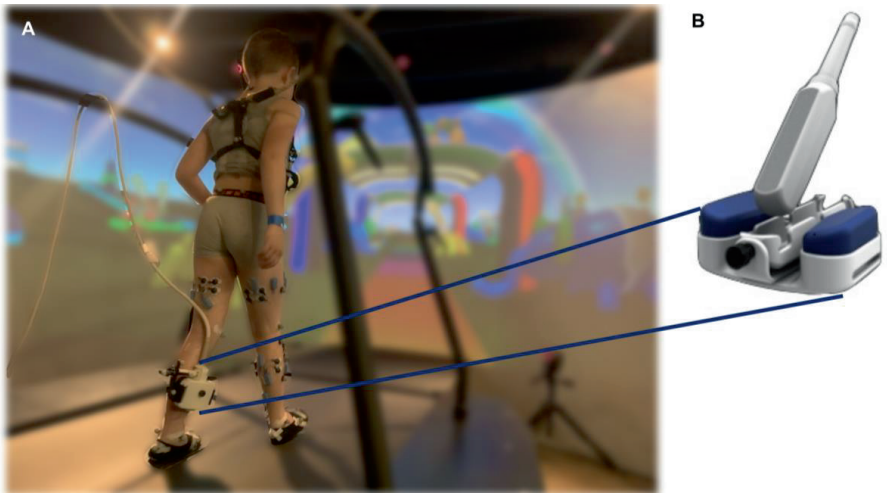


Figure 3.1. (A) Ultrasound probe placement on the gastrocnemius muscle-tendon junction. (B) Probefix Dynamic, USONO, The Netherlands.

3D marker data were processed with Vicon Nexus (v2.3, Oxford, UK). The spatiotemporal parameters step length, step width and stride time were computed using the Gait Off-line Analysis Tool (GOAT v4.2, Motek Medical, The Netherlands). Since MG length is mostly affected by flexion-extension, only sagittal plane hip, knee and ankle kinematics, and ankle kinetics over time-normalized gait cycles were computed using GOAT. MG MTU length was calculated with musculoskeletal modeling software (OpenSim 4.2[16]). First, a generic gait model (GAIT2392) was scaled to the participant using marker data of a standing calibration trial. Second, the inverse kinematic tool was used to track the marker data of the walking trials with the scaled model.[16] MG MTU length was extracted with the muscle analysis tool.

A two-way repeated measures ANOVA was conducted to study the effect of group (CP/TD) and condition (BASE/MTJ/FAS) on the spatiotemporal parameters. For kinematics, kinetics, and MTU lengths, a two-way repeated measures ANOVA using statistical non-parametric mapping (SnPM) with 1000 permutations[17] was applied.[18] As this test requires equal group sizes, we conducted it ten times,

each time comparing all 16 TD children to 16 randomly selected participants with CP. An effect was considered significant if >50% of the tests were significant. Post-hoc paired t-tests with Bonferroni correction (SnPM with 10000 permutations for kinematics/kinetics) were applied. This was done for TD and CP separately in case of an interaction effect, and otherwise, for the BASE/MTJ/FAS main effect over both groups combined. The average root mean square difference (RMSd) was calculated as effect size for all significantly different phases in the gait cycle.

3.3 Results

Kinetics of four children with CP were excluded from analysis due to poor data quality. All other data could be included. Step width showed an interaction effect (p<0.05) and was overall increased by wearing the probe (CP: p=0.001; TD: p=0.015). Children with CP walked with significantly wider steps during the FAS condition (18.9±4.49cm) compared to BASE (16.89±4.36cm, p=0.004) and MTJ (17.5±4.53cm, p=0.006). TD children took significantly wider steps during the MTJ condition (16.9±3.17cm) compared to BASE (15.4±3.17cm, p=0.007). Step length (p=0.135) and stride time (p=0.155) were not affected by the probe.

Hip, knee, ankle kinematics, and ankle power were affected by wearing the probe (p<0.05; Fig. 3.2; Fig. 3.3; Fig. 3.4). Hip flexion showed a significant interaction effect (p<0.05) with reduced hip extension during terminal stance during MTJ compared to FAS in TD (RMSd 2.7°, p=0.002), but not CP. Both groups showed less knee flexion in swing with both probe placements compared to BASE (FAS/BASE 4.9°, p<0.001; MTJ/BASE 4.0°, p<0.001). Especially in the FAS condition, a large portion of the swing phase showed less knee flexion (60-93% gait cycle). Furthermore, knee flexion in a small part of swing was lower during FAS compared to the MTJ condition (4.1°, p=0.003; 75-89% gait cycle). Wearing the probe also significantly reduced ankle plantarflexion around toe-off (FAS/BASE 3.0°, p<0.001; FAS/MTJ 3.3° and 2.4° for the two significant regions, p<0.001). Additionally, slightly more power absorption was found with the probe in loading response (FAS/BASE 0.12W/kg, p=0.007; MTJ/BASE 0.12W/kg, p=0.006).

The alterations in the gait pattern coincided with less shortening of the MG MTU in swing when the probe was worn for both TD and CP (FAS/BASE 3.6mm, p<0.001; MTJ/BASE 3.7mm, p<0.001, Fig. 3.2). Additionally, MG MTU length was slightly less shortened (1.7mm, p=0.007) in loading response during the MTJ condition compared to BASE.

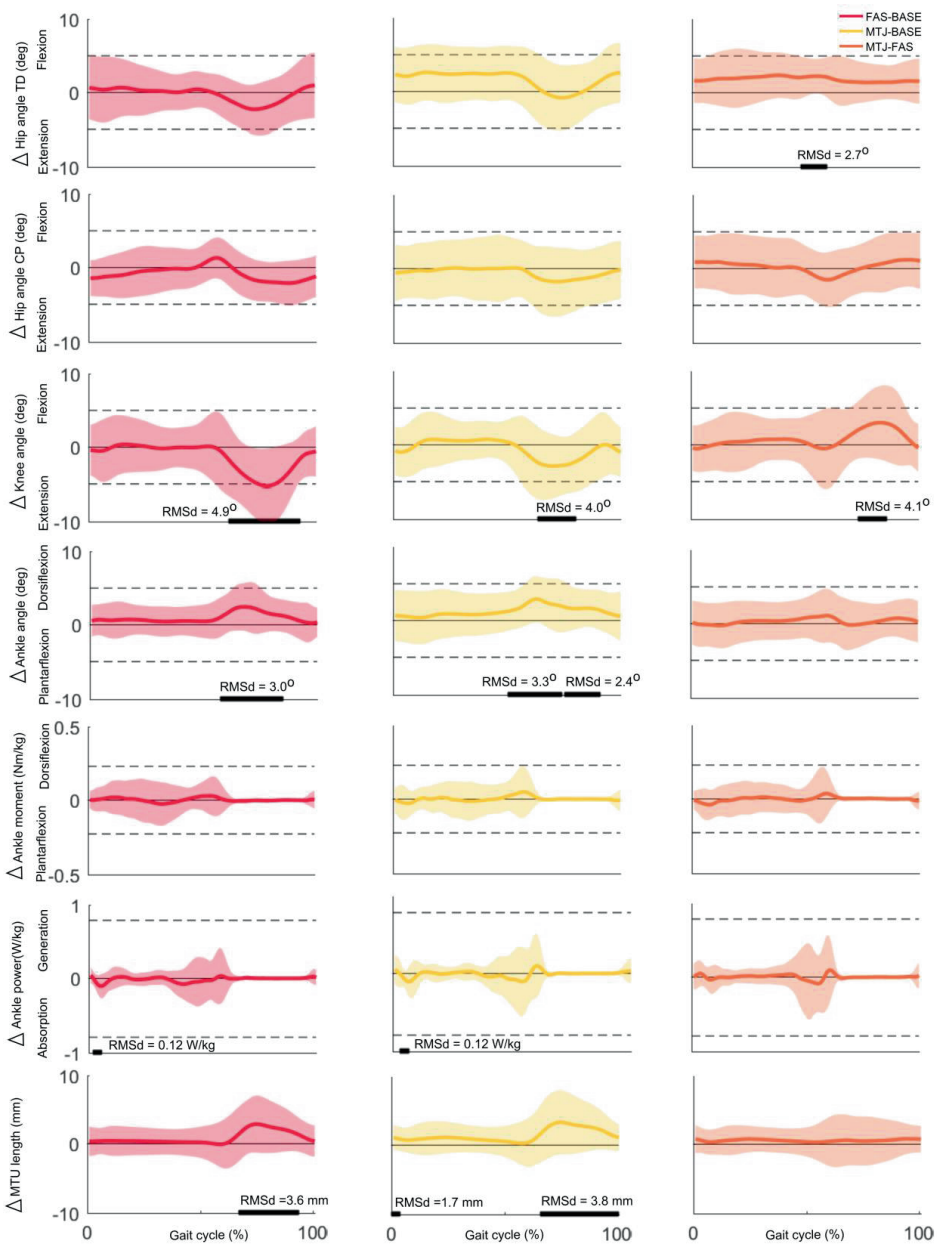


Figure 3.2. Average difference with standard deviation between gait conditions, with post-hoc SnPM results. Significant ($p<0.05$) parts of the curves are indicated with black bars with the corresponding average RMSd values. The dashed lines represent the acceptable error of 5° for kinematics,[19] and the standard error of measurement for kinetics.[20] The differences are presented for TD and CP separately in case of significant interactions, and otherwise, combined for TD and CP. Abbreviations; TD, typically developing children; CP, children with spastic cerebral palsy; RMSd, Root Mean Square Difference; MTU, muscle-tendon unit; FAS, gait condition of fascicle tracking; BASE, baseline gait condition; MTJ, gait condition of muscle-tendon junction tracking.

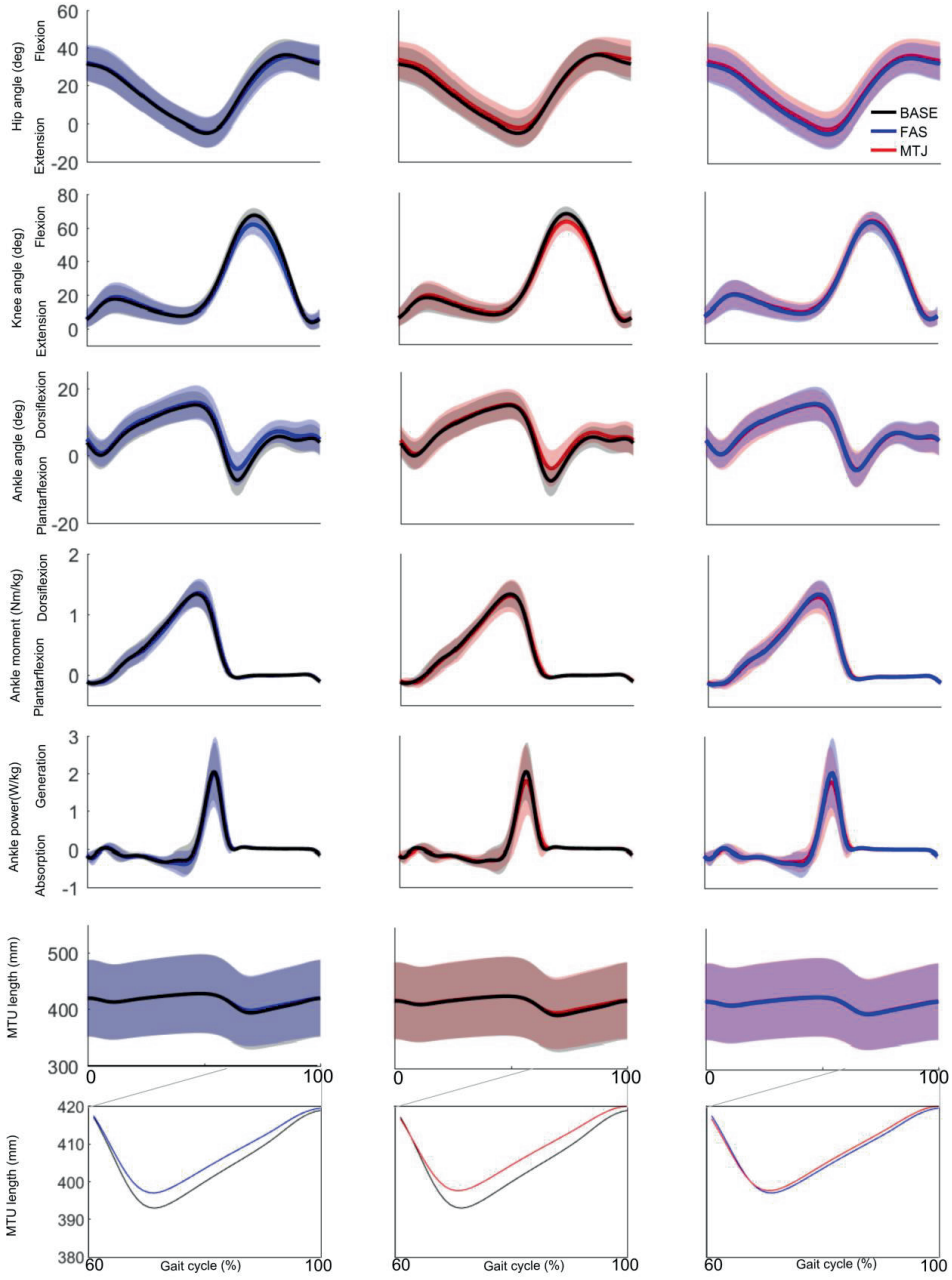


Figure 3.3. Average kinematic, kinetic and MTU length curves with standard deviations for TD children. The last row shows zoomed in figures of MTU length without standard deviations. Abbreviations; TD, typically developing children; MTU, muscle-tendon unit; FAS, gait condition of fascicle tracking; BASE, baseline gait condition; MTJ, gait condition of muscle-tendon junction tracking.

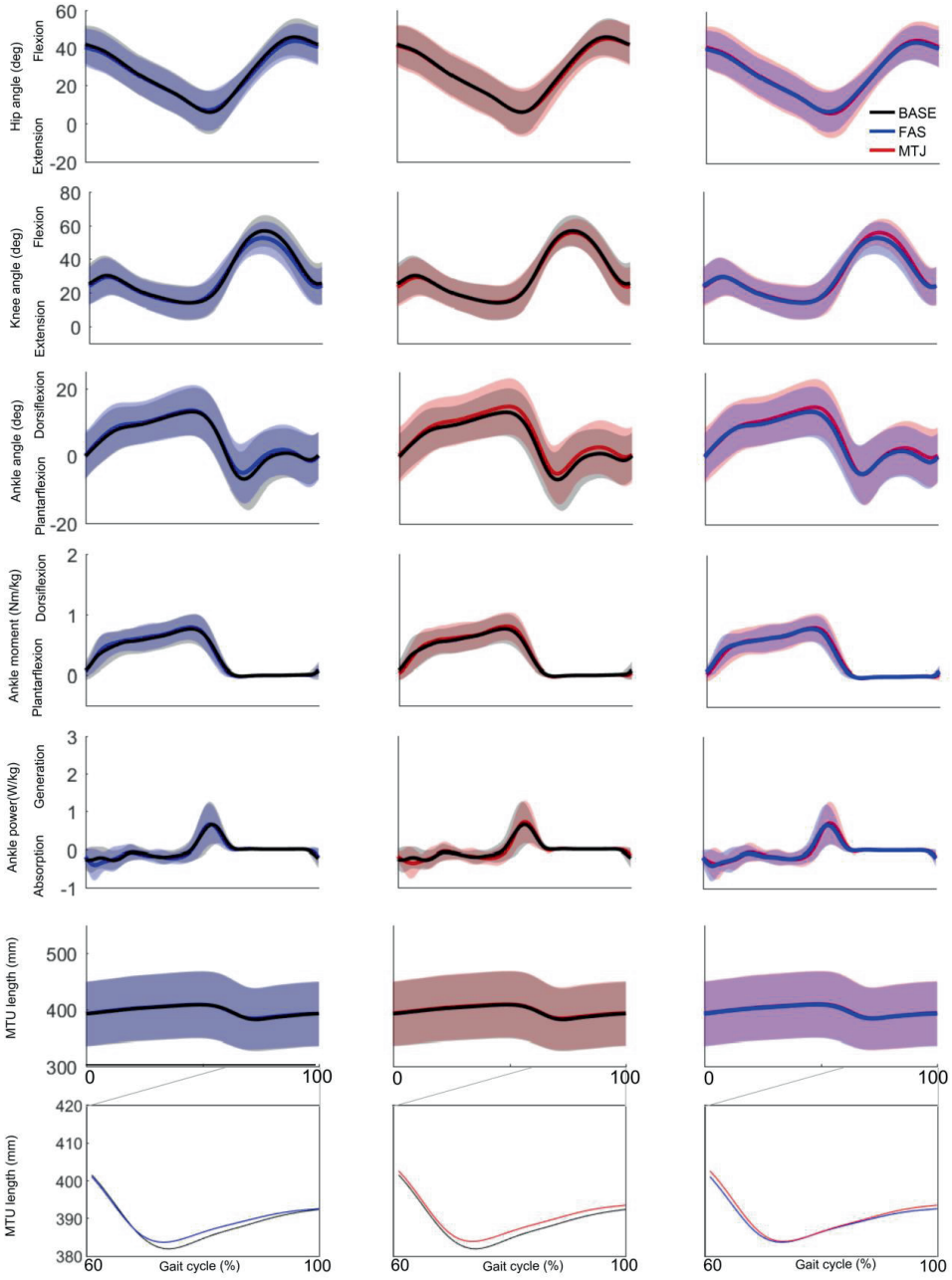


Figure 3.4. Average kinematic, kinetic and MTU length curves with standard deviations for children with CP. The last row shows zoomed in figures of MTU length without standard deviations. Abbreviations; CP, children with spastic cerebral palsy; MTU, muscle-tendon unit; FAS, gait condition of fascicle tracking; BASE, baseline gait condition; MTJ, gait condition of muscle-tendon junction tracking.

3.4 Discussion

As hypothesized, wearing an US probe with probe holder leads to slightly increased step width and sagittal plane lower-limb gait alterations. Specifically, reduced plantarflexion around toe-off, reduced knee flexion in initial and mid-swing, and more ankle power absorption in loading response were observed when wearing an US probe. These kinematic alterations coincided with decreased shortening of MG MTU in swing. The effects of the probe on kinematics can be considered relatively small, since they are similar to the measurement error (<5°) inherent to clinical 3D gait analysis, and smaller than the clinically acceptable error (5°).[19]

Even though the gait alterations are small and, with the exception of minor differences in hip flexion and step width, similar between CP and TD, they occurred systematically and should therefore be considered when interpreting dynamic US imaging results. Reduced knee flexion in swing was probably caused by the US cable being placed close to the knee joint. This is particularly a problem in small children. The reduced ankle plantarflexion around toe-off and ankle power during loading response were possibly due to the added weight of the probe or due to more cautious gait to prevent the probe from slipping off the leg. The low placement over the MTJ made it difficult to avoid probe contact with the opposite leg, which may also explain the increased step width.

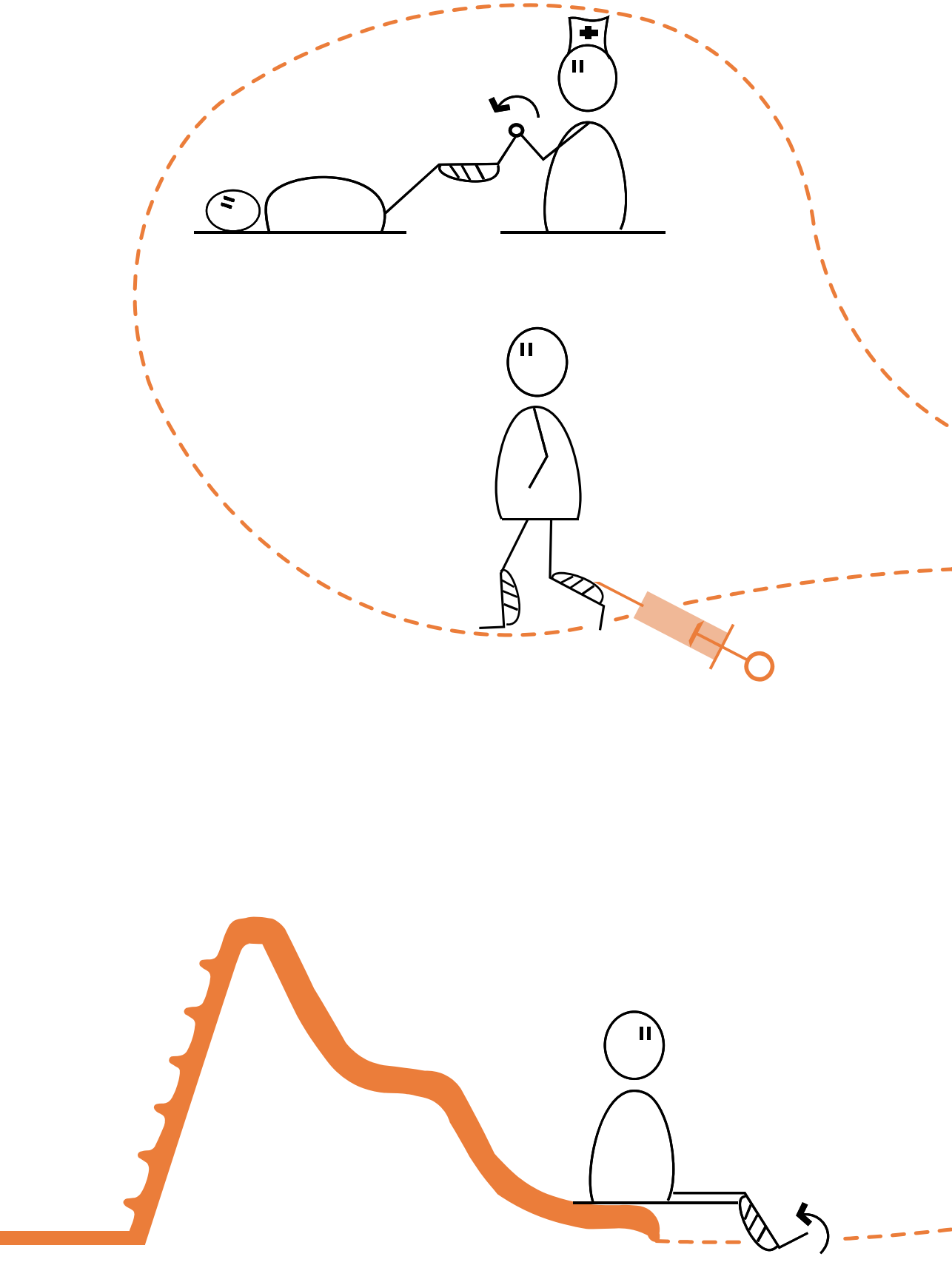
MG MTU length was slightly less shortened during swing when the probe was placed on the leg. Compared to age-matched TD children, children with spastic CP generally walk with increased plantarflexion and show reduced MG MTU length during swing.[21] Studies applying dynamic US imaging during gait also identified less muscle belly, tendon and fascicle lengthening during swing in CP compared to TD.[4–7] Our findings on the MTU indicate that shortening could have been underestimated in these dynamic US studies. However, as we did not find an interaction effect, the underestimation is likely to have occurred equally in both groups thus minimally affecting the conclusions drawn.

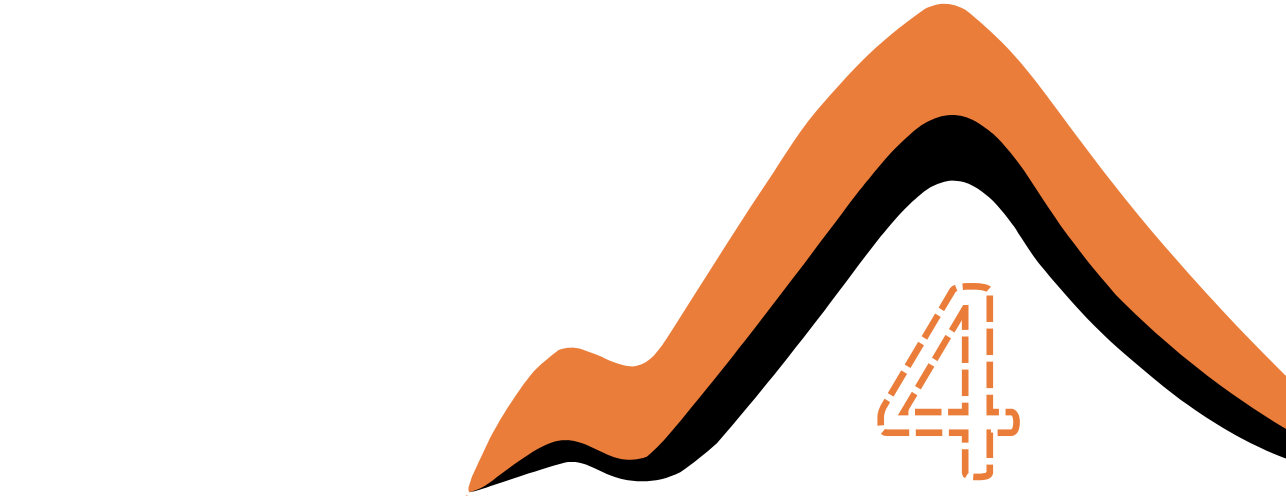
There are some limitations to this study. Our sample size is small and therefore it could be that we lacked power to find significant interaction effects. Additionally, our results pertain to the probe plus probe holder used in this study. Other dynamic US studies[1,4–7,22] used other types of probe holders with different weights and sizes, which may give different results. Furthermore, this study is performed during treadmill walking, which can result in small changes in kinematics[11] and larger changes in kinetics compared to overground walking.[23] Therefore, treadmill walking itself might also affect muscle dynamics to some extent.

Although effects of the probe are minor, alterations to the measurement equipment could further reduce the effects. For example, a lighter probe or probe holder can be used. Moreover, a more flexible cable may overcome the impediments during swing. In conclusion, minimal and similar gait alterations and MTU length changes due to the probe were found in TD children and children with CP.

References

1. Cronin NJ, Lichtwark G. The use of ultrasound to study muscle-tendon function in human posture and locomotion. Gait Posture. 2013; 37: 305–12.
2. Gillett JG, Barrett RS, Lichtwark G a. Reliability and accuracy of an automated tracking algorithm to measure controlled passive and active muscle fascicle length changes from ultrasound. *Comput Methods Biomech Biomed Engin* 2013; 16: 678–87.
3. Lichtwark GA, Wilson AM. Interactions between the human gastrocnemius muscle and the Achilles tendon during incline, level and decline locomotion. *J Exp Biol* 2006; 209: 4379–88.
4. Kalsi G, Fry NR, Shortland AP. Gastrocnemius muscle–tendon interaction during walking in typically-developing adults and children, and in children with spastic cerebral palsy. *J Biomech* 2016; 49: 3194–9.
5. Barber L, Carty C, Modenese L, Walsh J, Boyd R, Lichtwark G. Medial gastrocnemius and soleus muscle-tendon unit, fascicle, and tendon interaction during walking in children with cerebral palsy. *Dev Med Child Neurol* 2017; 59: 843–51.
6. Hösl M, Böhm H, Arampatzis A, Keymer A, Döderlein L. Ultrasound imaging of the medial gastrocnemius in flexed knee gait of children with cerebral palsy. *Gait Posture* 2016; S49: 50.
7. Bar-On L, Flux E, van der Krogt MM, et al. Medial gastrocnemius muscle and tendon interaction during gait in typically developing children and children with cerebral palsy. In: Virtual meeting of the European Society of Movement Analysis in adults and Children 2020. 2020.
8. van Hooren B, Teratsias P, Hodson-Tole EF. Ultrasound imaging to assess skeletal muscle architecture during movements: A systematic review of methods, reliability, and challenges. *J Appl Physiol* 2020; 128: 978–99.
9. Leitner C, Hager PA, Penasso H, et al. Ultrasound as a Tool to Study Muscle–Tendon Functions during Locomotion: A Systematic Review of Applications. *Sensors 2019, Vol 19, Page 4316* 2019; 19: 4316.
10. Dussault-Picard C, Mohammadyari SG, Arvisais D, Robert MT, Dixon PC. Gait adaptations of individuals with cerebral palsy on irregular surfaces: A Scoping Review. *Gait Posture* 2022.
11. van der Krogt MM, Sloot LH, Harlaar J. Overground versus self-paced treadmill walking in a virtual environment in children with cerebral palsy. *Gait Posture* 2014; 40: 587–93.
12. Flux E, van der Krogt MM, Cappa P, Petrarca M, Desloovere K, Harlaar J. The Human Body Model versus conventional gait models for kinematic gait analysis in children with cerebral palsy. *Hum Mov Sci* 2020; 70: 102585.
13. Van Den Bogert AJ, Geijtenbeek T, Even-Zohar O, Steenbrink F, Hardin EC. A real-time system for biomechanical analysis of human movement and muscle function. *Med Biol Eng Comput* 2013; 51: 1069–77.
14. Matsas A, Taylor N, Mcburney H. Knee joint kinematics from familiarised treadmill walking can be generalised to overground walking in young unimpaired subjects. *Gait Posture* 2000; 11: 46–53.
15. Scholtes VAB, Dallmeijer AJ, Becher JG. The Spasticity Test: a clinical instrument to measure spasticity in children with cerebral palsy. In: The Effectiveness of Multilevel Botulinum Toxin Type A and Comprehensive Rehabilitation in Children with Cerebral Palsy. Citeseer, 2007: 29–64.
16. Delp SL, Anderson FC, Arnold AS, et al. OpenSim: open-source software to create and analyze dynamic simulations of movement. *IEEE Trans Biomed Eng* 2007; 54: 1940–50.
17. Marozzi M. Some remarks about the number of permutations one should consider to perform a permutation test. *Statistica* 2004; 64: 193–201.
18. Pataky TC, Vanrenterghem J, Robinson MA. Zero-vs. one-dimensional, parametric vs. non-parametric, and confidence interval vs. hypothesis testing procedures in one-dimensional biomechanical trajectory analysis. *J Biomech* 2015; 48: 1277–85.
19. McGinley JL, Baker R, Wolfe R, Morris ME. The reliability of three-dimensional kinematic gait measurements: A systematic review. *Gait Posture* 2009; 29: 360–9.
20. Meldrum D, Shouldice C, Conroy R, Jones K, Forward M. Test–retest reliability of three dimensional gait analysis: Including a novel approach to visualising agreement of gait cycle waveforms with Bland and Altman plots. *Gait Posture* 2014; 39: 265–71.
21. Van der Krogt MM, Doorenbosch CAM, Becher JG, Harlaar J. Walking speed modifies spasticity effects in gastrocnemius and soleus in cerebral palsy gait. *Clin Biomech* 2009; 24: 422–8.
22. Ishikawa M, Komi P V. The role of the stretch reflex in the gastrocnemius muscle during human locomotion at various speeds. *J Appl Physiol* 2007; 103: 1030–6.
23. Van der Krogt MM, Sloot LH, Buizer AI, Harlaar J. Kinetic comparison of walking on a treadmill versus over ground in children with cerebral palsy. *J Biomech* 2015; 48: 3577–83.





EXAMINING THE ROLE OF INTRINSIC AND REFLEXIVE CONTRIBUTIONS TO ANKLE JOINT HYPER-RESISTANCE TREATED WITH BOTULINUM TOXIN-A

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Abstract

Background. Spasticity, i.e. stretch hyperreflexia, increases joint resistance similar to symptoms like hypertonia and contractures. Botulinum neurotoxin-A (BoNT-A) injections are a widely used intervention to reduce spasticity. BoNT-A effects on spasticity are poorly understood, because clinical measures, e.g. modified Ashworth scale (MAS), cannot differentiate between the symptoms affecting joint resistance. This paper distinguishes the contributions of the reflexive and intrinsic pathways to ankle joint hyper-resistance for participants treated with BoNT-A injections. We hypothesized that the overall joint resistance and reflexive contribution decrease 6 weeks after injection, while returning close to baseline after 12 weeks.

Methods. Nine participants with spasticity after spinal cord injury or after stroke were evaluated across three sessions: 0, 6 and 12 weeks after BoNT-A injection in the calf muscles. Evaluation included clinical measures (MAS, Tardieu Scale) and motorized instrumented assessment using the instrumented spasticity test (SPAT) and parallel-cascade (PC) system identification. Assessments included measures for: (1) overall resistance from MAS and fast velocity SPAT; (2) reflexive resistance contribution from Tardieu Scale, difference between fast and slow velocity SPAT and PC reflexive gain; and (3) intrinsic resistance contribution from slow velocity SPAT and PC intrinsic stiffness/damping.

Results. Individually, the hypothesized BoNT-A effect, the combination of a reduced resistance (week 6) and return towards baseline (week 12), was observed in the MAS (5 participants), fast velocity SPAT (2 participants), Tardieu Scale (2 participants), SPAT (1 participant) and reflexive gain (4 participants). On group-level, the hypothesis was only confirmed for the MAS, which showed a significant resistance reduction at week 6. All instrumented measures were strongly correlated when quantifying the same resistance contribution.

Conclusions. At group-level, the expected joint resistance reduction due to BoNT-A injections was only observed in the MAS (overall resistance). This observed reduction could not be attributed to an unambiguous group-level reduction of the reflexive resistance contribution, as no instrumented measure confirmed the hypothesis. Validity of the instrumented measures was supported through a strong association between different assessment methods. Therefore, further quantification of the individual contributions to joint resistance changes using instrumented measures across a large sample size are essential to understand the heterogeneous response to BoNT-A injections.

4.1 Introduction

Botulinum neurotoxin-A (BoNT-A) injections are currently the most frequently used clinical intervention for focal spasticity.[1-3] Spasticity is a common symptom after various brain and neural injuries, such as spinal cord injury (SCI) or stroke, referring to an exaggerated stretch reflex, i.e. stretch hyperreflexia.[4,5] Spasticity is perceived as an increased joint resistance to movement, i.e. joint hyper-resistance. BoNT-A injections are used clinically to reduce muscle activity and hence spasticity.[1] BoNT-A injections reduce muscle activity by inhibiting the release of acetylcholine at the neuromuscular junction, which chemically denervates the exposed muscle fibers. BoNT-A effects reduce after 2 to 4 months due to nerve sprouting and muscle re-innervation.[1]

Clinical evaluation of BoNT-A injections has shown a significant reduction in joint resistance after 2–8 weeks using the modified Ashworth scale (MAS).[6-8] With the MAS, currently a common clinical test, clinicians evaluate overall joint resistance, which can physiologically include tissue characteristics, and tonic and reflexive muscle activity.[5,9-11] For the MAS, a single passive movement profile is repeatedly applied, whereas movements with varying characteristics, e.g. slow and fast velocities, are required to unravel joint resistance contributions. Therefore, the MAS can clinically only evaluate spasticity indirectly and cannot distinguish between spasticity and other symptoms as involuntary background activity, shortened soft tissue, contractures and muscle fibrosis.[4,12,13] Furthermore, the MAS has a questionable reliability, especially when applied at the lower limb.[11,14] Hence, the clinical effect of BoNT-A injections on spasticity is poorly understood, while BoNT-A injections are a frequently used clinical intervention for spasticity.

Quantification of the intrinsic and reflexive contributions to joint hyper-resistance is essential to understand the beneficial and adverse effects of BoNT-A injections and support clinical decision making. BoNT-A injections can, for example, have side-effects and should ideally only be administered to patients who suffer from increased reflexive contributions to joint hyper-resistance.[15] Objective information on both intrinsic and reflexive joint resistance can support clinical decision making and help evaluate treatment effects.[5] The intrinsic resistance represents the combination of tissue-related non-neural and tonic neural contributions to joint resistance.[10] The reflexive resistance, representing the phasic neural contributions, can be used as measure for spasticity. Model-based processing of neuromechanical responses can be used to unravel and quantify the intrinsic and reflexive contributions.[10,16-20] Furthermore, instrumentation and motorization using robotic devices can improve precision, consistency and objectivity of the applied movements and measurements.[21-23]

Model-based evaluation of BoNT-A effects on joint hyper-resistance contributions have been applied using neuromechanical models.[24-27] These studies showed conflicting results on BoNT-A effects with either no change or a significant reduction of the reflexive resistance observed after injection. The neuromechanical modelling approaches used limited experimental datasets measured over the full passive range of motion (pROM), similar to current clinical measures. The subsequent joint resistance estimation primarily relies on a priori knowledge and simplifying assumptions. As a result, these methodologies are sensitive to incomplete model definitions and imperfect a priori knowledge.[17,18,20] Furthermore, the lack of a gold standard complicates interpretation of the reported conflicting results.[5,28,29] Besides the selected model, differences in reported BoNT-A effects may also be influenced by participant heterogeneity, the experimental setup, and the assessed joint. Given the conflicting

results and lack of a gold standard, investigating fundamentally different approaches to assess joint hyper-resistance is of interest to improve understanding of BoNT-A effects.

An alternative approach to assess BoNT-A effects on joint hyper-resistance contributions is data-driven modelling. Data-driven modelling evaluation of BoNT-A effects on joint hyper-resistance contributions could be executed using system identification.[10,16,30,31] For example, the parallel-cascade (PC) system identification technique has shown the ability to discriminate spastic participants from controls and paretic from non-paretic joints.[30,32] The PC technique has also shown good group-level responsiveness during the evaluation of several clinical treatments, like functional electrical stimulation-assisted walking, Tizanidine and robot-assisted gait training.[33-35] Currently, no system identification results have been reported on BoNT-A effects. Contrary to neuromechanical modelling, the system identification techniques previously tested in a clinical setting used rich experimental datasets measured over only a limited portion of the pROM.[30-35] As intrinsic and reflexive joint resistance depend on joint angle, the obtained joint resistance estimates do not characterize the full pROM.[36]

The goal of this paper was to distinguish the contribution of intrinsic and reflexive ankle joint resistance for participants treated with BoNT-A injections to reduce spasticity. We hypothesized that reflexive joint resistance decreases 6 weeks after injection, while returning close to baseline after 12 weeks.[24,25] Due to the reduced reflexive joint resistance, we also expected the overall joint resistance to decrease 6 weeks after injection, while returning close to baseline after 12 weeks.[6-8] In absence of a gold standard, the joint resistance contributions were assessed using multiple joint resistance measures with different characteristics and limitations. Joint resistance contributions were estimated using clinical measures (MAS/Tardieu Scale),[9,37] an instrumented spasticity test (SPAT)[22,23] and a parallel-cascade (PC) system identification technique.[10,30] To support validity of the measures used, the linear association between the various outcome measures was investigated.

4.2 Methods

Participant and study schedule

Six people with SCI and three stroke survivors participated in the study: age 54.4 ± 11.1 year, 2 women, see Table 4.1. Patients treated at the Sint Maartenskliniek, Nijmegen were assessed for eligibility by their rehabilitation physician. Inclusion criteria were: (1) adult, older than 18 year; (2) stable neurological condition in chronic phase, minimum 6 months post-lesion/-stroke; (3) a MAS or Tardieu score ≥ 1 for any of the m. triceps surae; (4) treatment of any of the m. triceps surae with BoNT-A injections aimed at spasticity reduction; and (5) pROM of the affected ankle joint in the sagittal plane ≥ 20°. Participants were excluded if BoNT-A injections were combined with other treatments aimed at reducing spasticity. Note, included participants did typically receive the BoNT-A injections in combination with home stretching exercises in line with usual care. Participants gave written informed consent before definitive inclusion.

Figure 4.1. Experimental Setup. Participants were seated on an adjustable chair for the instrumented evaluations. The manipulator connected to the adjustable chair applied dorsiflexion, ramp-and-hold perturbations around the ankle joint, while measuring the biomechanical response. If the left foot was measured, the right leg was supported with a right lower leg support inserted into the chair frame (not shown).

Table 4.1.

Participant demographic, clinical and BoNT-A injection characteristics (N = 9)

Age	Gender	Diagnosis	Meas. side	Months post stroke/SCI	AIS (SCI)	BoNT-A injection	BoNT-A brand	BoNT-A dose per muscle (units)
54	M	Stroke (Ischaemic)	R	12		4th	Dysport	GM (300) GL (300)
58	M	Stroke (Ischaemic)	L	69		5th	Allergan	SOL (50) GM (50) GL (50)
49	M	Stroke (Hemorrhagic)	L	64		1st	Dysport	SOL (400) GM (200) GL (200)
67	M	SCI (C5–C7)	L	30	D	8th	Dysport	SOL (300)
62	F	SCI (T7–T12)	L	54	B	13th	Dysport	SOL (400) GM (200) GL (200) TP (200)
29	M	SCI (T7–T12)	R	25	A	4th	Dysport	SOL (200) GM (200) GL (100)
51	M	SCI (T7–T12)	R	183	C	3rd	Dysport	SOL (300) GM (200) GL (200)
59	M	SCI (L1)	L	144	C	7th	Dysport	SOL (150) GM (160) GL (160)
61	F	Cauda equina syndrome (L4–L5)	R	17		1st	Dysport	SOL (300) GM (200) GL (200) TP (300)

The (most) affected side with a pROM ≥ 20° was selected as measured side during experiments. Abbreviations: AIS, American Spinal Injury Association (ASIA) Impairment Scale; BoNT-A, Botulinum Neurotoxin type-A; GM, Gastrocnemius Medialis; GL, Gastrocnemius Lateralis; SCI, Spinal Cord Injury; SOL, Soleus; TP, Tibialis Posterior

In this exploratory longitudinal study, ankle joint resistance was evaluated across three sessions: a baseline (week 0) measurement on the same day as BoNT-A injection and two post-intervention measurements at 6 and 12 weeks after BoNT-A injection. The week 12 evaluation was usually measured on the same day as a new BoNT-A injection, as BoNT=A injections were repeated every three months. In each session the clinical evaluation was executed by the same trained physiotherapist (WO, non-blinded), whereas the instrumented evaluation was executed by a researcher (RV, EF or EA) using a robotic manipulator, see Fig. 4.1.

Instrumented experimental setup

The instrumented evaluations (SPAT and PC technique) were performed with participants seated on an adjustable chair, see Fig. 4.1. The (most) affected side in compliance with the inclusion/exclusion criteria was measured. The measured foot was placed on a rigid footplate and secured using Velcro straps. The rigid footplate was part of the robotic manipulator fixed onto the frame of the adjustable chair. The chair supported the participant's back and upper leg to achieve a fixed posture with 70° hip and 30° knee flexion. The hip and knee angles were selected to be attainable by all participants and to avoid muscle slack in order to allow for proper elicitation of the stretch reflex even with the small amplitude (2°) perturbations used for the PC technique.[30,36] For each participant, the chair was adjusted to these hip and knee angles in the first session. For subsequent sessions, the chair was re-adjusted to the position of the first session to ensure constant posture across sessions. As such, the upper leg was firmly supported across all sessions to minimize movement of the leg that could introduce bias and variability in the instrumented measures. The ankle and manipulator axes of rotation were visually aligned by minimizing knee translation in the sagittal plane while rotating the footplate.

The robotic manipulator used a one degree-of-freedom actuator (MOOG, Nieuw-Vennep, the Netherlands) to apply the desired joint perturbations in the sagittal plane. Ankle angle and angular velocity were measured using an encoder situated at the actuator axis. Ankle torque was measured using a torque sensor placed between the actuator and footplate. The ankle angle, velocity and torque were recorded at 2048 Hz with the dorsiflexion direction defined as positive. For ankle angle, the neutral (0°) angle was determined using a goniometer at 0° dorsiflexion/plantarflexion. For safety, manipulator movement was restricted to the maximal ankle pROM, which was re-evaluated every session, using adjustable hardware endstops. Measurements over full pROM (SPAT) were executed with a 2° margin at both endstops. Measurements over a limited pROM (PC technique) started 10° below the dorsiflexion endstop to avoid slack of the calf muscles. As pROM was re-evaluated every session, anatomical angles for both instrumented measurements could vary across sessions. At the start of each measurement, mean torque was measured over a 1 s period to determine the neutral (0 Nm) torque for that measurement.

Experimental protocol

The same protocol was executed in all three sessions. A clinical evaluation was executed with participants lying supine on an examination table to obtain scores for the MAS (overall joint resistance)[9] and Tardieu Scale (reflexive joint resistance).[37] During clinical evaluation, the knee was supported by a cushion to achieve 30° knee flexion, similar to the instrumented setup. For the MAS, the ankle joint was rotated three times over the full pROM in 1 s.[9] The MAS was scored on an ordinal six-point scale from 0, no increase in muscle tone, to 4, affected part(s) rigid in flexion or extension. For the Tardieu Scale, the ankle joint was rotated over the full pROM at three different velocities: V1, as slow as possible; V2, velocity approximately equal to limb falling under gravity; and V3, as fast as possible.[37] The quality (TS_Q) of the joint response was scored for all velocities on an ordinal five-point scale from 0, no resistance throughout the movement, to 4, infatigable clonus at a precise angle.[37]

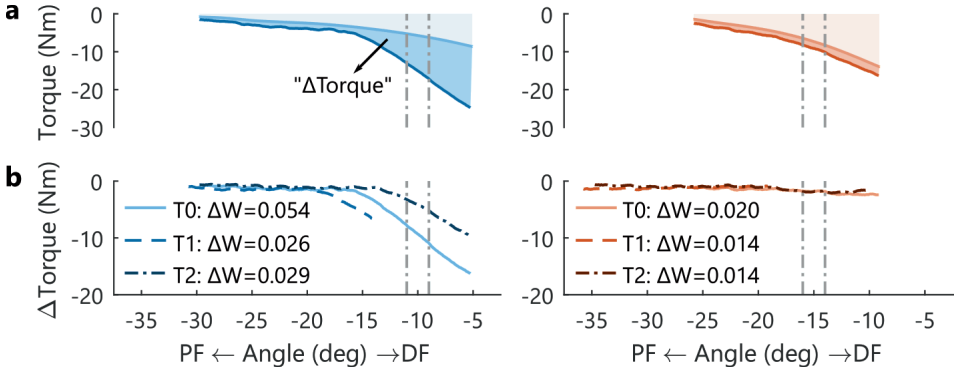


Figure 4.2. Instrumented SPAT assessment for two representative participants with a clear (left) and little (right) reflexive response. A) Ensemble averaged (3 repetitions) torque-angle curves for the instrumented SPAT at both slow (light solid line) and fast (dark solid line) velocity at week 0 (T_0). The work delivered by the ankle joint is highlighted for the slow velocity trial (light-shaded area) and the difference (ΔTorque) between the fast and slow velocity trials (dark-shaded area). The instrumented SPAT was analyzed from 10 to 90% pROM with the limited pROM used for the PC technique demarcated (dash-dotted verticals). B) Ensemble averaged difference in torque (ΔTorque) between the fast and slow velocity SPAT at each session: week 0 (T_0), 6 (T_1) and 12 (T_2) after BoNT-A injection. Torque differences were computed by interpolating the slow velocity torque data onto the exact angles measured in the fast velocity dataset, visualized by the dark-shaded area in A. Manipulator movement was restricted to the maximal ankle pROM each session for safety, reflected by the varying pROM depicted across sessions.

The instrumented SPAT evaluation consisted of two measurements at different velocities emulating V1 and V3 of the Tardieu Scale, see Fig. 4.2A.[22,23] First, three slow (10°/s) dorsiflexion perturbations over the full pROM were applied. Second, three fast (150°/s) dorsiflexion perturbations over the full pROM were applied. At both velocities, repetitions were separated by 20s of rest. The maximum dorsiflexion angle was held for 1s before returning towards plantarflexion with an opposite profile to the dorsiflexion perturbation. Participants were instructed to relax and not respond to the perturbations. The PC technique evaluation consisted of two measurement blocks (2min) with 1min rest in between. In each block, a series of small (2° amplitude) ramp-hold-return perturbations were continuously applied, see Fig. 4.3A.[38] These ramp-and-hold perturbations had a 125°/s max. velocity, 15800°/s2 max. acceleration and 40ms duration. Perturbations randomly switched between 'steps', i.e. the maximum dorsiflexion angle was held for 580 ms, and 'pulses', i.e. no hold period at the maximum dorsiflexion angle.[39] The manipulator returned towards plantarflexion with an opposite profile to the dorsiflexion perturbation. Participants were again instructed to relax and not respond to the perturbations.

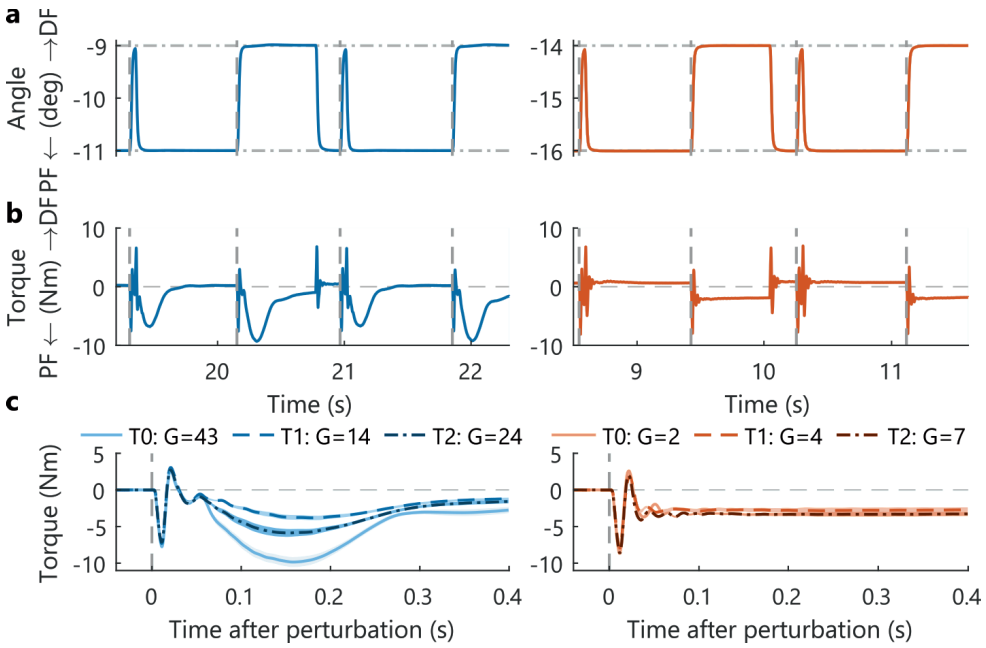


Figure 4.3. PC technique assessment for two representative participants with a clear (left) and little (right) reflexive response. A) Four consecutive dorsiflexion perturbations (onset at dashed verticals) used for the PC technique at week 0 (T_0). Perturbation signals were randomly generated, hence the different time-axes used to visualize a similar sequence of pulse and step perturbations. B) The subsequent ankle joint response, measured as torque, elicited through each dorsiflexion perturbation. C) Ensemble averaged (± SD, single measurement block) torque response at each session. The torque ensemble averages were created by aligning all step perturbations at the perturbation onset (dashed verticals). The reflexive gain G (Nm s/rad) shows the quantified reflexive contribution at each session. To enhance visualization, torque ensembles were normalized to zero torque at perturbation onset.

Data analysis

All data was analyzed using Matlab 2017b (Mathworks, Natick, MA, USA). For the instrumented SPAT, the work, i.e. product of force and displacement, around the ankle was used to quantify joint resistance.[22,23] Work was computed as area under the torque-angle curve, ranging from 10% to 90% pROM. The torque-angle curve was corrected for gravitational effects of the footplate and foot. Work was computed as measure of: (1) intrinsic joint resistance from the slow velocity trials W_{slow}; (2) overall joint resistance from the fast velocity trials W_{fast}; and (3) reflexive joint resistance from the difference between the fast and slow trials ΔW. All values of work were normalized for body weight (kg) and pROM. Due to a calibration issue, instrumented SPAT outcomes for the session at week 12 of one participant were removed.

For the PC technique, intrinsic and reflexive joint resistance parameters were estimated using a time-invariant algorithm modified from the original algorithm by Kearney et al.[10] The algorithm consisted of the following steps:

1. The measured angle, velocity and torque signals were anti-alias filtered (2nd-order, 65.8 Hz, critically damped) and downsampled to 146.3 Hz.

2. Measured acceleration was extracted from the state vector of the velocity low-pass filter and also downsampled to 146.3 Hz.

3. Non-parametric estimation of intrinsic, reflexive and voluntary torque contributions were obtained via an iterative procedure. Iterations continued until variance accounted for (%VAF) did not improve (<0.005%) or reached max. 10 iterations.

(a) Residual intrinsic torque was computed by subtracting reflexive and voluntary torque from the net torque. (1st iteration) Reflexive and voluntary torque were set to zero.

(b) A 35 ms intrinsic impulse response function (IRF) was estimated using a correlation-based method between angle and residual intrinsic torque. A pseudo-inverse approach based on minimum description length was used to retain only significant terms.[40]

(c) Residual reflexive torque was computed by subtracting voluntary and intrinsic torque, i.e. the convolved intrinsic IRF with angle, from the net torque.

(d) A 650 ms reflexive IRF was estimated between half-wave rectified velocity and residual reflexive torque using the same correlation-based method.

(e) Residual voluntary torque was computed by subtracting intrinsic and reflexive torque, i.e. the convolved reflexive IRF with half-wave rectified velocity, from net torque.

(f) Voluntary torque was estimated as the low-pass filtered (2nd-order, 0.5 Hz, Butterworth) residual voluntary torque.

4. The intrinsic inertia I (acceleration-component), damping B (velocity-component) and stiffness K (angle-component) were estimated using linear least squares between acceleration, velocity and angle, and intrinsic torque.

5. The reflexive IRF was fit between half-wave rectified velocity and reflexive torque with both signals lowpass filtered (2nd-order, 14.6 Hz, critically-damped).

6. The reflexive delay δ was estimated via a grid search (35–65 ms, 1 ms increments), coupled to a nonlinear least squares fit on the reflexive IRF of reflexive gain G, damping ζ and frequency ω.

Statistical analysis

The statistical analysis was performed using Matlab 2017b and R3.6.2 (R Foundation for Statistical Computing, Vienna, Austria). The outcome measures included two clinical measures (MAS, TS_Q), three instrumented SPAT measures (W_{fast}, ΔW, W_{slow}) and three PC technique measures (G, K, B). TSQ was evaluated based on the highest velocity (V3) assessment of the Tardieu Scale only, as this velocity was closest to the instrumented evaluations.

On an individual level, the hypothesized longitudinal BoNT-A effects were evaluated by comparing the measured resistance between baseline and week 6, as well as between week 6 and week 12. For each outcome measure, we considered the hypothesized BoNT-A effect observed, if a reduced resistance compared to baseline was measured at week 6 in combination with a return towards baseline at week 12. On group-level, the hypotheses on the longitudinal BoNT-A effects were evaluated using the Friedman non-parametric one-way repeated measures analysis for all outcome measures.[27] Post-hoc multiple comparison tests between sessions were executed for significant Friedman test results. For each multiple comparison, p-values were adjusted using the Bonferroni correction. Significance level was set at $\alpha = 0.05$.

To support reliability of the longitudinal BoNT-A evaluation, repeatability of the instrumented measures was assessed using the intraclass correlation coefficient (ICC).[41] ICCs were computed with a two-way mixed effects model, assessing absolute agreement between single repetitions. ICC robustness was investigated using the 95% confidence interval (CI) constructed via a non-parametric bootstrap procedure using the bias corrected and accelerated (BCa) method.[42] Minimal Detectable Difference (MDD) was calculated using the IC according to Weir.[43]

Validity of the outcome measures was assessed based on linear associations. We expected strong ($r > 0.7$) linear associations between outcome measures estimating the same contribution, i.e. between the overall measures (MAS, W_{fast}), the reflexive measures (TS_Q , ΔW, G) and the intrinsic measures (W_{slow}, K, B). Furthermore, we expected no or weak linear associations between outcome measures estimating different contributions. The non-parametric Spearman's rank correlation coefficient ρ was used for associations involving the ordinal clinical measures. Pearson's correlation coefficient r was used for associations involving only instrumented measures. Robustness of ρ and r were investigated using the 95% CI based on a BCa bootstrap procedure.

4.3 Results

We investigated BoNT-A effects on the intrinsic and reflexive contributions to ankle joint hyper-resistance in nine participants at three sessions: week 0 (T_0), 6 (T_1) and 12 (T_2) after BoNT-A injection. Joint resistance was assessed using common clinical measures, i.e. MAS, Tardieu Scale (TS_Q), an instrumented SPAT (W_{fast}, ΔW, W_{slow}) and PC system identification technique (G, K, B).

Qualitative analysis of instrumented measures

The reflexive response elicited during the instrumented evaluation strongly varied between participants. For example, some participants showed a clear reflexive response in both instrumented measures, whereas other participants showed a small or no reflexive response, see Figs. 4.2A, 4.3B. This heterogeneity in the reflexive response was observed both before and after BoNT-A injection, see Figs. 4.2B, 4.3C. For the instrumented SPAT, the reflexive response was mainly present in the part of the pROM close to maximum dorsiflexion, see dark-shaded area Fig. 4.2A. For the PC technique, the reflexive response was observed 100–300ms after each dorsiflexion perturbation, see Fig. 4.3B, C.

The observed intrinsic response also varied between participants. For the instrumented SPAT, variation of the intrinsic response was seen over the full pROM, see light-shaded area Fig. 4.2A. For the PC technique, variation of the intrinsic response was visible in the sustained plantarflexion torque response after step perturbations (i.e. a 580 ms hold period at maximum dorsiflexion), see Fig. 4.3B. This spring-like behavior around the joint, especially visible in the absence of a reflexive response, was interpreted as the elastic intrinsic resistance, i.e. intrinsic stiffness.

Longitudinal evaluation of BoNT-A injections

The longitudinal evaluation of the BoNT-A effect on joint resistance showed a heterogeneous response across all participants, see Fig. 4.4 and Table 4.2. For overall joint resistance, the MAS showed a reduced resistance in 6 of 9 participants at T_1 with 5 out of these 6 participants returning to baseline value at T_2. The instrumented SPAT overall resistance measure (W_{fast}) only showed reduced resistance in 4

participants at T_1 with 2 out of these 4 participants returning towards baseline value at T_2. On average, both MAS and W_{fast} showed a reduced resistance at T_1 with MAS returning close to baseline at T_2, whereas W_{fast} showed a further reduction. Only the MAS showed the hypothesized longitudinal BoNT-A effect on group-level ($\chi^2 = 6.91$, $p = 0.03$)[2], with post-hoc comparisons showing a significant reduction between T_0 and T_1 ($t = 2.41$, $p = 0.05$). The pROM over which the instrumented assessments were measured changed across sessions in 5 participants. For 2 participants the dorsiflexion pROM was reduced (10°) at T_1, whereas for 3 participants the full pROM shifted (10°) either towards dorsiflexion (2 participants) or plantarflexion (1 participant). The changes in pROM remained at T_2 for 3 participants, whereas 2 participants had a pROM in T_2 equal to T_0.

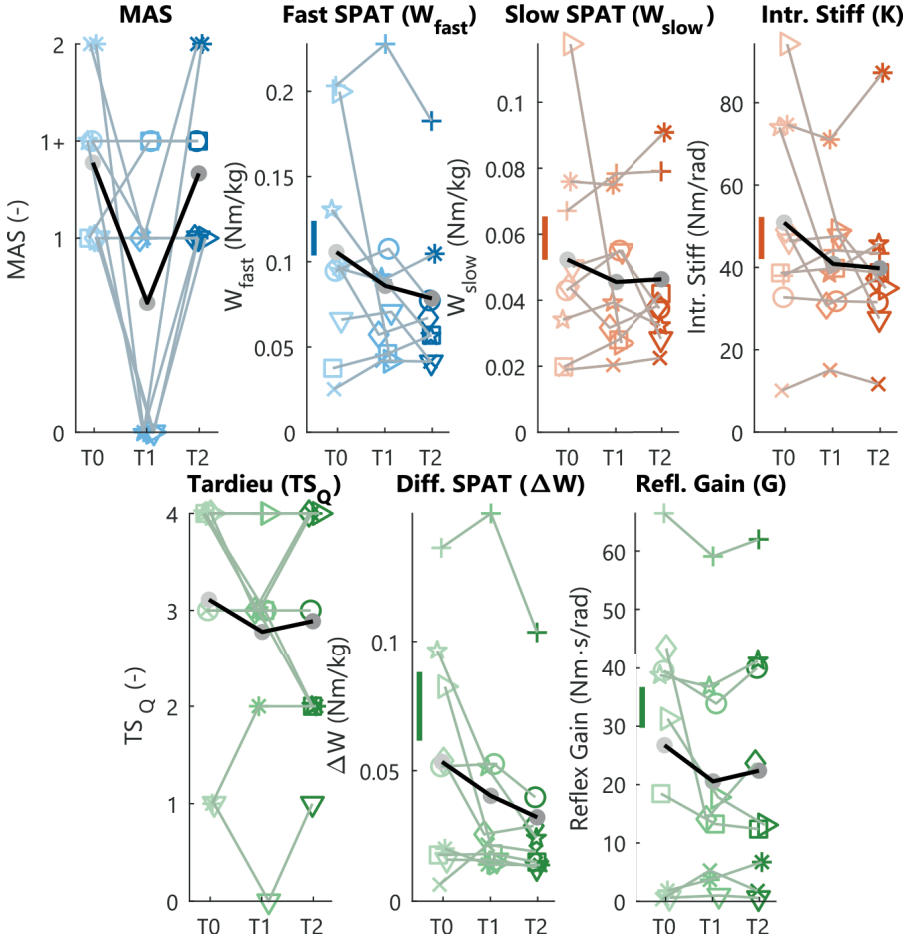


Figure 4.4. Longitudinal BoNT-A effect on joint resistance contributions for all participants. The quantified joint resistance contributions are shown for each participant (lines) at each session (unique symbol per participant across plots): week 0 (T_0, light), week 6 (T_1, medium) and week 12 (T_2, dark). The mean values across all participants is shown at each session (grey dots, bold black lines). The BoNT-A effect on overall joint resistance is shown for the MAS (clinical) and W_{fast} (SPAT) (blue). The BoNT-A effect on intrinsic resistance is shown for (red): W_{slow} (SPAT) and intrinsic stiffness (K, PC). Finally, the BoNT-A effect on reflexive resistance is shown for (green): the Tardieu Scale (TS_Q, clinical), ΔWork (SPAT) and reflexive gain (G, PC). The best-case minimal detectable difference (MDD) (vertical line) is depicted for reference, see Supplementary Materials 4.1, Table S4.1.

For reflexive joint resistance, the Tardieu Scale (TS_Q) showed a reduced resistance in 4 participants with 2 out of 4 of these participants returning to baseline value at T_2, see Fig. 4.4 and Table 4.2. Regarding the instrumented measures a reduction in reflexive resistance at T_1 was observed in: 5 participants for ΔW, 6 participants for G, and 3 participants for both G and ΔW. Out of these participants with reduced resistance at T_1, an increase towards baseline value at T_2 was observed in: 1 of 5 participants for ΔW, 4 of 6 participants for G, and 1 of 3 participants for both G and ΔW. The participants that did not show a reduction in G at T_1 had the lowest values for G at baseline, see Fig. 4.4. Combined with the MAS, 4 participants showed reduced resistance at T_1 for both MAS and ΔW and 3 participants showed a reduction for both MAS and G. On average, all reflexive resistance measures showed a reduction at T_1 with both TS_Q and G returning towards baseline at T_2, whereas ΔW showed a further reduction. A significant longitudinal BoNT-A effect on reflexive resistance was only found for the ΔW ($\chi^2 = 11.9$, $p = 0.003$)[2], although post-hoc comparisons did not find any significant differences between sessions.

For intrinsic joint resistance, a reduced resistance at T_1 was observed in: 3 participants for W_{slow}, 5 participants for K, and 3 participants for both K and W_{slow}, see Fig. 4.4 and Table 4.2. Out of these participants with reduced resistance at T_1, an increase towards baseline value at T_2 was observed in: 2 of 3 participants for W_{slow}, 3 of 5 participants for K, and 2 of 3 participants for both K and W_{slow}. On average, both intrinsic resistance measures showed a reduction at T_1 with W_{slow} returning towards baseline at T_2 and K showing a further reduction. No significant longitudinal BoNT-A effect on intrinsic resistance was found.

The subject heterogeneity likely introduces a confounding effect with the measured BoNT-A injection effect. Participants with high baseline values, i.e. high intrinsic and/or reflexive resistance, show larger responses to the BoNT-A injection than participants with low baseline values, see Fig. 4.5 with reflexive gain as example outcome measure. Across all measures, moderate to strong correlations between baseline value and measured BoNT-A injection effect were observed, see Table 4.3. Small baseline values provide little room to observe the hypothesized reduction in joint resistance.

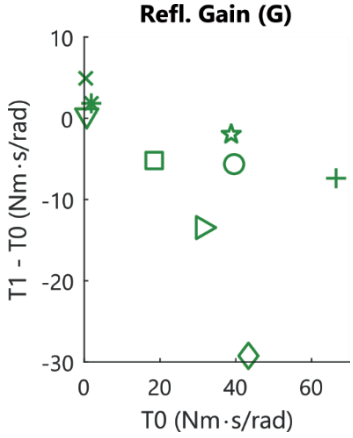


Figure 4.5. BoNT-A injection effect in relation to baseline value for reflexive joint resistance. The BoNT-A injection effect (difference between week 6 and week 0) (y-axis) is shown in relation to the baseline value (x-axis) for reflexive gain (G, PC). Each symbol represents a single participant, corresponding the symbols used in Fig. 4.4.

Table 4.2

Pre- and post-BoNT-A injection outcome measures for the intrinsic and reflexive contributions to ankle joint resistance *(N=9)*

	T_0 (Week 0)	T_1 (Week 6)	T_2 (Week 12)	Friedman test
MAS (-)	1.5 [1,1.6]	1 [0,1.1]*	1 [1,1.6]	p = **0.03**
Fast SPAT W_{fast} (Nm/kg)	0.096 [0.059,0.148]	0.071 [0.045,0.095]	0.062 [0.049,0.091]	p = 0.48
Tardieu TS_Q (-)	4 [2.5 4]	3 [2.75,3.25]	3 [2,4]	p = 0.52
Diff. SPAT W (Nm/kg)	0.052 [0.017,0.086]	0.022 [0.015,0.052]	0.022 [0.014,0.034]	p = **0.003**
Refl. Gain G (Nm s/rad)	31 [1.5,40]	14 [4.9,35]	13 [5.5,40]	p = 0.31
Slow SPAT W_{slow} (Nm/kg)	0.044 [0.031,0.069]	0.039 [0.028,0.060]	0.038 [0.030,0.061]	p = 0.26
Intr. Stiffness K (Nm/rad)	46 [37,74]	40 [32,48]	37 [31,44]	p = 0.67

The median [25th, 75th percentile] across participants are reported. Longitudinal differences across all sessions were evaluated using the Friedman test. Significant Friedman tests (ΔW , MAS) were investigated using a multiple comparisons test adjusted with the Bonferroni correction. Sessions with a significant difference compared with week 0 are indicated (*)

Table 4.5

Spearman's/Pearson's correlation coefficients and their 95% confidence intervals (N = *26–27*)

	MAS	Tardieu TS_Q	Refl. Gain G	Intr. Stiffness K	Intr. Damping B
Fast SPAT W_{fast}	0.05 [− 0.38,0.40]	0.24 [− 0.22,0.61]	0.73 [0.48,0.87]	0.46 [0.16,0.78]	0.83 [0.73,0.90]
Diff. SPAT ΔW	− 0.01 [− 0.42, 0.41]	0.60 [0.23,0.81]	0.86 [0.71,0.93]	0.17 [− 0.15,0.51]	0.72 [0.43,0.86]
Refl. Gain G	− 0.08 [− 0.48, 0.33]	0.57 [0.21,0.80]			
Slow SPAT W_{slow}	− 0.03 [− 0.44,0.40]	− 0.09 [− 0.50,0.31]	0.27 [− 0.12,0.59]	0.74 [0.39,0.90]	0.71 [0.41,0.83]
Intr. Stiffness K	− 0.20 [− 0.62,0.27]	− 0.07 [− 0.44,0.40]			
Intr. Damping B	− 0.04 [− 0.46,0.36]	0.38[− 0.07,0.67]			

Correlations between the clinical measures (MAS, TSQ), instrumented SPAT (W_{fast}), ΔW, W_{slow}, and PC technique measures (reflexive gain G, intrinsic stiffness K and intrinsic damping B). Spearman's rank correlation coefficient ρ was used for all correlations involving the ordinal clinical measures, whereas Pearson's correlation coefficient r was used otherwise. The 95% Cis were constructed using a non-parametric BCa bootstrap procedure.

Table 4.3
Spearman's/Pearson's correlation coefficient between baseline value (T_0) and BoNT-A injection effect (T_1–T_0; N=8/9)

Outcome Measure	ρ/r
MAS	− 0.51
Fast SPAT W_{fast}	− 0.53
Slow SPAT W_{slow}	− 0.77
Intr. Stiffness *K*	− 0.81
Tardieu TS_Q	− 0.38
Diff. SPAT W	− 0.34
Refl. Gain *G*	− 0.57

Spearman's rank correlation coefficient ρ was used for all correlations involving the ordinal clinical measures, whereas Pearson's correlation coefficient r was used otherwise.

Table 4.4
Intraclass correlation coefficient (ICC) and their 95% confidence intervals *(N = 54/78)*

Outcome Measure	ICC
Fast SPAT W_{fast}	0.98 [0.96,1.00]
Diff. SPAT W	0.94 [0.88,0.98]
Refl. Gain *G*	0.98 [0.97,0.99]
Slow SPAT W_{slow}	0.96 [0.89,0.99]
Intr. Stiffness *K*	0.97 [0.87,1.00]
Intr. Damping *B*	0.99 [0.97,1.00]

ICCs for the instrumented assessment based on three repetitions per session for the instrumented SPAT and two repetitions per session for the PC technique. The 95% CIs were constructed using a non-parametric bootstrap procedure.

Linear associations and repeatability of joint resistance measures

Excellent ICC values were observed for both the instrumented SPAT (r = [0.98, 0.94, 0.97]) and PC technique (r = [0.98, 0.97, 0.99]) measures, see Table 4.4. The 95% CIs lower bounds did show relatively high uncertainty for ΔW (0.88), W_{slow} (0.89) and K (0.87). The reported ICCs represent a best-case scenario for optimal experimental conditions, as only short 20–60 s breaks were included between repetitions and participants were not taken out of the instrumented setup between repetitions.

The PC technique model showed a good model fit effectiveness for the overall model and the specific intrinsic and reflexive parameters. Regarding the overall fit (i.e. Step 1–6 of the algorithm), a median variance accounted for (%VAF) of 92.5% [(Q1,Q3) 90.9%, 96.2%] was obtained by the complete model on the measured data, similar to previous PC studies.[36] For the parameterized intrinsic pathway (i.e. Step 4 of the algorithm), a median %VAF of 91.4% [89.0%, 93.1%] was obtained, whereas for the parameterized reflexive pathway (i.e. Step 6 of the algorithm), a median %VAF of 84.4% [77.7%, 87.6%] was obtained.

Most clinical and instrumented assessments quantifying the same resistance contribution showed a positive correlation as expected, see Table 4.5. For overall resistance, the MAS was not correlated with the SPAT W_{fast} (r = 0.05). For the reflexive resistance, the Tardieu Scale showed a moderate positive correlation with the instrumented measures ΔW and G (r = 0.60/0.57), whereas both instrumented measures showed a strong correlation (r = 0.86). For the intrinsic resistance, the SPAT W_{slow} showed a strong correlation with both PC technique outcomes of stiffness K (r = 0.74) and damping B (r = 0.71).

Most clinical and instrumented assessments quantifying a different resistance contribution were not correlated as expected, see Table 4.5. For the overall resistance, MAS was not correlated with intrinsic/reflexive measures (r = [0.19, 0.01]), whereas the SPAT W_{fast} did show strong correlation with the PC reflexive gain G and intrinsic damping B. For the reflexive resistance, the Tardieu Scale was not or weakly correlated with non-reflexive measures (r = [0.09, 0.38]). The reflexive gain G showed strong

correlation with SPAT W_{fast} and the SPAT ΔW showed strong correlation with PC intrinsic damping G. For the intrinsic resistance, only PC technique intrinsic damping B showed strong correlations as reported above.

4.4 Discussion

This paper studied the intrinsic and reflexive ankle joint resistance within participants treated with BoNT-A injections to reduce spasticity. We hypothesized that both reflexive and overall joint resistance would decrease 6 weeks after BoNT-A injection, while returning close to baseline value after 12 weeks. Three fundamentally different joint resistance assessments were used: (1) clinical tests (MAS, Tardieu Scale); (2) instrumented SPAT measured over the full pROM with elementary processing; and 3) data-driven PC system identification measured over a limited pROM with model-based processing. Individually, the hypothesized BoNT-A effect (reduction at week 6, return to baseline week 12) was observed in the MAS (5 participants), W_{fast} SPAT (2 participants), Tardieu Scale (2 participants), ΔW SPAT (1 participant) and G (4 participants). On group-level, our hypothesis was only confirmed for the MAS, a measure of overall joint resistance, which showed a significant reduced resistance at week 6. Regarding validity, all instrumented outcome measures showed a strong correlation when quantifying the same resistance contribution.

Longitudinal evaluation of BoNT-A injections

On group-level, only the MAS showed the hypothesized effect of reduced joint resistance at week 6 with a return close to baseline at week 12. Our MAS results are in line with larger clinical trials evaluating BoNT-A effects with the MAS.[6-8] The MAS should be interpreted with care as the scale is subjective and a non-blinded rater scored the participants.[14,44] Contrary to the MAS, all instrumented measures showed a more heterogeneous response and did not capture a significant reduction on group-level 6 weeks after injection. Thus, either there was indeed no significant reduction (true negative) or, as implied by the MAS results and previous clinical trials, we were not able to correctly measure the significant reduction (false negative).

Previous studies using instrumented measures to investigate BoNT-A effects over the full pROM also reported heterogeneity between participants.[24-27] Moreover, a mix of positive/negative results were reported 4–6 weeks after injection for these instrumented assessment studies. The studies executed with a device assessing the wrist (Neuroflexor) and estimating resistance components using a biomechanical wrist model with low complexity did report a reduced reflexive response. The study executed with a device assessing the ankle (MOOG manipulator, similar to our study) and estimating resistance with a neuromechanical ankle model with higher complexity did not report a reduction. Therefore, differences in the reported results may be influenced by participant heterogeneity (such as age, severity of impairment, time since impairment, number of previous BoNT-A injections, BonT-A injection dose), the experimental setup, the assessed joint and the model used for resistance estimation.

The heterogeneous response among the study population complicated group-level evaluation of the BoNT-A effect. For example, the PC technique showed a reflex reduction in 6 out of 9 participants at week 6. The 3 participants without reflex reduction had the lowest reflexive response at baseline. Therefore, these 3 participants had little potential to further reduce the reflexive response and also

limited a potential group effect. These 3 participants also had a relatively limited dorsiflexion pROM at baseline and 2 of these 3 participants showed an improved dorsiflexion pROM at week 6. As such, BoNT-A injections may result in better outcomes within people with high reflexive activity and/or clonus than people with only high resistance to passive joint motion. Interpretation of the population heterogeneity was also convoluted by different outcomes for the instrumented measures. A reflex reduction was observed in 5 participants for the SPAT and 6 participants for the PC technique, yet only 3 participants showed a reduction in both outcome measures.

As the reflexive response depends on joint angle and pROM, the full and limited pROM used during assessments could potentially explain these differences.[36,45] Both methods simplified this complex dependency through averaging over the full pROM (SPAT) or assessing a limited pROM (PC). As a result, neither method controlled for variations in the reflexive response due to observed changes in pROM and potential underlying changes in e.g. muscle slack length. Quantitative analysis of the measured individual effects is desired to increase understanding of the heterogeneous response.

Quantitative analysis of individual effects would require a larger participant group and insight into the minimal detectable difference (MDD), which have currently not been reported yet. To illustrate such an analysis, the PC technique showed a reflex reduction larger than a best-case scenario MDD (6.9 Nm s/rad) for 3 of 9 participants at week 6. Only best-case scenario MDDs could be computed as experimental conditions were optimal regarding repeatability. Clinically relevant MDDs would require a test-retest reliability design with longer breaks between repetitions, measurements on separated days and removing participants from the measurement device between repetitions.[19,28,46] The best-case results did indeed show that both instrumented SPAT and PC technique had excellent ICC between r = [0.94, 0.99], whereas typically reported values are between r = [0.85, 0.95] for similar instrumented measures.[19,28,46-48] Overall, the BoNT-A effect on the reflexive contributions remains ambiguous.

Linear associations of joint resistance measures

In absence of a gold standard, the validity of the instrumented measures was shown through linear association between the methodologies.[5,28,29] As expected, most measures quantifying the same resistance contribution (e.g. ΔW and G) showed moderate to strong correlations. Strong correlations were observed between the instrumented measures, whereas a similar study found moderate similarity between two instrumented measures.[29] However, Andringa et al.[29] compared methodologies using a different experimental setup (Neuroflexor and Wristalyzer) and different data processing approaches (low complexity biomechanical and higher complexity neuromechanical model).[18,49] In our study, the results were obtained using the same device, which may explain part of the relatively strong correlations observed. Only between the MAS and SPAT (W_{fast}), both measures of overall joint resistance, no correlation was observed. While both measures compute an overall resistance effect, the characteristics of the applied perturbation differed between the relatively slow velocity of the MAS (20–30 deg/s) and fast velocity of the SPAT, W_{fast} (150 deg/s). Changing perturbation characteristics could affect the relative magnitude of the intrinsic and reflexive contributions within the measured overall response, as both contributions contain velocity- and acceleration-dependent components.[10,50,51] Therefore, the lack of association between MAS and fast velocity SPAT could potentially be explained by the different perturbation profiles used. In addition, the MAS, which is a subjective measure, was scored by a non-blinded rater and has questionable reliability, which could all have influenced the observed correlation.

Besides, a general lack of correlation was observed across joint resistance measures quantifying a different resistance contributions, although unexpected correlations were observed between a couple of outcome measures. The reflexive measures of the instrumented SPAT (ΔW) did show a strong correlation with the intrinsic damping (viscous) contribution of the PC technique (B). Note, the reflexive instrumented SPAT measure was computed as the difference in work between a fast and slow passive movement. Thus, ΔW was considered fully velocity-dependent, which can be attributed to either a reflexive or viscous intrinsic contribution.[10] This could explain the observed commonality with intrinsic damping of the PC technique. The commonality of the reflexive SPAT measures with an intrinsic outcome measure illustrated that the separation of joint resistance contributions could be improved. On the one hand, additional information from an extended experimental dataset might improve the ability to disentangle joint resistance. On the other hand, detailed model-based processing, such as neuromechanical models or data-driven processing, could improve the ability to disentangle joint resistance.[10,24-27] Andringa et al.[29] did show that despite the use of these type of neuromechanical models, weak correlations between reflexive and intrinsic contribution may remain. Overall, at group-level the quantified intrinsic and reflexive resistance outcome measures matched well, supporting the validity towards clinical application.

Study limitations and clinical application

First, the clinical evaluations in this study were all performed by a non-blinded, trained physiotherapist. Therefore, knowledge of the hypotheses of this study combined with information about the specific session (week 0, 6 or 12) could have biased the MAS and Tardieu Scale scores. Second, spasticity is a complex symptom, which can manifest itself differently within a the passive experimental environment compared with an active or functional environment.[4] Therefore, BoNT-A effects as experienced in daily life and functional tasks may not necessarily be captured in the clinical and instrumented assessments used. In addition, the full complexity of spasticity is difficult to capture within the limited number of participants included in the study. Third, a low reflexive resistance magnitude at baseline before BoNT-A injection was observed in 3 participants, which limited their potential to show a reflex reduction. Scientifically, future studies evaluating longitudinal BoNT-A effects could avoid this limitation by determining a threshold magnitude, e.g. based on MDD, for inclusion of participants in the data analysis. Clinically, these 3 participants illustrate the relevance of adding instrumented measures to enable differentiation between patients with similar MAS values in support of clinical decision making. Fourth, the instrumented evaluations were limited due to natural variations in the pROM shown by multiple participants across sessions. Small variations in pROM were exacerbated in our protocol, because the adjustable hardware endstops restricting manipulator movement for safety could only be adjusted per 10°. For both instrumented measures, variability in the pROM likely translated to additional variability in outcome measures across sessions, as joint resistance depends on joint angle and pROM.[36,45] Due to simplification in both instrumented measures, the added variability of the pROM could not be controlled for, which reduced the ability to detect BoNT-A effects.

Despite these limitations and heterogeneous results, clinical studies of instrumented measures distinguishing the relative contributions to joint hyper-resistance remain important. First, our results again confirm that the MAS, on which many clinical evaluations of BoNT-A effects are based, does not correlate well with instrumented measures specifically aimed at quantifying the reflexive joint resistance or spasticity. Second, further research into the diagnostic properties of the instrumented

measures is of interest to potentially support clinical decision making. For example, previous studies showed that the PC technique could discriminate spastic participants from controls and paretic from non-paretic joints.[30,32] Towards clinical application, additional investigation into diagnostic properties like the reliability (MDD) and normative data are desired to enable clinical decision making based on the quantified joint resistance contributions. Moreover, investigating the relation between instrumented measures and functional or goal-oriented outcomes, like motor recovery level or goal attainment scale (GAS),[52] is of interest. This relation is important: given the lack of a gold standard to evaluate the instrumented measures against; to examine the role of increased intrinsic and reflexive joint resistance on reduced functionality; and to provide extended clinical context on the indication and evaluation of BoNT-A treatment.

4.5 Conclusions

Our group-level hypothesis of a reduced joint resistance 6 weeks after injection with a return close to baseline at week 12 was only observed in the MAS (overall joint resistance). This observed reduction could not be attributed to an unambiguous group-level reduction of the reflexive or intrinsic resistance as no instrumented measures confirmed the hypothesis. Several individuals did show the hypothesized BoNT-A effect in the reflexive or intrinsic contributions. A moderate to strong correlation between all reflexive measures and a strong correlation between the intrinsic measures supported the validity of the used instrumented measures. Ultimately, objective and reliable joint resistance quantification would improve clinical decision making in prescription of BoNT-A and unravel the effect of BoNT-A injections on spasticity.

References

1. Davis EC, Barnes MP. Botulinum Toxin and Spasticity. *J Neurol Neurosurg Psychiatry*. 2000;69(2):143–7. https://doi.org/10.1136/jnnp.69.2.143.
2. Brashear A, Lambeth K. Spasticity. Curr Treat Options Neurol. 2009;11(3):153–61. https://doi.org/10.1007/s11940-009-0018-4.
3. Chang E, Ghosh N, Yanni D, Lee S, Alexandru D, Mozaffar T. A review of spasticity treatments: pharmacological and interventional approaches. Crit Rev Phys Rehabil Med. 2013;25(1–2):11–22. https://doi.org/10.1615/ CritRevPhysRehabilMed.2013007945.A.
4. Dietz V, Sinkjær T. Spastic movement disorder: impaired reflex function and altered muscle mechanics. Lancet Neurol. 2007;6(8):725–33. https:// doi.org/10.1016/S1474-4422(07)70193-X.
5. van den Noort JC, Bar-On L, Aertbeliën E, Bonikowski M, Braendvik SM, Broström EW, et al. European consensus on the concepts and measure- ment of the pathophysiological neuromuscular responses to passive muscle stretch. Eur J Neurol. 2017;24(7):981–91. https://doi.org/10.1111/ ene.13322.
6. Shaw L, Rodgers H, Price C, van Wijck F, Shackley P, Steen N, et al. BoTULS. A multicentre randomised controlled trial to evaluate the clinical effec- tiveness and cost-effectiveness of treating upper limb spasticity due to stroke with botulinum toxin type A. Health Technol Assessment. 2010. https://doi.org/10.3310/hta14260.
7. Baker JA, Pereira G. The efficacy of botulinum toxin A for spasticity and pain in adults. A systematic review and meta-analysis using the grades of recommendation, assessment, development and evaluation approach. Clin Rehabil. 2013;27(12):1084–96. https://doi.org/10.1177/0269215513 491274.
8. Gracies JM, Brashear A, Jech R, McAllister P, Banach M, Valkovic P, et al. Safety and efficacy of abobotulinumtoxin A for hemiparesis in adults with upper limb spasticity after stroke or traumatic brain injury: a double-blind randomised controlled trial. Lancet Neurol. 2015;14(10):992–1001. https:// doi.org/10.1016/S1474-4422(15)00216-1.
9. Bohannon RW, Smith MB. Interrater reliability of a modified Ashworth scale of muscle spasticity. Phys Ther. 1987;67(2):206–7.
10. Kearney RE, Stein RB, Parameswaran L. Identification of intrinsic and reflex contributions to human ankle stiffness dynamics. IEEE Trans Biomed Eng. 1997;44(6):493–504. https://doi.org/10.1109/10.581944.
11. Fleuren JFM, Voerman GE, Erren-Wolters CV, Snoek GJ, Rietman JS, Hermens HJ, et al. Stop using the Ashworth Scale for the assessment of Spasticity. J Neurol Neurosurg Psychiatry. 2010;81:46–52. https://doi.org/ 10.1136/jnnp.2009.177071.
12. Katz RT, Rymer WZ. Spastic hypertonia: mechanisms and measurement. Arch Phys Med Rehabil. 1989;70(2):144–55.
13. Burne JA, Carleton VL, O'Dwyer NJ. The spasticity paradox: movement disorder or disorder of resting limbs? J Neurol Neurosurg Psychiatry. 2005;76(1):47–54. https://doi.org/10.1136/jnnp.2003.034785.
14. Pandyan AD, Johnson GR, Price CIM, Curless RH, Barnes MP, Rodgers H. A review of the properties and limitations of the Ashworth and modified Ashworth scales as measures of spasticity. Clin Rehabil. 1999;13(5):373–83. https://doi.org/10.1191/026921599677595404.
15. Joshi TN, Joshi S. Adverse effects of botulinum neurotoxin A in spasticity management. Int J Nutr Pharmacol Neurol Dis. 2011;1(2):126. https://doi. org/10.4103/2231-0738.84202.
16. van der Helm FCT, Schouten AC, de Vlugt E, Brouwn GG. Identification of intrinsic and reflexive components of human arm dynamics during postural control. J Neurosci Methods. 2002;119(1):1–14. https://doi.org/ 10.1016/S0165-0270(02)00147-4.
17. de Vlugt E, de Groot JH, Schenkeveld KE, Arendzen JH, van der Helm FCT, Meskers CGM. The relation between neuromechanical parameters and Ashworth score in stroke patients. J Neuroeng Rehabil. 2010;7(1):35. https://doi.org/10.1186/1743-0003-7-35.
18. Lindberg PG, Gäverth J, Islam M, Fagergren A, Borg J, Forssberg H. Valida- tion of a new biomechanical model to measure muscle tone in spastic muscles. Neurorehabil Neural Repair. 2011;25(7):617–25. https://doi.org/ 10.1177/1545968311403494.
19. Sloot LH, van der Krogt MM, de Gooijer-van de Groep KL, van Eesbeek S, de Groot JH, Buizer AI, et al. The validity and reliability of modelled neural and tissue properties of the ankle muscles in children with cerebral palsy. Gait Posture. 2015;42(1):7–15. https://doi.org/10.1016/j.gaitpost.2015.04. 006.

20. Wang R, Herman PA, Ekeberg Ö, Gäverth J, Fagergren A, Forssberg H. Neural and non-neural related properties in the spastic wrist flexors: an optimization study. Med Eng Phys. 2017;47:198–209. https://doi.org/10. 1016/j.medengphy.2017.06.023.
21. Voerman GE, Burridge JH, Hitchcock RA, Hermens HJ. Clinometric properties of a clinical spasticity measurement tool. Disabil Rehabil. 2007;29(24):1870–80. https://doi.org/10.1080/09638280601143752.
22. Bar-On L, Aertbeliën E, Wambacq H, Severijns D, Lambrecht K, Dan B, et al. A clinical measurement to quantify spasticity in children with cerebral palsy by integration of multidimensional signals. Gait Posture. 2013;38(1):141–7. https://doi.org/10.1016/j.gaitpost.2012.11.003.
23. Sloot LH, Bar-On L, van der Krogt MM, Aertbeliën E, Buizer AI, Desloovere K, et al. Motorized versus manual instrumented spasticity assessment in children with cerebral palsy. Dev Med Child Neurol. 2017;59(2):145–51. https://doi.org/10.1111/dmcn.13194.
24. Gäverth J, Eliasson AC, Kullander K, Borg J, Lindberg PG, Forssberg H. Sensitivity of the neuroflexor method to measure change in spastic- ity after treatment with botulinum toxin A in wrist and finger muscles. J Rehabil Med. 2014;46(7):629–34. https://doi.org/10.2340/16501977-1824.
25. Wang R, Gäverth J, Herman PA. Changes in the neural and non-neural related properties of the spastic wrist flexors after treatment with botuli- num toxin A in post-stroke subjects: an optimization study. Front Bioeng Biotechnol. 2018. https://doi.org/10.3389/fbioe.2018.00073.
26. de Gooijer-van de Groep KL, Meskers CGM, de Vlugt E, Arendzen JH, de Groot JH. Estimating the Effects of Botulinum Toxin A Therapy Post- Stroke. Evidence for Reduction of Background Muscle Activation. In: Identification of Neural and Non-Neural Contributors to Joint Stiffness in Upper Motor Neuron Disease. Leiden, Netherlands: Leiden University Medical Center (LUMC); 2019. p. 119–136.
27. Andringa A, van Wegen E, van de Port I, Guit L, Polomski W, Kwakkel G, et al. The Effect of Botulinum Toxin-A on Neural and Non-neural Compo- nents of Wrist Hyper-Resistance in Adults with Stroke or Cerebral Palsy. PM &R: The Journal of Injury, Function and Rehabilitation. 2021;p. 1–10.
28. Andringa A, van Wegen E, van de Port I, Kwakkel G. Measurement proper- ties of the neuroflexor device for quantifying neural and non-neural components of wrist hyper-resistance in chronic stroke. Front Neurol. 2019;10:730.
29. Andringa A, Meskers CGM, van de Port I, Zandvliet S, Scholte L, de Groot J, et al. Quantifying neural and non-neural components of wrist hyper-resistance after stroke: comparing two instrumented assessment methods. Med Eng Phys. 2021;98:57–64. https://doi.org/10.1016/j.meden gphy.2021.10.009.
30. Mirbagheri MM, Barbeau H, Ladouceur M, Kearney RE. Intrinsic and reflex stiffness in normal and spastic, spinal cord injured subjects. Exp Brain Res. 2001;141(4):446–59. https://doi.org/10.1007/s00221-001-0901-z.
31. Schouten AC, de Vlugt E, van Hilten JJ, van der Helm FCT. Quantifying proprioceptive reflexes during position control of the human arm. IEEE Trans Biomed Eng. 2008;55(1):311–21. https://doi.org/10.1109/TBME. 2007.899298.
32. Mirbagheri MM, Alibiglou L, Thajchayapong M, Rymer WZ. Muscle and reflex changes with varying joint angle in hemiparetic stroke. J NeuroEng Rehabil. 2008. https://doi.org/10.1186/1743-0003-5-6.
33. Mirbagheri MM, Ladouceur M, Barbeau H, Kearney RE. The effects of long term FES-assisted walking on intrinsic and reflex dynamic stiff- ness in spinal cord injured patients. IEEE Trans Neural Syst Rehabil Eng. 2002;10(4):280–9. https://doi.org/10.1109/TNSRE.2002.806838.
34. Mirbagheri MM, Chen D, Rymer WZ. Quantification of the effects of an alpha-2 adrenergic agonist on reflex properties in spinal cord injury using a system identification technique. J NeuroEng Rehabil. 2010. https://doi. org/10.1186/1743-0003-7-29.
35. Mirbagheri MM, Kindig MW, Niu X. Effects of robotic-locomotor training on stretch reflex function and muscular properties in individuals with spinal cord injury. Clin Neurophysiol. 2015;126(5):997–1006. https://doi. org/10.1016/j.clinph.2014.09.010.
36. Mirbagheri MM, Barbeau H, Kearney RE. Intrinsic and reflex contributions to human ankle stiffness: variation with activation level and position. Exp Brain Res. 2000;135(4):423–36. https://doi.org/10.1007/s002210000534.
37. Gracies JM, Marosszeky JE, Renton R, Sandanam J, Gandevia SC, Burke D. Short-term effects of dynamic Lycra splints on upper limb in hemiplegic patients. Arch Phys Med Rehabil. 2000;81(12):1547–55. https://doi.org/10. 1053/apmr.2000.16346.

38. van't Veld RC, Schouten AC, van der Kooij H, van Asseldonk EHF. Neu- rophysiological validation of simultaneous intrinsic and reflexive joint impedance estimates. J NeuroEng Rehabil. 2021;18(1):36. https://doi.org/ 10.1186/s12984-021-00809-3.
39. Ludvig D, Kearney RE. Real-time estimation of intrinsic and reflex stiffness. IEEE Trans Biomed Eng. 2007;54(10):1875–84. https://doi.org/10.1109/ TBME.2007.894737.
40. Westwick DT, Kearney RE. Identification of Linear Systems. In: Identifica- tion of Nonlinear Physiological Systems. Hoboken: John Wiley & Sons, Inc.; 2003. p. 103–123. https://doi.org/10.1002/0471722960.ch5.
41. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. J Chiropr Med. 2016;15(2):155–63. https://doi.org/10.1016/j.jcm.2016.02.012.
42. Carpenter J, Bithell J. Bootstrap confidence intervals: when, which, what? A practical guide for medical statisticians. Stat Med. 2000;19(9):1141–64. https://doi.org/10.1002/(SICI)1097-0258(20000515)19:9%3c1141::AID- SIM479%3e3.0.CO;2-F.
43. Weir JP. Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. J Strength Conditioning Res. 2005;19(1):231–40. https://doi.org/10.1519/15184.1.
44. Platz T, Eickhof C, Nuyens G, Vuadens P. Clinical scales for the assessment of spasticity associated phenomena, and function: a systematic review of the literature. Disabil Rehabil. 2005;27(1–2):7–18. https://doi.org/10.1080/ 09638280400014634.
45. Fleuren JF, Nederhand MJ, Hermens HJ. Influence of posture and muscle length on stretch reflex activity in poststroke patients with spasticity. Arch Phys Med Rehabil. 2006;87(7):981–8. https://doi.org/10.1016/j.apmr. 2006.03.018.
46. Bar-On L, Desloovere K, Molenaers G, Harlaar J, Kindt T, Aertbeliën E. Identification of the neural component of torque during manually-applied spasticity assessments in children with cerebral palsy. Gait Posture. 2014;40(3):346–51. https://doi.org/10.1016/j.gaitpost.2014.04.207.
47. Mirbagheri MM, Kearney RE, Barbeau H. Quantitative, Objective Measure- ment of Ankle Dynamic Stiffness: Intrasubject Reliability and Intersubject Variability. In: 18th Annual International Conference of the IEEE Engineer- ing in Medicine and Biology Society. Amsterdam, Netherlands: IEEE; 1996. p. 585–586.
48. Gäverth J, Sandgren M, Lindberg PG, Forssberg H, Eliasson AC. Test- retest and inter-rater reliability of a method to measure wrist and finger spasticity. J Rehabil Med. 2013;45(7):630–6. https://doi.org/10.2340/16501 977-1160.
49. de Gooijer-van de Groep KL, de Vlugt E, van der Krogt HJ, Helgadóttir Á, Arendzen JH, Meskers CGM, et al. Estimation of tissue stiffness, reflex activity, optimal muscle length and slack length in stroke patients using an electromyography driven antagonistic wrist model. Clin Biomech. 2016;35:93–101. https://doi.org/10.1016/j.clinbiomech.2016.03.012.
50. Finley JM, Dhaher YY, Perreault EJ. Acceleration dependence and task- specific modulation of short- and medium-latency reflexes in the ankle extensors. Physiol Rep. 2013. https://doi.org/10.1002/phy2.51.
51. van't Veld RC, van Asseldonk EHF, van der Kooij H, Schouten AC. Disentan- gling acceleration-, velocity-, and duration-dependency of the short- and medium-latency stretch reflexes in the ankle plantarflexors. J Neurophys- iol. 2021;126(4):1015–29. https://doi.org/10.1152/jn.00704.2020.
52. Wissel J, Ri S. Assessment, goal setting, and botulinum neurotoxin a therapy in the management of post-stroke spastic movement disorder: updated perspectives on best practice. Expert Rev Neurother. 2022;22(1):27–42. https://doi.org/10.1080/14737175.2021.2021072.

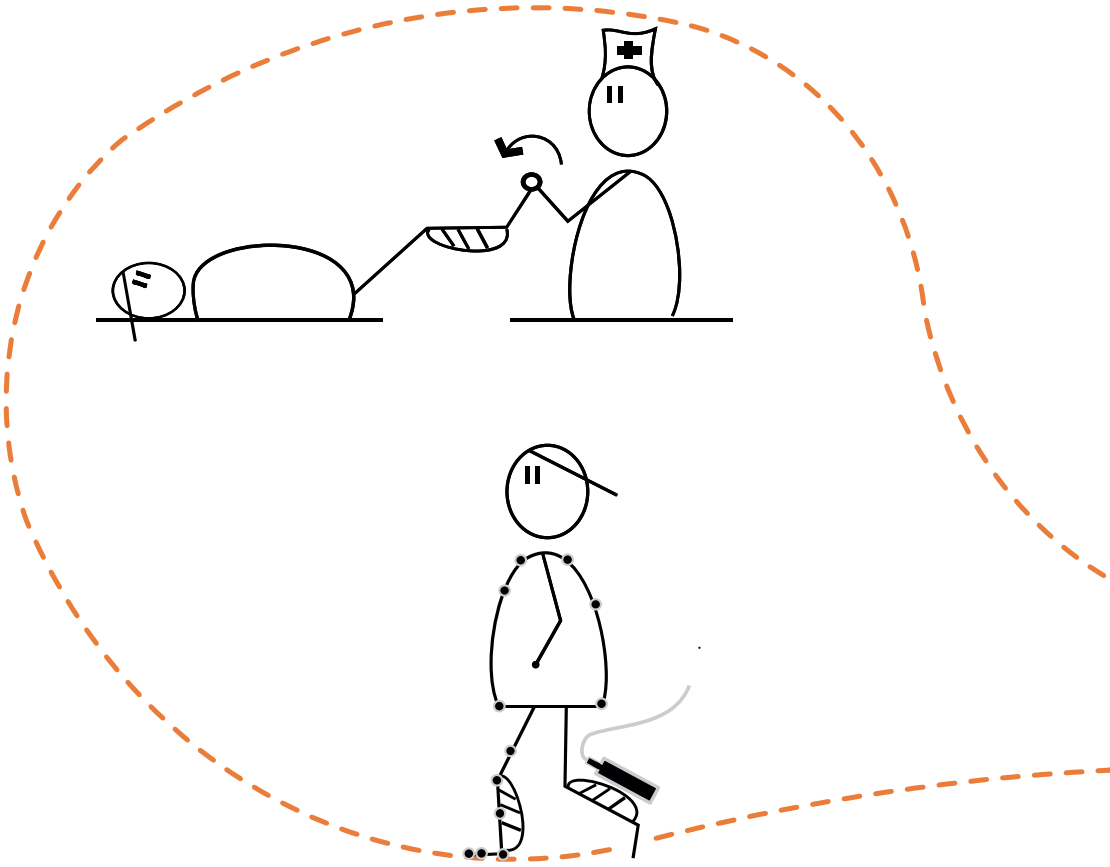
Supplementary Materials 4.1

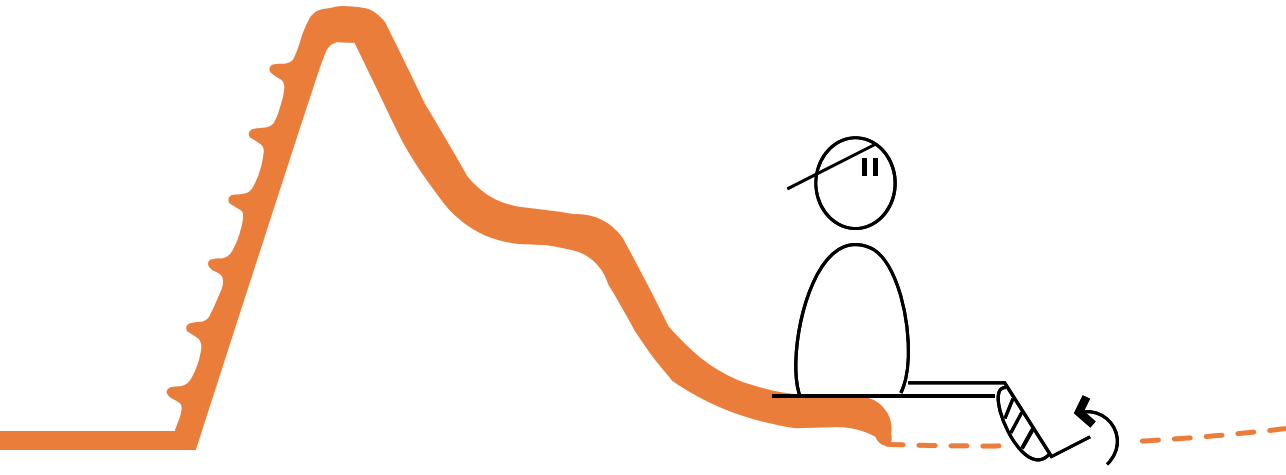
Table S4.1

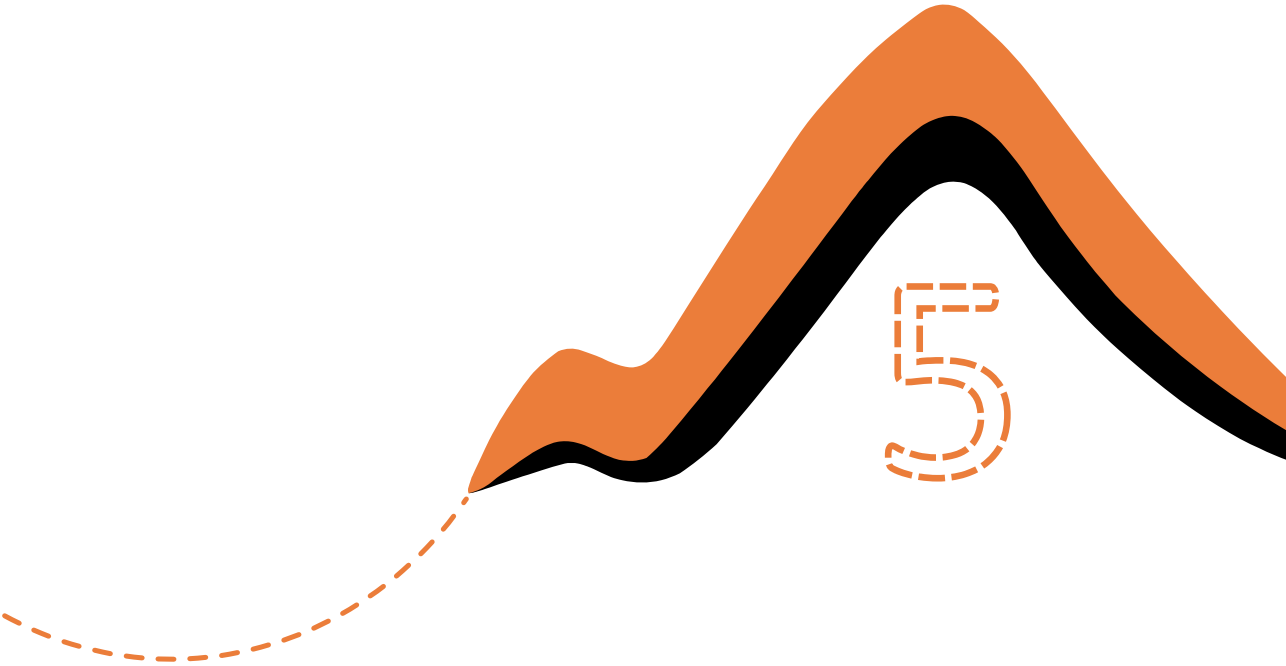
Best-case minimal detectable difference (MDD) (N = 54/78)

Outcome Measure	MDD
Diff. SPAT ΔW (Nm/kg)	0.026
Slow SPAT Wslow (Nm/kg)	0.013
Fast SPAT Wfast (Nm/kg)	0.021
Refl. Gain G (Nm s/rad)	6.9
Intr. Stiffness K (Nm/rad)	10
Intr. Damping B (Nm s/rad)	0.094

MDDs for the instrumented assessment outcomes based on the ICC values in Table 4 [52]. The MDD present a best-case scenario as the repeatability was tested under most optimal circumstances.







5

A COMPARISON OF DIFFERENT METHODS TO QUANTIFY STRETCH REFLEXES IN CHILDREN WITH CEREBRAL PALSY

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In preparation

Abstract

Background. Joint hyper-resistance is a common impairment in children with spastic cerebral palsy (CP) and can limit activities such as walking. Stretch hyperreflexia is the main neural component of joint hyper-resistance. Several assessment methods have been developed to assess stretch hyperreflexia in the ankle joint. However, the methods have not been validated as a gold standard is lacking. Furthermore, although most methods are designed based on the same concept of stretch hyperreflexia, it is unknown whether they give similar outcomes. Therefore, this study aimed to compare several currently available methods for assessing stretch hyperreflexia.

Method. Five assessment methods were compared in 18 children with spastic CP, four tests applying passive stretches to evoke reflexes, and one functional test analyzing stretch reflexes during walking. The spasticity test (SPAT) is a currently used clinical test based on manual perturbations of the ankle joint to evoke calf muscle stretch. Three different motorized tests, a motorized SPAT, an eight-degree stretch reflex test, and a system-identification method, assessed the responses to perturbations around the ankle joint, with perturbations applied using a robotic device. The functional assessment method consists of analyzing stretch reflexes during the swing and stance phase of gait, by calculating the ratio between muscle activation and fascicle lengthening velocity. The outcomes of the different methods were compared using Spearman and Pearson correlations and a bootstrapping method to define 95% confidence intervals (CI).

Results. The eight-degree stretch reflex was strongly correlated with the motorized SPAT (r=0.70, CI 0.00 - 0.96) and with the system-identification method (r=0.84, CI 0.48 - 0.98), but the motorized SPAT was not correlated with the system-identification method (r=0.10, CI -0.24 - 0.80). In addition, neither the SPAT, nor the functional assessments were correlated to any other assessment method.

Interpretation. Although different assessment methods are based on similar concepts, they cannot be used interchangeably. Population-specific limitations, such as anxiety for motorized assessments, can partly explain these results. Furthermore, the magnitude of stretch reflexes likely differs between passive and active conditions. In addition, the concept of stretch hyperreflexia might be too complex to capture with one assessment method.

5.1 Introduction

Increased joint resistance, i.e. joint hyper-resistance, is a common impairment in patients with neurological disorders such as cerebral palsy (CP) and spinal cord injury. The hyper-resistance limits the joints' active and passive range of motion (RoM). Moreover, it can severely impair walking and independent function in ambulatory patients.[1] Joint hyper-resistance can be caused by non-neural and neural impairments (Fig. 5.1),[1] and treatment can be adjusted to the underlying causes of hyper-resistance.

Non-neural impairments are caused by altered tissue properties, and often treated by lengthening the muscle through stretching, casting, or surgery. Neural impairments include involuntary background muscle activation and exaggerated stretch reflexes, i.e. stretch hyperreflexia (Fig. 5.1). Therefore, treatment of neural impairments focuses on reducing muscle activation by botulinum toxin injections, baclofen, or selective dorsal rhizotomy. Besides, several studies have shown some improvements in reducing stretch hyperreflexia using biofeedback,[2–6] but this has not yet been implemented in clinical care.

It is important to correctly assess joint hyper-resistance to guide treatment dosage and evaluate treatment outcome. In clinical settings, this is most often done using manual passive stretching of the muscle and rating the perceived resistance, for example with the modified Ashworth Scale,[7] the (Modified) Tardieu Scale[8,9], or the Spasticity Test (SPAT)[10]. It is now well established, from various studies, that these clinical tests have several limitations.[7,11–14] First, both the applied movement and judging the amount of hyper-resistance require subjective assessor interpretations, resulting in poor reliability. Second, it is difficult to distinguish the different causes of perceived resistance. Third, all manual clinical scales possess low resolution due to the use of ordinal scales, which are insufficient for most treatment evaluations and inadequate for biofeedback applications.

To counteract the limitations of the clinical tests, several standardized methods have been developed to assess joint hyper-resistance. These involve motorization using robotic manipulators to impose a standardized movement, and instrumentations such as torque sensors and EMG to measure the responses. These methods eliminate the subjectivity of the applied movement and perceived

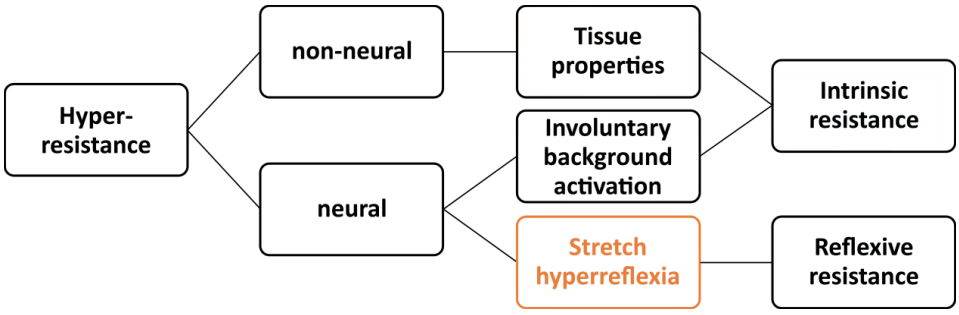


Figure 5.1. Terminology of joint hyper-resistance. Figure adapted from Van den Noort et al. [15]

resistance and can generate higher-resolution outcomes. Outcome measures can be either biomechanical or neurological. The biomechanical response, i.e. the resulting joint torque, is most often incorporated in instrumented and motorized stretch reflex assessments and most closely resembles the currently used clinical tests. Nevertheless, neurological measures, for instance as quantified using electromyography (EMG), are more closely related to the reflex pathways and allow for discrimination between muscle contributions. On the other hand, the biomechanical measures have better repeatability than neurological measures[16] and do not require normalization, which is particularly difficult in children with CP.[17] However, most torque-based methods cannot distinguish between the neural and non-neural velocity-dependent components, i.e. distinguishing stretch hyperreflexia from the velocity-dependent viscoelastic component of the musculo-tendon unit, which is possible with the neurological response.

Available motorized assessment methods to assess stretch hyperreflexia around the ankle joint differ in the applied movement profiles and outcome calculations.[18–21] For example, a robotic device can apply slow and fast joint rotations around the ankle joint over a large RoM as done in a motorized version of the SPAT.[22] The biomechanical resistance during slow rotations indicates the intrinsic resistance, i.e., a combination of the non-neural component of hyper-resistance and the background muscle activity. The additional resistance during fast rotations indicates the reflexive resistance, i.e., stretch hyperreflexia (see Fig. 5.1 for terminology). Other methods consist of smaller movement profiles, such as fast eight-degree perturbations to evoke stretch reflexes.[2,4] The resulting resistance during the perturbation can be used as an overall measure of joint hyper-resistance, and the resulting muscle activation can be used as a neurological outcome to estimate stretch hyperreflexia. Finally, several model-based methods exist to disentangle intrinsic and reflexive joint resistance, for example using a parallel-cascade system identification technique. Movement profiles of system identification techniques can be performed over full RoM or restricted ranges, such as two-degree ramp-and-hold perturbations. [2,23]

A limitation of the aforementioned clinical and motorized assessments is their questionable representation of joint hyper-resistance during a functional task, such as walking. Studies assessing h-reflexes - the electrical analog of the stretch reflex - show that modulation of reflex magnitudes between different activities is impaired in children with CP.[24,25] Several methods have been proposed to assess reflexive resistance during walking.[24–31] Most studies analyzing gait of children with CP found evidence for increased reflexive resistance in children with CP, which can occur in the late swing and/or early stance phase of gait.[27–30] Yet, it is unknown whether children having larger stretch hyperreflexia as measured with motorized passive stretches also experience larger stretch hyperreflexia during gait.

All proposed methods are developed to assess the same concept of stretch hyperreflexia. However, it is unclear if the various methods are indeed correlated. Validity of stretch hyperreflexia methods is often limited to comparing patients with controls.[28,29,32–34] Comparing stretch hyperreflexia methods with each other can provide additional information regarding the validity. Our group previously showed that the motorized SPAT and system identification methods correlated strongly in patients with SCI/stroke.[22] Still, it is unknown if the different assessment methods are correlated in children with CP.

Therefore, this study compares the outcomes of four passive and one functional assessment method for stretch hyperreflexia in children with CP (Fig. 5.2). The passive tests consist of one clinically used manual assessment method, the SPAT ($SPAT_{man}$), and three motorized assessment methods, the motorized SPAT ($SPAT_{mot}$), eight-degree stretch reflex ($8°SR_{torque}$, and $8°SR_{EMG}$), and parallel-cascade system identification (SI). The functional test consists of analyzing the ratio between muscle stretch and muscle activation during walking in the stance ($Func_{stance}$) and swing phase ($Func_{swing}$).

5.2 Methods

Participants

Eighteen children diagnosed with uni- or bilateral spastic CP or related forms of spastic paresis (further referred to as 'CP' for the whole group) participated in this cross-sectional study. Children were aged 6-17, classified with gross motor function classification system (GMFCS[35]) level I/II, and had a SPAT score > 0 in the m. soleus or m. gastrocnemius medialis indicating the presence of stretch hyperreflexia.[10] Additionally, children had to be able to follow simple instructions and walk for approximately half an hour in total with sufficient rest. Exclusion criteria were orthopedic surgery on the legs (<12 months), lower limb botulinum toxin-A injections (<6 months), selective dorsal rhizotomy surgery, frequent epilepsy, behavioral problems, and comorbidities affecting gait. The study protocol was approved by the local medical ethics committee of the VU University Medical Center (NL65846.029.18) and conformed to the Declaration of Helsinki guidelines. Parents of participants under sixteen and all participants twelve years and older provided written informed consent.

Experimental protocol

The experimental protocol consisted of performing the manual SPAT, three motorized assessments using an ankle dynamometer (see Fig. 5.3) and a functional measurement on a treadmill (Fig. 5.2), all performed on the same day, with motorized and functional assessments in a randomized order. All tests on the dynamometer were performed in the same room, with at least two minutes of rest and additional breaks provided when necessary between measurements. EMG electrodes were placed on the m. gastrocnemius medialis, m. soleus and m. tibialis anterior and remained on the lower leg between the assessments to ensure identical positioning.

The functional protocol is extensively described in our previous work.[29] Shortly, 3D gait analysis was performed while children walked on a split-belt instrumented treadmill at a self-selected walking speed. M. gastrocnemius medialis EMG was captured, and fascicle stretch was assessed through dynamic ultrasound imaging and differentiated to obtain fascicle lengthening velocity. Reflective markers were placed according to the Human Body Model markerset[36] and used to define strides from initial contact to next initial contact.[37]

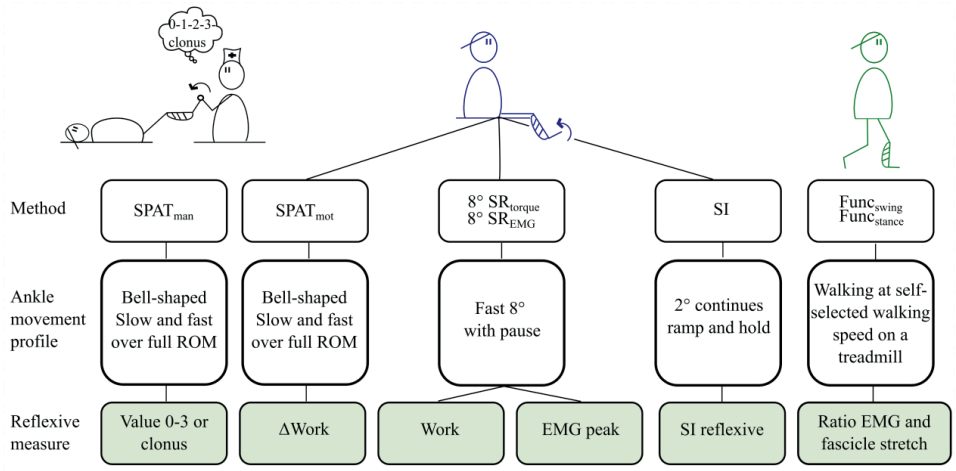


Figure 5.2. Different methods to assess stretch hyperreflexia. The first four measurements were performed while the participants remained passive. The manual SPAT ($SPAT_{man}$) was performed by manually rotating the ankle joint. The motorized SPAT ($SPAT_{mot}$), eight-degree stretch reflex (8°SR), and system identification (SI) were performed by a motorized rotation of the footplate of the dynanometer. The functional measurement ($Func_{swing}$ and $Func_{stance}$) as performed on a split-belt instrumented treadmill.

Manual SPAT

Passive levels of stretch hyperreflexia were manually determined using the SPAT[10] ($SPAT_{man}$), by applying slow (≥3 seconds) and fast (<1 second) angular dorsiflexion rotations around the ankle joint with extended knee to stretch the m. gastrocnemius medialis. The clinician graded the intensity of the felt muscle resistance during the fast velocity stretch on a 0–3 scale[10] or as the presence of clonus, which was labeled as level four for further analysis.

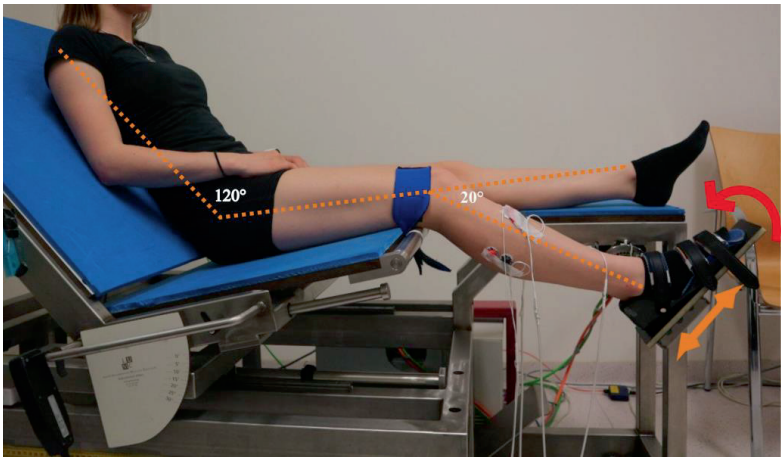


Figure 5.3. Experimental setup. Subjects were seated with a fixed 120° hip angle and a knee angle adjusted to their maximum knee extension angle minus 20°. The knee and foot were supported with Velcro straps to prevent knee movements during ankle perturbations. The position of the footplate was adjusted in the direction of the orange arrow to align the plantarflexion rotation axis optimally.

Ankle dynamometer

The three motorized passive assessments were performed on an ankle dynamometer, a single-axis actuator (MOOG, Nieuw-Vennep, the Netherlands) designed to apply rotations around the ankle joint in the sagittal plane (Fig. 5.3). The chair was individually adjusted to ensure similar conditions for all participants. The most affected leg, as clinically diagnosed, was attached to a rigid footplate with Velcro straps. The footplate position was adjusted to align the ankle's plantarflexion rotation axis with the actuator's rotation axis. Alignment was controlled by minimizing movement in the knee joint during ankle manipulation. Furthermore, the upper leg was supported with Velcro straps to prevent knee movements. Children were seated with a fixed 120° hip angle and knee angle set at maximum knee extension minus 20°.

Similar to the knee joint, and ankle joint alignments were standardized to help accommodate individual joint configurations, as is often deviating in children with CP. Footplate movements were restricted using hardware pins around the ankle joint's maximum passive RoM (pRoM) as indicated by the child. All movements kept at least 2° margin with respect to the maximum pRoM limits. Furthermore, the maximum torque exerted by the footplate was constrained to 20 Nm (children <12 years) or 40 Nm (children >13 years). The $SPAT_{mot}$ was performed over the entire RoM minus the 2° margin. The starting ankle angle for the SI and eight-degree stretch reflex was set at that angle where a resistance torque of body weight * 0.05 was measured, i.e. at torques between 1 and 3.6 Nm, similar to torques applied in previous studies.[38] The angle corresponding to this torque was determined by applying slow movements around the ankle joint and assessing the resistance. In case of absent reflexes at the starting angle, the ankle angle was increased incrementally with 10 degrees towards dorsiflexion, within the RoM limits. All joint angles were noted and kept similar between assessments.

Footplate angle, angular velocity, and torque were measured at 2048 Hz, with the positive angle and torque defined in the dorsiflexion direction. The resulting torque was measured and corrected for the gravitational effects of the footplate and foot. Electromyographic (EMG) activity of the m. gastrocnemius medialis, m. soleus and m. tibialis anterior was recorded with a porti EMG system (TMSi, Oldenzaal, the Netherlands), also at 2048 Hz, with electrodes placed according to the SENIAM guidelines (http://www.seniam.org/). EMG signals were high-pass filtered (2^{nd} order Butterworth, 5 Hz), rectified, and normalized to the average EMG activity during treadmill walking.[39] During the perturbations, children were instructed to relax and ignore any movements of the footplate. Muscle activation levels were monitored during the experiments, and children were encouraged to relax if unexpected increases in muscle activity were visible in either the m. gastrocnemius medialis, m. soleus or m. tibialis anterior.

Motorized SPAT

The experiment started with a motorized version of the SPAT ($SPAT_{mot}$), as described in our previous work.[22] Shortly, the experiment consisted of two slow (10°/s) and six fast (max 1a50°/s) movements around the ankle joint, with a one-second hold at the maximum dorsiflexion angle. Based on previous research, a bell-shaped movement profile was chosen to better mimic ankle movement profiles during the passive clinical tests and gait.[21,40] Movements were separated by at least 10s rest. They were repeated in case of unexpected increased voluntary muscle activity or torque before or during the movements, as visually determined by the researcher. The work (area between the torque-angle

curves) around the ankle was used to quantify joint resistance during the slow and fast movements. Work was calculated from the starting angle at torque bodyweight * 0.05 up to 90% pRoM.

Eight-degree stretch reflex

The eight-degree stretch reflex[2] consisted of perturbations of 8 degrees maximum amplitude, maximum angular velocity of 190°/s, maximum angular accelerations of 8000 °/s^2 and 66 ms duration. The ankle remained dorsiflexed for 300 ms before slowly returning to the starting angle. Perturbations were separated with short resting periods in which children had to return to baseline torque levels.

The resulting resistance was assessed as the total work, calculated as the area under the curve over a 200ms window, starting 120ms after perturbation onset to avoid inertial influences. In line with our previous study on the conditioning of stretch reflex magnitudes, increases in muscle activation due to the eight-degree stretch reflex were also calculated. The EMG analysis allows for a better comparison with the functional measure, as the functional measure is also based on muscle activation. The root mean square (RMS) EMG activity was assessed during a manually determined 100 ms period, chosen to include the M1 and M2 activity. The RMS was corrected for baseline activity prior to the perturbation.

System-identification method

Reflexive resistance was calculated using a parallel-cascade SI algorithm.[22] Shortly, ramp-and-hold perturbations were applied for two minutes, with a 125 °/s max velocity, 15800 °/s^2 maximum acceleration and 40ms duration. The measured angle, velocity and torque signals were used as input of a system identification algorithm (calculations correspond to the first five steps described in our previous work[22]) to compute the reflexive response. Consequently, the reflexive resistance was calculated as the integral of the reflexive response. This deviates from the protocol in our previous study, where the reflexive response is calculated with a non-linear least squares fit,[22] as several children showed low reflex magnitudes, resulting in high standard error of measurements when fitting the reflexive resistance.

Functional measure: stretch reflex magnitude during gait

The stretch reflex magnitude during gait was assessed by relating m. gastrocnemius medialis activation to stretch of the fascicles, as described extensively in our previous work. Shortly, peak muscle activation was assessed by calculating RMS EMG activity during the late swing and early stance phase of gait. Consequently, maximum fascicle lengthening velocity was calculated in a window 40-120 ms before the EMG activity, considering the stretch reflex delays (Fig. 5.4). The ratio between the peak muscle activation and peak fascicle lengthening velocity was considered a measure of the magnitude of the stretch reflexes and calculated separately during stance and swing.

Statistical analysis

The different measures were compared using a Spearman rank correlation for the relation of the $SPAT_{man}$ with the other measures, given the nominal scale of this test, and Pearson correlations for all other comparisons. Correlations >0.8 were considered very strong; 0.60–0.80 strong; 0.40–0.60 moderate; 0.20–0.40 fair; and <0.20 weak.[41] Furthermore, 95% confidence intervals were calculated using a non-parametric bootstrap procedure, using the bias-corrected and accelerated method.[42] Correlations were considered significant when 0 was not within the 95% bootstrap confidence interval.

5.3 Results

Eighteen children with CP participated, eight female and ten male, with an average age of 11±3 y, height of 1.5±0.2 m, and 39±15 kg. Nine were classified as GMFCS level I and nine as level II; ten had unilateral and eight bilateral CP; six were most affected on the left side, and 12 on the right. Three children (all GMFCS level II and clonus) could not perform the $SPAT_{mot}$ movement due to anxiety and were further excluded from comparisons involving the $SPAT_{mot}$. One child requested an adjusted ROM during the $SPAT_{mot}$ because of feeling uncomfortable, but remained included as this ROM included the end dorsiflexion, resulting in 15 included participants for the $SPAT_{mot}$. Four children were excluded from the functional assessment due to technical issues,[29] resulting in 14 children for the functional assessment.

The included children's average ankle range of motion was 40.9±8.7 degrees, ranging from 27-58 degrees. The SI and 8°SR were performed at the starting angle at which a resistance of 0.05*bodyweight Nm was encountered for ten children (average 16 degrees ankle plantarflexion angle). For eight other children, the starting angle at 0.05*bodyweight Nm did not result in visible responses in the torque (Fig. 5.5L) during the SI perturbations; therefore the dorsiflexion angle was increased within the possibilities of the pRoM. For six children, the starting angle was moved 10 degrees towards dorsiflexion (3 $SPAT_{man}$ I and 3 $SPAT_{man}$ II, average zero degrees ankle plantarflexion angle), and for two children this was plus 20 degrees ($SPAT_{man}$ I and clonus, average 1.5 degrees dorsiflexion angle). The average ankle angle for all participants was 8.8±11.7 degrees plantar flexion.

The stretch reflex magnitudes, as measured using the five assessment methods, strongly varied between participants and between assessment methods. For example, some children showed a clear reflexive response in one measurement, but barely any response to other types of perturbations, whereas other children scored high on all tests. Two participants with generally high and generally low scores on the different assessments are presented in Fig. 5.4 and Fig. 5.5. The participant presented on the left generally scored high on the motorized assessments, but the participant on the right scored higher on the clinical test.

In general, most comparisons did not reveal significant correlations, mostly due to very large confidence intervals. Overall, the $8°SR_{torque}$ showed a strong correlation with the SI (r=0.84, [0.48-0.98]) and the $SPAT_{mot}$ (r=0.70, [0.00, 0.96]; Fig. 5.6, Table 5.1). There was no correlation between the SI and the $SPAT_{mot}$ (r=0.34, [-0.24, 0.80]). $Func_{swing}$ and $Func_{stance}$ only showed a moderate correlation (r=0.50, [0.02, 0.82]). All other parameters showed no significant correlation (see Table 5.1 and Fig. S5.1).

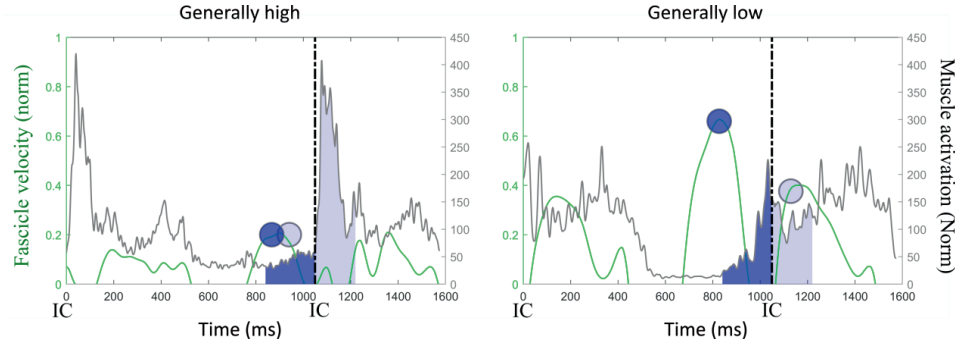


Figure 5.4. Example data of the same two children as Fig. 5.5, showing one and a half strides for fascicle lengthening velocity (normalized to tibia length) and m. gastrocnemius medialis activation (normalized to average EMG during a stride). Functional stretch hyperreflexia was calculated as the ratio between the RMS EMG activity (presented in dark blue for late swing and light blue for early stance) and the peak lengthening velocity (presented by the circles) of the fascicles (shown in green) prior to the EMG peak, within a window of 40-120 ms. The left participant only showed increased activation in early stance. This increase can be triggered by the peak fascicle velocity encountered during late swing, resulting in a high EMG/stretch ratio and high reflexive resistance according to the $Func_{stance}$. According to the motorized assessments, the participant presented on the right had generally low reflexive resistance, but did experience increased activity during both late swing and early stance. The muscle activation was preceded by high fascicle velocity, as is expected for children with lower stretch reflexes. Therefore, the resulting reflexive resistance was lower in the stance phase for this participant compared to the participant with generally high scores on reflexive resistance. Nevertheless, both children appeared to encounter stretch reflex activity while walking.[29]

Figure 5.5: On the right page: Example data of two children, with generally high (left) and low reflexive resistance (right), corresponding to the upward and downward blue triangles respectively in Fig. 5.6. Light blue shaded areas indicate the area over which a parameter is calculated. Angle/torque are the estimated anke angle and torque and EMG regards muscle activation of the m. gastrocnemius medialis. $SPAT_{man}$ for the m. gastrocnemius medialis is level III for the child presented on the left and clonus for the child presented on the right. **(A,B)** $SPAT_{mot}$ is the area between the fast and slow ankle angle-torque curves, ranging from the starting angle, calculated as 0.05*bodyweight, up till 90% of pRoM. **(C,D)** The 8°SR ankle angle profile, performed around the same starting angle as used in the calculation of the $SPAT_{mot}$ (e.g. 5° plantarflexion; see B,D),. When no reflexes are present, the starting angle was performed in a more dorsiflexed position (e.g. 5° plantarflexion, compared to the 25° plantarflexion starting angle used for the $SPAT_{mot}$; see A,C). **(E,F)** $8°SR_{torque}$ is the area under the ankle torque curve over a 200ms window, starting 120ms after perturbation onset. **(G,H)** $8°SR_{EMG}$ is the RMS EMG (corrected for baseline activity) calculated over a manually determined participant-specific 100ms window. **(I,J)** SI ankle movement profile, around same starting angle as 8°SR (C,D). **(K,L)** Torque from the SI method was used as input for the parallel-cascade system identification, where patients with large reflexive resistance showed a clear increase in torque after the perturbation (K). In contrast, patients with low reflexive resistance showed no increases in torque shortly after the perturbations (L).

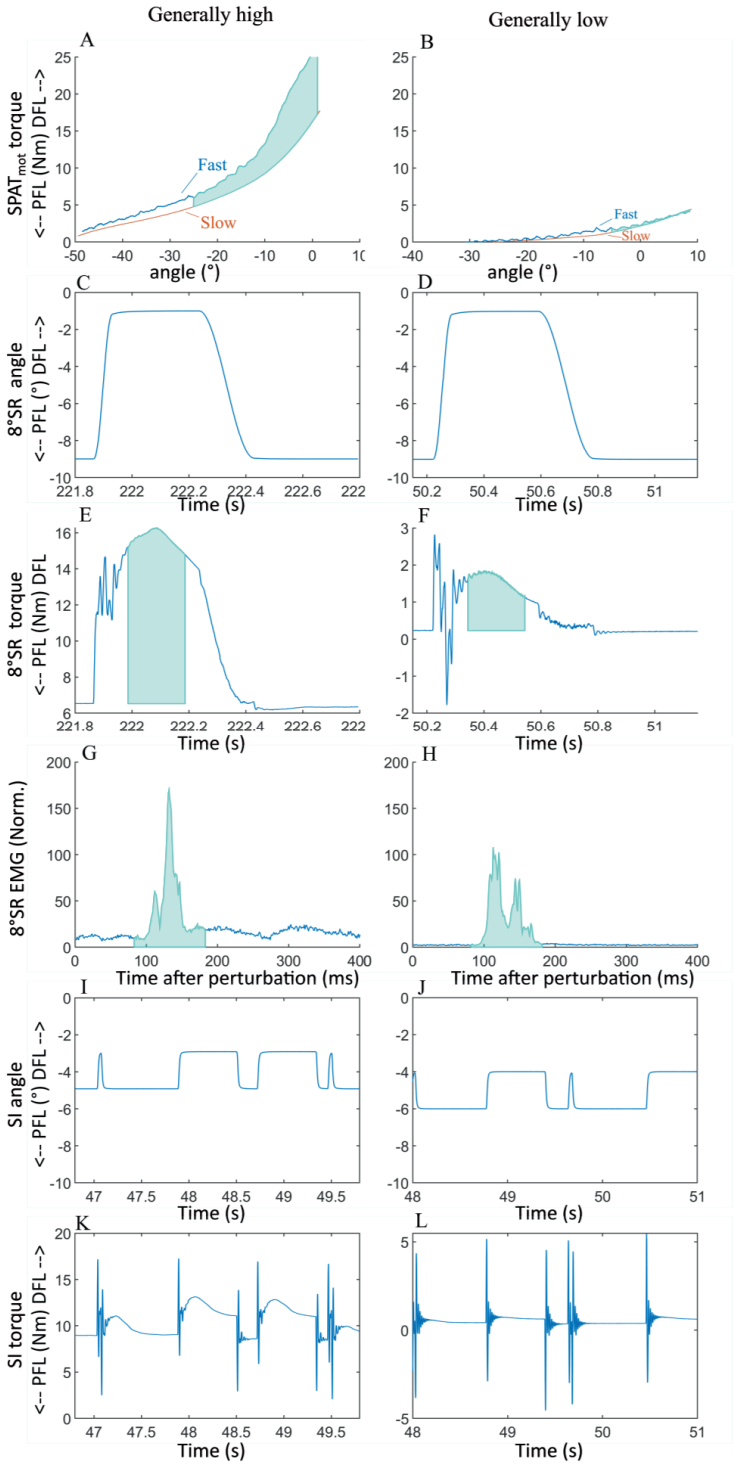

Generally high
Generally low
A
B
SPAT$_{mot}$ torque
<-- PFL (Nm) DFL -->
Fast
Slow
angle (°)
C
D
8°SR angle
<-- PFL (°) DFL -->
Time (s)
E
F
8°SR torque
<-- PFL (Nm) DFL
G
H
8°SR EMG (Norm.)
Time after perturbation (ms)
I
J
SI angle
<-- PFL (°) DFL -->
K
L
SI torque
<-- PFL (Nm) DFL -->

Table 5.1. Correlation coefficient values between the different parameters

		$SPAT_{mot}$	$8°SR_{torque}$	$8°SR_{EMG}$	SI	$Func_{swing}$	$Func_{stance}$
$SPAT_{man}$	ρ	-0.40	-0.06	0.25	0.18	-0.06	-0.23
	CI	[-0.77, 0.20]	[-0.63, 0.50]	[-0.43, 0.72]	[-0.36, 0.61]	[-0.65, 0.55]	[-0.71, 0.52]
	$SPAT_{mot}$	r	**0.70**	-0.32	0.34	0.04	0.58
		CI	**[0.00, 0.96]**	[-0.78, 0.47]	[-0.24, 0.80]	[-0.53, 0.71]	[-0.16, 0.89]
		$8°SR_{torque}$	r	0.10	**0.84**	-0.03	0.63
			CI	[-0.55, 0.65]	**[0.48, 0.98]**	[-0.69, 0.78]	[-0.64, 0.98]
			$8°SR_{EMG}$	r	0.02	0.25	-0.29
				CI	[-0.54, 0.50]	[-0.50, 0.81]	[-0.72, 0.20]
				SI	r	-0.24	0.34
					CI	[-0.75, 0.60]	[-0.85, 0.96]
					$Func_{swing}$	r	**0.50**
						CI	**[0.02, 0.82]**

Note. Spearman's rank correlation coefficients rho were calculated for the $SPAT_{man}$ (first row) and Pearson's r correlation coefficients for all other tests. 95% confidence intervals are shown below the rho/r values. Bold values indicate correlations that were significant based on the bootstrap interval.

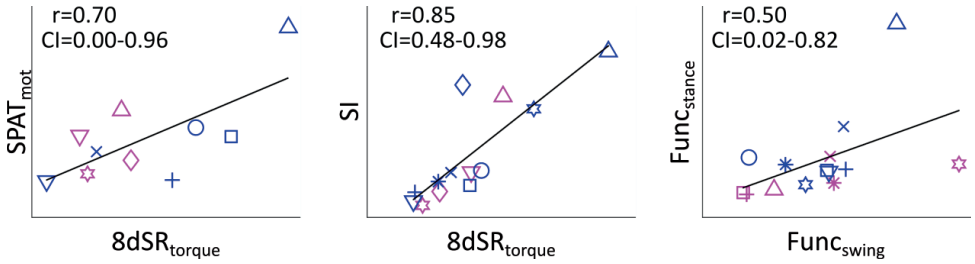


Figure 5.6. Correlation plots of the three significant correlations. Individual participants are presented with their subject-specific symbol-color combination. All non-significant correlation plots are presented in the Supplementary Materials 5.1, Fig. S5.1.

5.4 Discussion

This study compared five methods to evaluate stretch hyperreflexia in children with spastic CP. For the passive assessment methods, we found strong correlations between the motorized $8°SR_{torque}$, the SI and the $SPAT_{mot}$, but not with the currently used clinical test, the $SPAT_{man}$. Furthermore, the functional assessment method showed no correlation with any of the four passive assessment methods. Although all assessment methods aim to asses stretch hyperreflexia, results were often uncorrelated.

The poor correlation of passive clinical test scores such as the $SPAT_{man}$ with passive motorized assessments aligns with previous literature.[14,18,19,43–45] This may be because clinical tests have poor reliability, repeatability, and sensitivity,[1,12,14,46] leading to variation in measurements which does not represent true variations in stretch reflex magnitudes. Instrumentation of these tests, i.e. through the assessment of torque or muscle activation, decreases objectivity, increases specificity, and generally increases reliability.[16] A remaining source of variation regards the imposed movement profile, and this variation can be reduced through motorization of assessments.[16,21] Sloot et al.[21] showed that manual

and motorized assessments have different movement profiles, potentially explaining differences in neurological outcomes. Upon their suggestion, we replaced the ramp-and-hold rotations with a bell-shaped movement profile during the $SPAT_{mot}$, to better mimic ankle movement during the $SPAT_{man}$ and regular walking.[21] Nevertheless, the $SPAT_{man}$ and $SPAT_{mot}$ remained uncorrelated.

There are several population-specific limitations of the motorized assessment methods that can explain the poor correlations in the current study. First, three children in our study, all clinically presenting with clonus in the calf muscles, experienced anxiety during the $SPAT_{mot}$ and were excluded from this assessment. Sloot et al.[47] showed the feasibility of this test in 13 children with CP before, yet none of them had clonus. Although not assessed, some anxiety might be present in the remaining children, which could have influenced the reflex magnitude.[48] Graser et al.[49] reported similar issues when assessing upper limb function in children with CP using an exoskeleton device. They opted that several children showed increased resistance as they experienced the moving exoskeleton as frightening. Therefore, anxiety appears to be a critical limiting factor for the clinical feasibility of motorized assessment methods. A second limitation is that assessing the pRoM in a robotic device is difficult, especially for children with CP. Anxiety for the robotic device can decrease the measured pRoM. Bony deformities can further limit the measured pRoM due to incorrect alignment of the plantar/dorsiflexion angle with the actuator of the robotic device. The assessed pRoM was additionally reduced in some participants due to safety regulations from the robotic manipulator, as mechanical end-stops could only be adjusted in increments of ten degrees and were always positioned within the actual pRoM. The motorized assessments may underestimate the reflexive resistance due to insufficient pRoM. Particularly, the $SPAT_{mot}$ depends on the maximum dorsiflexion angle, and underestimating the pRoM decreases the measured stretch reflex magnitude.[49,50] Therefore, the population-specific limitations in determining the pRoM limit accuracy of the motorized assessments.

The motorized assessment methods showed stronger mutual correlations: the $8°SR_{torque}$ strongly correlated with the SI and the $SPAT_{mot}$, indicating that these assessment methods measure similar aspects of the concept of stretch hyperreflexia. We did not find a correlation between the SI and the $SPAT_{mot}$. The motorized assessments differ in movement profiles, with different starting angles and movement ranges. The SI and 8°SR methods showed higher feasibility in terms of anxiety, but only assessed responses around a limited range within the full joint RoM, as opposed to the large RoM of the $SPAT_{mot}$. Reflexive resistance magnitudes highly depend on the analyzed angle,[51,52] muscle pre-activation level, and movement history.[53,54] This can explain the lack of correlation of the $SPAT_{mot}$ with the SI method and the large confidence interval (0.00-0.96) of the correlation with the $8°SR_{torque}$ method. Besides, the dependency on starting angle[51,52] can explain why the SI method was correlated with the $8°SR_{torque}$ method, both performed from the same starting angle, but not with the $SPAT_{mot}$. We conclude that generalization of the SI and $8°SR_{torque}$ method to reflexes experienced in the entire ROM appears low. Both tests only sample the hypersensitivity at one specified angle of the complete range. A more complete picture could be obtained when these would be assessed over multiple angles throughout the pRoM.

In contrast, we previously found a very strong positive correlation between the SI and $SPAT_{mot}$ in a similar research setup for patients with spinal cord injury or stroke.[22] The population-specific limitations regarding the anxiety and the starting angles can cause the absence of a positive correlation in our study. An increase in resistance to passive movements, for example due to anxiety,[49] increases

torque levels, influencing the torque-based starting angle. Alternatively, starting angles can be based on pRoM, as in our previous study,[22] but this does not eliminate the population-specific limitations. Notwithstanding, the chosen starting angles in our study correspond to the angles at which reflexive resistance is generally increased in adults with stroke[51] and children with CP.[55] Therefore, the motorized assessments were expected able to capture reflexive resistance. Potentially, differences due to starting angles exceed the between-subject differences in stretch reflexes, despite the heterogeneous population included in our study.

We did not find a correlation between the neurological ($8°SR_{EMG}$) and biomechanical ($8°SR_{torque}$) versions of the 8°SR. This agrees with previous studies relating torque and EMG activation in the m. gastrocnemius medialis in children with CP[33] or the wrist muscles in stroke,[56] who found no consistent relation between neurological and biomechanical outcomes. Relating EMG and torque presents with several limitations. First, the resulting torque is generated by a combination of muscles and passive connective tissues around the joint and therefore not directly linked to the activity of a single muscle. Secondly, normalization of EMG is challenging in children with CP, as it is difficult to perform a maximum voluntary contraction due to reduced selective motor control.[57] Erroneous normalization will result in erroneous scaling of the ratio and more variability. Moreover, muscle-tissue properties are often altered in children with CP,[58,59] resulting in different muscle-activation – force relations. Torque- and EMG-based methods might capture complementary information on the magnitude of stretch hyperreflexia, but cannot be used interchangeably.

This study adds that the clinical test score also relates poorly to a functional measure of stretch hyperreflexia during gait. Therefore, scores on clinical tests do not appear to be informative of the stretch reflex magnitudes encountered during daily life. Additionally, the lack of correlation between this study's passive motorized and functional methods adds to the evidence that stretch reflex values obtained in passive conditions do not relate to those obtained in functional tasks.[53,60–62] Poor correlations between EMG-based functional method and torque-based motorized assessment can be caused by the poor correlation between neurological and biomechanical methods, as stated previously. Yet, the $8°SR_{EMG}$ also showed no correlation with the EMG-based functional assessment method. Explanations are the impaired modulation of the reflex loop between activities[24] and that stretch reflexes magnitudes are susceptible to many variables such as initial muscle activation,[53] antagonistic muscle activation,[63] muscle force,[64] and movement history.[53] Therefore, most currently used passive stretch hyperreflexia assessments might not reflect limitations encountered during activities of daily life, such as walking.

The functional assessment method can also not be considered the gold standard, and several limitations are present for the functional assessment of stretch hyperreflexia. For example, this study assessed stretch hyperreflexia as the ratio between muscle activation and fascicle lengthening velocity during gait. However, other pathways might be involved in triggering stretch reflexes.[29] Furthermore, the passive methods are based on the responses to external perturbations, whereas the functional assessment method is based on muscle activation during regular walking. Therefore, it cannot be said with certainty that pathological muscle activation during gait is caused by stretches evoked on the muscles. Adding perturbations, for example by means of treadmill perturbations,[28] increases this certainty, and such analysis might therefore show a stronger correlation with passive assessment methods. However, even though such analysis might better show hypersensitive reflexes during

perturbed walking, it still needs to be shown how these relate to measures during unperturbed walking.

Results from this study provide several implications. First, the absence of correlations between passive and functional assessment methods does not disregard the potential of either type of measurement. Passive assessments can provide valuable information for non-ambulatory patients. Furthermore, passive motorized assessments have the potential to assess the main contributor underlying the joint hyper-resistance, i.e. intrinsic or reflexive resistance,[65] which is more difficult during gait, and can therefore provide information on the underlying pathology. When particularly interested in optimizing the gait pattern, it might be more valuable to assess the reflex magnitude using a functional measure directly. From the results of this study, we conclude that the $SPAT_{mot}$ is unsuitable for children with CP, given the population-specific limitations, including anxiety. An instrumented SPAT in which rotations are carried out manually will likely reduce anxiety levels and may be preferred for full pRoM assessments.[21,33] The parallel-cascade system identification method and the eight-degree stretch reflex method might provide valuable information when assessed over several angles within the pRoM to provide more generalized information regarding reflexive resistance over a functional RoM. Passive and functional assessment methods can provide complementary information, and neurological and biomechanical outcome measures can also be complementary. Generally, the concept of stretch hyperreflexia might be too difficult to capture with one assessment method.

References

1. van den Noort JC, Bar-On L, Aertbeliën E, et al. European consensus on the concepts and measurement of the pathophysiological neuromuscular responses to passive muscle stretch. *Eur J Neurol* 2017; 24: 981-e38.
2. van 't Veld RC, Flux E, Schouten AC, van der Krogt MM, van der Kooij H, van Asseldonk EHF. Reducing the Soleus Stretch Reflex With Conditioning: Exploring Game- and Impedance-Based Biofeedback. *Front Rehabil Sci* 2021; 2: 1–13.
3. Thompson AK, Chen XY, Wolpaw JR. Acquisition of a Simple Motor Skill: Task-Dependent Adaptation Plus Long-Term Change in the Human Soleus H-Reflex. *J Neurosci* 2009; 29. DOI:10.1523/JNEUROSCI.4326-08.2009.
4. Mrachacz-Kersting N, Kersting UG, de Brito Silva P, et al. Acquisition of a simple motor skill: Task-dependent adaptation and long-term changes in the human soleus stretch reflex. *J Neurophysiol* 2019; 122: 435–46.
5. O'Dwyer N, Neilson P, Nash J. Reduction of spasticity in cerebral palsy using feedback of the tonic stretch reflex: a controlled study. *Dev Med Child Neurol* 1994; 35: 770–86.
6. Nash J, Neilson PD, O'Dwyer NJ. Reduing spasticity to control muscle contracture of children with cerebral palsy. *Dev Med Child Neurol* 1989; 31: 471–80.
7. Bohannon RW, Smith MB. Interrater reliability of a modified Ashworth scale of muscle spasticity. *Phys Ther* 1987; 67: 206–7.
8. Tardieu G. A la recherche d'une technique de mesure de la spasticite. *Rev neurol* 1954; 91: 143–4.
9. Boyd RN, Graham HK, Kerr Graham H. Objective measurement of clinical findings in the use of botulinum toxin type A for the management of children with cerebral palsy. *Eur J Neurol* 1999; 6: s23–35.
10. Scholtes VAB, Dallmeijer AJ, Becher JG. The Spasticity Test: a clinical instrument to measure spasticity in children with cerebral palsy. In: The Effectiveness of Multilevel Botulinum Toxin Type A and Comprehensive Rehabilitation in Children with Cerebral Palsy. Citeseer, 2007: 29–64.
11. Scholtes VAB, Becher JG, Lankhorst GJ. Clinical assessment of spasticity in children with cerebral palsy: a critical review of available instruments. *Dev Med Child Neurol* 2006; 48: 64–73.
12. Haugh AB, Pandyan AD, Johnson GR. A systematic review of the Tardieu Scale for the measurement of spasticity. *Disabil Rehabil* 2006; 28: 899–907.
13. Charalambous CP. Interrater reliability of a modified ashworth scale of muscle spasticity. In: Classic Papers in Orthopaedics. Springer-Verlag London Ltd, 2014: 415–7.
14. Fleuren JFM, Voerman GE, Erren-Wolters C V, et al. Stop using the Ashworth Scale for the assessment of spasticity. *J Neurol* 2009; 81: 46.
15. van den Noort JC, Steenbrink F, Roeles S, Harlaar J. Real-time visual feedback for gait retraining: toward application in knee osteoarthritis. *Med Biol Eng Comput* 2015; 53: 275–86.
16. Wood DE, Burridge JH, Wijck FM Van, et al. Biomechanical approaches applied to the lower and upper limb for the measurement of spasticity: a systematic review of the literature. *Disabil Rehabil* 2005; 27: 19–33.
17. Sousa ASP, Tavares JRMS. Surface electromyographic amplitude normalization methods: A review. In: Electromyography: new developments, procedures and applications. 2012. https://repositorio-aberto.up.pt/bitstream/10216/64430/2/67854.pdf (accessed 19 Aug 2021).
18. van 't Veld RC, Schouten AC, van der Kooij H, van Asseldonk EHF. Neurophysiological validation of simultaneous intrinsic and reflexive joint impedance estimates. *J Neuroeng Rehabil* 2021; 18: 1–12.
19. Bar-On L, Aertbeliën E, Molenaers G, Dan B, Desloovere K. Manually controlled instrumented spasticity assessments: a systematic review of psychometric properties. *Dev Med Child Neurol* 2014; 56: 932–50.
20. de Gooijer-van de Groep KL, de Vlugt E, de Groot JH, et al. Differentiation between non-neural and neural contributors to ankle joint stiffness in cerebral palsy. *J Neuroeng Rehabil* 2013; 10: 81.
21. Sloot LH, Bar-On L, van der Krogt MM, et al. Motorized versus manual instrumented spasticity assessment in children with cerebral palsy. *Dev Med Child Neurol* 2017; 59: 145–51.
22. van't Veld RC, Flux E, van Oorschot W, et al. Examining the role of intrinsic and reflexive contributions to ankle joint hyper-resistance treated with botulinum toxin-A. *J Neuroeng Rehabil* 2023; 20: 1–14.
23. Veld RC Van, Schouten AC, Kooij H Van Der, Asseldonk EHF Van. Validation of Online Intrinsic and Reflexive Joint Impedance Estimates using Correlation with EMG Measurements. 2018.
24. Hodapp M, Klisch C, Mall V, Vry J, Berger W, Faist M. Modulation of Soleus H-Reflexes During Gait in Children With Cerebral Palsy. *J Neurophysiol* 2007; 98: 3263–8.

25. Hodapp M, Klisch C, Berger W, Mall V, Faist M. Modulation of soleus H-reflexes during gait in healthy children. *Exp Brain Res* 2007; 178: 252–60.
26. Andersen JBJ, Sinkjaer T. An Actuator System for Investigating Electrophysiological and Biomechanical Feature Around the Human Ankle Joint During Gait. *IEEE Trans Rehabil Eng* 1995; 3: 299–306.
27. Van der Krogt MM, Doorenbosch CAM, Becher JG, Harlaar J. Dynamic spasticity of plantar flexor muscles in cerebral palsy gait. *J Rehabil Med J Rehabil Med J Rehabil Med* 2010; 42: 656–63.
28. Flux E, van der Krogt MM, Harlaar J, Buizer AI, Sloot LH. Functional assessment of stretch hyperreflexia in children with cerebral palsy using treadmill perturbations. *J NeuroEngineering Rehabil 2021 181* 2021; 18: 1–17.
29. Flux E, Mooijekind B, Bar-On L, van Asseldonk E, Buizer AI, van der Krogt MM. Relation between gastrocnemius medialis muscle-tendon stretch and muscle activation during gait in children with cerebral palsy. In: Stretch hyperreflexia in children with cerebral palsy: assessment, contextualization and modulation. 2023.
30. Crenna P. Spasticity and 'Spastic' Gait in Children with Cerebral Palsy. *Neurosci Biobehav Rev* 1998; 22: 571–8.
31. Lamontagne A, Malouin F, Richards CL. Locomotor-Specific measure of spasticity of plantarflexor muscles after stroke. *Arch Phys Med Rehabil* 2001; 82: 1696–704.
32. van der Velden LL, de Koff MAC, Ribbers GM, Selles RW. The diagnostic levels of evidence of instrumented devices for measuring viscoelastic joint properties and spasticity; a systematic review. *J Neuroeng Rehabil* 2022; 19: 1–8.
33. Bar-On L, Aertbeliën E, Wambacq H, et al. A clinical measurement to quantify spasticity in children with cerebral palsy by integration of multidimensional signals. *Gait Posture* 2013; 38: 141–7.
34. Sloot LH, van der Krogt MM, Groep KL de G de, et al. The validity and reliability of modelled neural and tissue properties of the ankle muscles in children with cerebral palsy. *Gait Posture* 2015; 42: 7–15.
35. Palisano R, Rosenbaum P, Bartlett D, et al. Gross Motor Function Classification System. *Dev Med Child Neurol* 1997; 39: 214–23.
36. Flux E, van der Krogt M, P C, et al. The Human Body Model versus conventional gait models for kinematic gait analysis in children with cerebral palsy. *Hum Mov Sci* 2020; 70. DOI:10.1016/J.HUMOV.2020.102585.
37. Zeni JA, Richards JG, Higginson JS. Two simple methods for determining gait events during treadmill and overground walking using kinematic data. *Gait Posture* 2008; 27: 710–4.
38. Bénard MR, Jaspers RT, Huijing PA, Becher JG, Harlaar J. Reproducibility of hand-held ankle dynamometry to measure altered ankle moment-angle characteristics in children with spastic cerebral palsy. *Clin Biomech* 2010; 25: 802–8.
39. Flux E, Bar-On L, Buizer AI, Harlaar J, van der Krogt MM. Electromyographic biofeedback-driven gaming to alter calf muscle activation during gait in children with spastic cerebral palsy. *Gait Posture* 2023; 102: 10–7.
40. Rabita G, Dupont L, Thevenon A, Lensel-Corbeil G, Pérot C, Vanvelcenaher J. Differences in kinematic parameters and plantarflexor reflex responses between manual (Ashworth) and isokinetic mobilisations in spasticity assessment. *Clin Neurophysiol* 2005; 116: 93–100.
41. Altman DG. Practical statistics for medical research. CRC press, 1990.
42. Carpenter J, Bithell J. Bootstrap confidence intervals: when, which, what? A practical guide for medical statisticians. *Stat Med* 2000; 19: 1141–64.
43. Alhusaini AAA, Dean CM, Crosbie J, Shepherd RB, Lewis J. Evaluation of spasticity in children with cerebral palsy using Ashworth and Tardieu Scales compared with laboratory measures. *J Child Neurol* 2010; 25: 1242–7.
44. Bar-On L, Aertbeliën E, Wambacq H, et al. A clinical measurement to quantify spasticity in children with cerebral palsy by integration of multidimensional signals. *Gait Posture* 2013; 38: 141–7.
45. Alibiglou L, Rymer WZ, Harvey RL, Mirbagheri MM. The relation between Ashworth scores and neuromechanical measurements of spasticity following stroke. *J Neuroeng Rehabil* 2008; 5: 1–14.
46. Bar-On L, Molenaers G, Aertbeliën E, et al. Spasticity and its contribution to hypertonia in cerebral palsy. Biomed Res. Int. 2015; 2015. DOI:10.1155/2015/317047.
47. Sloot LH, Weide G, van der Krogt MM, et al. Applying Stretch to Evoke Hyperreflexia in Spasticity Testing: Velocity vs. Acceleration. *Front Bioeng Biotechnol* 2021; 8: 1–10.
48. Sibley KM, Carpenter MG, Perry JC, Frank JS. Effects of postural anxiety on the soleus H-reflex. *Hum Mov Sci* 2007; 26: 103–12.
49. Graser J V., Prospero L, Liesch M, Keller U, van Hedel HJA. Test–retest reliability of upper limb robotic

exoskeleton assessments in children and youths with brain lesions. *Sci Reports 2022 121* 2022; 12: 1–15.
50. Klingels K, De Cock P, Molenaers G, et al. Disability and Rehabilitation Upper limb motor and sensory impairments in children with hemiplegic cerebral palsy. Can they be measured reliably? Upper limb motor and sensory impairments in children with hemiplegic cerebral palsy. Can they be measured reliably? 2010. DOI:10.3109/09638280903171469.
51. Mirbagheri MM, Barbeau H, Kearney RE. Intrinsic and reflex contributions to human ankle stiffness: Variation with activation level and position. *Exp Brain Res* 2000. DOI:10.1007/s002210000534.
52. Mirbagheri MM, Alibiglou L, Thajchayapong M, Rymer WZ. Muscle and reflex changes with varying joint angle in hemiparetic stroke. *J Neuroeng Rehabil* 2008; 5: 1–15.
53. De Groote F, Blum KP, Horslen BC, Ting LH. Interaction between muscle tone, short-range stiffness and increased sensory feedback gains explains key kinematic features of the pendulum test in spastic cerebral palsy: A simulation study. *PLoS One* 2018; 13: e0205763.
54. Willaert J, Desloovere K, Van Campenhout A, Ting LH, De Groote F. Movement history influences pendulum test kinematics in children with spastic cerebral palsy. *Front Bioeng Biotechnol* 2020; 8: 920.
55. van den Noort JC, Scholtes VA, Harlaar J. Evaluation of clinical spasticity assessment in cerebral palsy using inertial sensors. *Gait Posture* 2009; 30: 138–43.
56. Malhotra S, Cousins E, Ward A, et al. An investigation into the agreement between clinical, biomechanical and neurophysiological measures of spasticity. *Clin Rehabil* 2008; 22: 1105–15.
57. Fowler EG, Staudt L a, Greenberg MB. Lower-extremity selective voluntary motor control in patients with spastic cerebral palsy: increased distal motor impairment. *Dev Med Child Neurol* 2010; 52: 264–9.
58. Lieber RL, Fridén J. Spasticity causes a fundamental rearrangement of muscle-joint interaction. *Muscle Nerve* 2002; 25: 265–70.
59. Dayanidhi S, Dykstra PB, Lyubasyuk V, McKay BR, Chambers HG, Lieber RL. Reduced satellite cell number in situ in muscular contractures from children with cerebral palsy. *J Orthop Res* 2015; 33: 1039–45.
60. Capaday C, Stein RB. Amplitude modulation of the soleus H-reflex in the human during walking and standing. *J Neurosci* 1986; 6: 1308–13.
61. Faist M, Ertel M, Berger W, Dietz V. Impaired modulation of quadriceps tendon jerk reflex during spastic gait: differences between spinal and cerebral lesions. *Brain* 1999; 122: 567–79.
62. Mynark RG, Koceja DM. Down training of the elderly soleus H reflex with the use of a spinally induced balance perturbation. *J Appl Physiol* 2002; 93: 127–33.
63. Edamura M, Yang JF, Stein RB. Factors that Determine the Magnitude and Time Course of Human H-Reflexes in Locomotion. *J Neurosci* 1991; 17: 420–7.
64. Falisse A, Bar-On L, Desloovere K, Jonkers I, De Groote F. A spasticity model based on feedback from muscle force explains muscle activity during passive stretches and gait in children with cerebral palsy. *PLoS One* 2018; 13: 1–20.
65. van't Veld R. Integrated Spasticity Assessment and Treatment Using Disentangled Joint Resistance. Enschede: University of Twente, 2022 DOI:10.3990/1.9789036553919.

Supplementary Materials 5.1

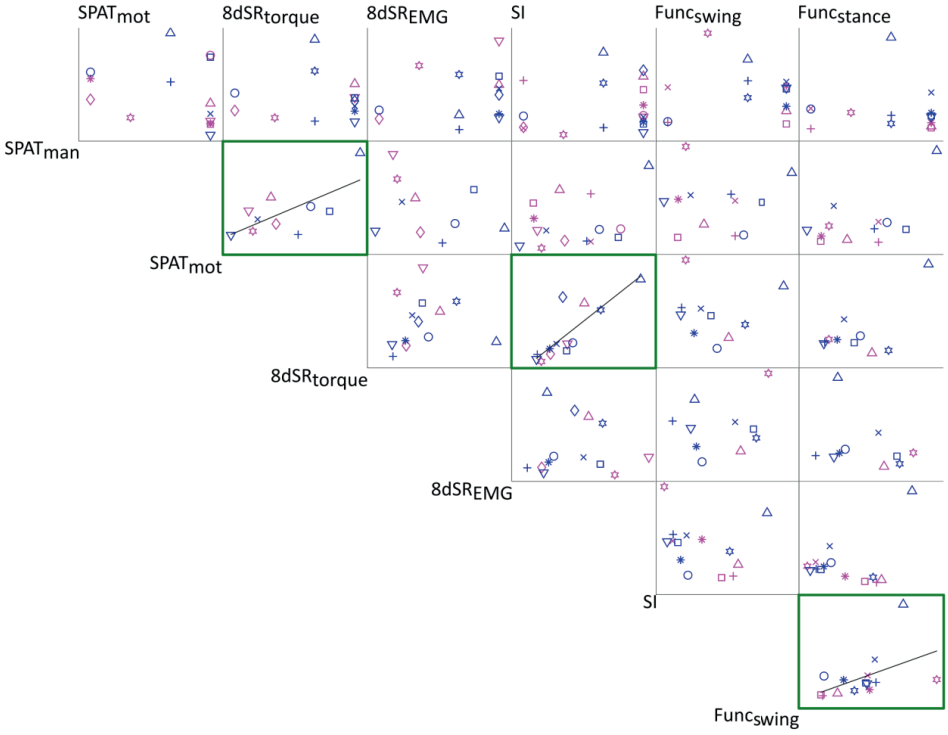
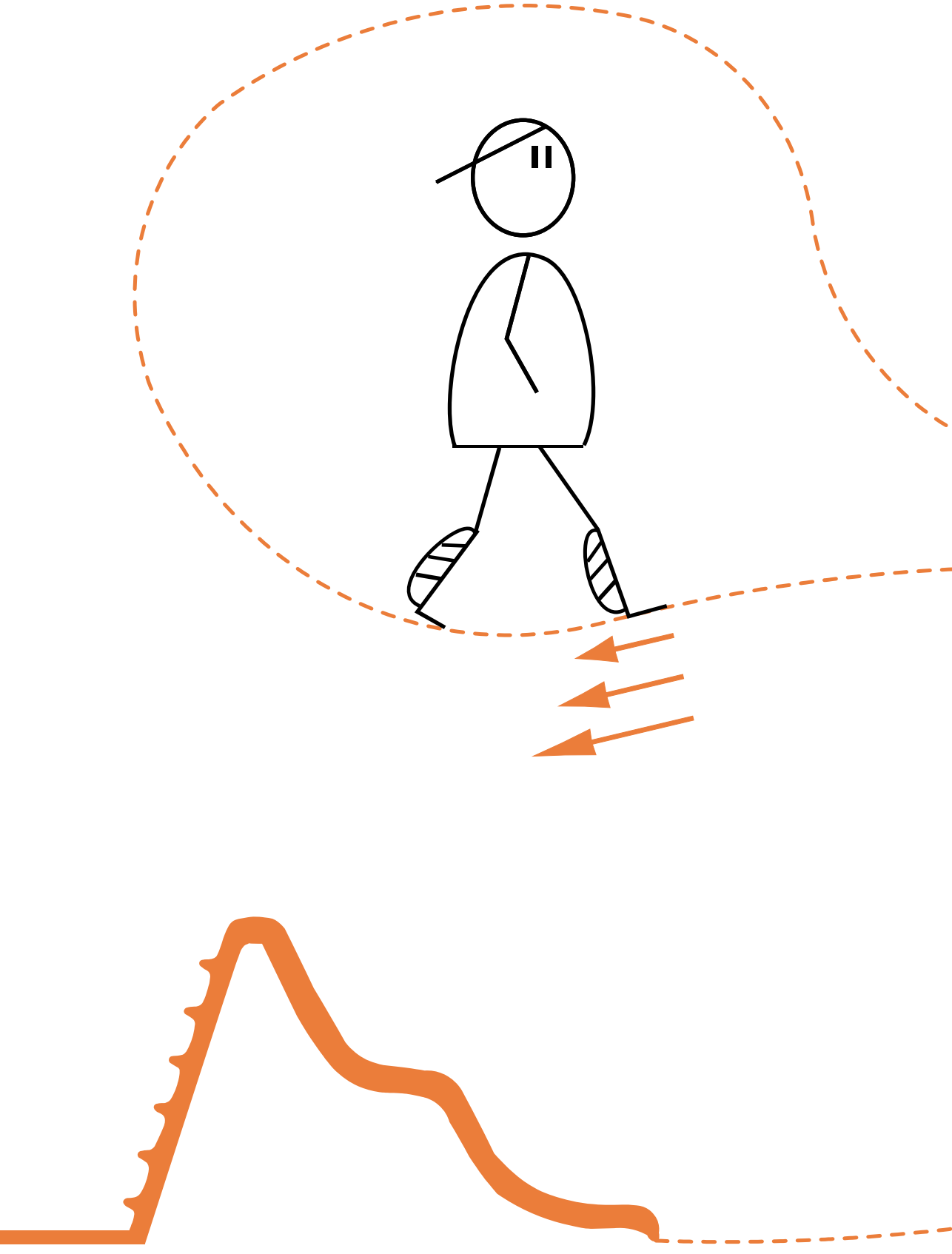
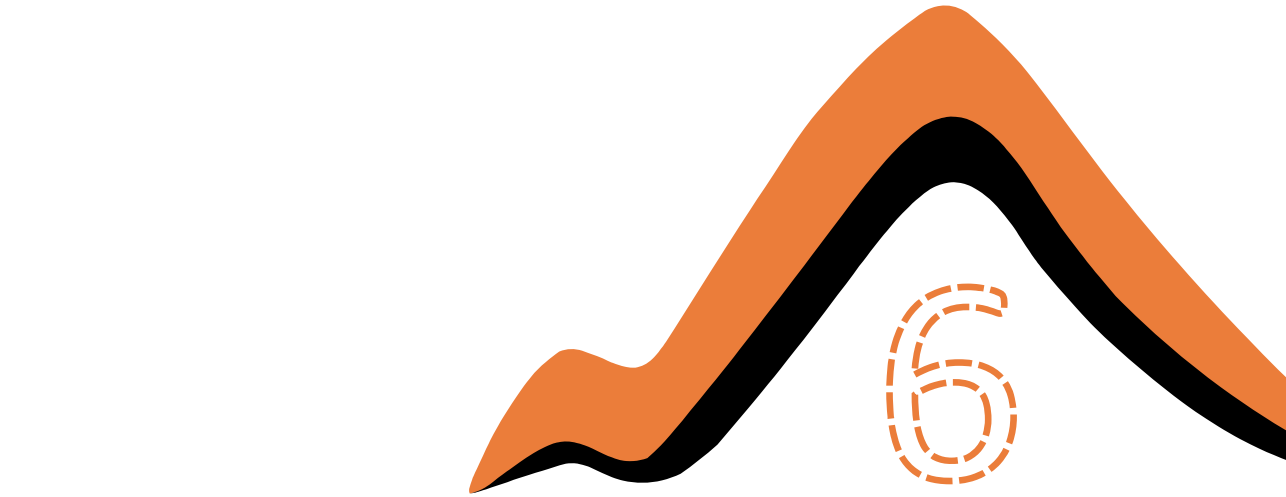


Figure S5.1. All correlations between the different stretch reflex measures. A green square outlines significant correlations and include the regression line. Confidence intervals and Spearman's rho (top line) and Pearson's r are shown in Table 1 in the main paper. Abbreviations; *SPAT$_{man}$*, spasticity assessment; *SPAT$_{mot}$*, motorized SPAT; *8°SR* = eight-degree stretch reflex; SI, parallel cascade system identification technique; Func, Functional measure calculated as the ratio between m. gastrocnemius medialis RMS EMG and muscle fascicle lengthening velocity during swing and stance.





FUNCTIONAL ASSESSMENT OF STRETCH HYPERREFLEXIA IN CHILDREN WITH CEREBRAL PALSY USING TREADMILL PERTURBATIONS

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Abstract

Background. As hyperactive muscle stretch reflexes hinder movement in patients with central nervous system disorders, they are a common target of treatment. To improve treatment evaluation, hyperactive reflexes should be assessed during activities as walking rather than passively. This study systematically explores the feasibility, reliability and validity of sudden treadmill perturbations to evoke and quantify calf muscle stretch reflexes during walking in children with neurological disorders.

Methods: We performed an observational cross-sectional study including 24 children with cerebral palsy (CP; 6-16 years) and 14 typically developing children (TD; 6-15 years). Short belt accelerations were applied at three different intensities while children walked at comfortable speed. Lower leg kinematics, musculo-tendon lengthening and velocity, muscle activity and spatiotemporal parameters were measured to analyze perturbation responses.

Results: We first demonstrated protocol feasibility: the protocol was completed by all but three children who ceased participation due to fatigue. All remaining children were able to maintain their gait pattern during perturbation trials without anticipatory adaptations in ankle kinematics, spatiotemporal parameters and muscle activity. Second, we showed the protocol's reliability: there was no systematic change in muscle response over time ($p=0.21$-0.54) and a bootstrapping procedure indicated sufficient number of perturbations, as the last perturbation repetition only reduced variability by ~2%. Third, we evaluated construct validity by showing that responses comply with neurophysiological criteria for stretch reflexes: perturbations superimposed calf muscle lengthening ($p<0.001$ for both CP and TD) in all but one participant. This elicited increased calf muscle activity (359±190% for CP and 231±68% for TD, both $p<0.001$) in the gastrocnemius medialis muscle, which increased with perturbation intensity ($p<0.001$), according to the velocity-dependent nature of stretch reflexes. Finally, construct validity was shown from a clinical perspective: stretch reflexes were 1.7 times higher for CP than TD for the gastrocnemius medialis muscle ($p=0.017$).

Conclusions: The feasibility and reliability of the protocol, as well as the construct validity - shown by the exaggerated velocity-dependent nature of the measured responses - strongly support the use of treadmill perturbations to quantify stretch hyperreflexia during gait. We therefore provided a framework which can be used to inform clinical decision making and treatment evaluation.

6.1 Background

Stretch hyperreflexia, also known as spasticity, is considered one of the key impairments in upper motor neuron syndromes such as cerebral palsy, which is the main cause of childhood-onset disability.[1] Stretch hyperreflexia is commonly defined as exaggerated velocity dependent stretch reflexes[2] and likely caused by supraspinal disinhibition of the stretch reflex loop.[3] These overactive reflexes cause muscle contractions that often limit lengthening of muscles, leading to significant restrictions in motion of the joints. The abnormal muscle activity patterns affect daily life activities, such as walking.[4] Stretch hyperreflexia is often associated with other neural and non-neural impairments, such as increased background muscle activation (neural) and altered tissue properties (non-neural).[5] Such tissue alterations include stiffer extracellular matrices, due to for instance increased collagen, which can lead to impaired muscle length and bony deformities.[6–8] The type of impairment guides the appropriate treatment. Neural impairments, including stretch hyperreflexia, can be treated with botulinum toxin injections, oral or intrathecal baclofen, or selective dorsal rhizotomy,[9–11] with exclusively the latter directly targeting stretch hyperreflexia by mechanically intervening the stretch-reflex loop. Non-neural impairments, on the other hand, are often treated with stretching, corrective casting, splinting or surgery to lengthen the muscle.[10,12] To select the appropriate treatment, diagnostic tests need to correctly identify the underlying neuromuscular problems.

The current clinical standard to assess stretch hyperreflexia is to apply manual rotations around a relaxed joint in a passive patient, and rate the perceived resistance to fast stretch of the muscle-tendon complex according to one of several available clinical scales.[13,14] Unfortunately, the subjective and qualitative nature of these tests does not allow for sufficient and reliable discrimination between underlying causes to inform treatment.[2,15,16] One could for example easily misinterpret increased passive tissue stiffness as neurological driven stiffness, or vice versa, as both present with increased resistance to stretch. Furthermore, assessment of stretch hyperreflexia in relaxed limbs for interpretation in activities like walking is criticized,[17,18] as the magnitude of reflexes is known to be centrally regulated based on the activity. For instance, reflex magnitudes decrease from sitting to standing [19] and further decrease when walking[20,21] and even adapt to the phase of the gait cycle.[4,22,23] Therefore, stretch hyperreflexia is best directly assessed during activities such as walking.

Several approaches have been suggested to assess reflexes during gait.[24–29] An actuated ankle orthosis for example has been shown to stretch and thus evoke calf muscle stretch reflexes during the stance phase of gait, [22,25,30] but it is unknown to what extent the mass and movement restrictions of such an orthosis alter gait. Similar to the ankle orthosis, treadmill perturbations can be applied to evoke (hyper)reflexes in the lower leg muscles during walking.[26,29,31] In these perturbation methods, the running treadmill belt under the standing foot is momentarily decelerated[26,29] or accelerated,[26,31] shortly increasing ankle plantar- or dorsiflexion and thus stretching the lower leg muscles, and evoking stretch reflexes. Up to now, treadmill perturbations have only been studied in able-bodied adults.[26,29,31] The next step is to evaluate this approach in patients with stretch hyperreflexia, such as children with spastic cerebral palsy. Children with cerebral palsy often present with abnormal gait patterns, and the altered ankle kinematics and lower gait stability[32] could interfere with the feasibility of the protocol and the effectiveness of evoking reflexes.

Therefore, the aim of our study is to analyze whether the treadmill perturbation protocol can evoke and quantify calf muscle stretch reflexes in children with cerebral palsy and in typically developing controls. We focus on the calf muscles, as they are the largest contributor to impaired gait in most children with cerebral palsy.[33] This paper is composed of three aspects: 1) protocol feasibility; whether we can apply perturbations while children maintain their walking pattern, 2) reliability; whether repeated perturbations present similar results, and 3) construct validity; whether the evoked muscle responses comply with the neurophysiological characteristics of stretch reflexes,[26] and whether patients with spastic cerebral palsy can be distinguished from controls. We hypothesized that the perturbation protocol is feasible in all children, presents sufficient reliability and indeed evokes reflex activity. Furthermore, we anticipated larger stretch reflexes in children with cerebral palsy versus typically developing children.

6.2 Methods

Participants

Twenty-four children with spastic cerebral palsy and fourteen typically developing children participated (see Table 6.1) in this observational cross-sectional study. Inclusion criteria were: aged between 5 and 16 years, able to follow simple instructions and walk for approximately half an hour in total with sufficient rest. Specific inclusion criteria for the cerebral palsy group were: a diagnosis of uni- or bilateral spastic cerebral palsy with gross motor function classification system[34] level I-II. Children were excluded if they had recently received treatment that consisted of functional surgery on the legs or lower limb botulinum toxin-A injections in the past 6 months, had visual deficits, frequent epilepsy, behavioral problems or comorbidities that affect walking. Children who had undergone selective dorsal rhizotomy (SDR) were included if the recovery period of 12 months was satisfied. These children were analyzed separately in the clinical applicability section, due to the severe impact of the SDR surgery on reflex sizes. Passive levels of spasticity were determined using the SPAT,[13] by stretching the calf muscles at slow and fast velocities, grading the intensity of muscle resistance during the fast velocity on a 0-3 scale. The SPAT score could not be determined in the case of clonus. Specific exclusion criteria for the typically developing group consisted of a history of neurological or orthopedic diseases.

Protocol

Participants walked on a split-belt instrumented treadmill in a virtual reality environment (Fig. 6.1, GRAIL, Motek ForceLink BV, Amsterdam, Netherlands) following the protocol as described by Sloot et al.[26] Participants wore their own flat shoes and were secured by a non-weight-bearing safety harness attached to the ceiling. Ground reaction forces were measured at 1000 Hz by force sensors mounted underneath both treadmill belts (50x200 cm; R-Mill, Forcelink, The Netherlands). Motion data was captured at 100 Hz using a motion capture system (Vicon Motion Systems, Oxford, UK) and the Human Body Model marker set.[35,36] EMG electrodes (bipolar, Ø 15mm, 24 mm inter-electrode distance) were placed on the gastrocnemius medialis (GM), soleus (SO) and tibialis anterior (TA) muscle bellies of both legs according to SENIAM guidelines.[37] EMG was measured at 1000 Hz via a wireless system (Wave EMG system, Cometa, Italy).

Table 6.1
Participant characteristics

	GMFCS	Distribution	Gender	Age	Height	Weight	GM	SO	CWS
	I/II	Uni/bi	F/M	(y)	(m)	(kg)	0/I/II/III/CL	0/I/II/III/CL	(m/s)
TD	-	-	7/7	10.7±3.2	1.50±0.19	40.3±13.0			1.19±0.15
CP	9/9	4/14	6/12	10.1±3.1	1.49±0.19	37.2±14.2	7/1/-/2/8	8/1/-/3/8	0.67±0.19
SDR	0/3	0/3	2/1	11.3±3.5	1.51±0.16	43.0±18.8	3/-/-/-/-	3/-/-/-/-	0.62±0.24

Participant characteristics were included from all children who completed the protocol. Abbreviations: GMFCS = gross motor function classification system;[34] GM = Gastrocnemius Medialis muscle; SO = Soleus muscle, scores reflect values of spasticity according to the SPAT test;[13] Cl = clonus, no SPAT can be quantified for these participants; CWS = comfortable walking speed on the treadmill; TD = Typically developing; CP = Cerebral palsy; SDR = Selective dorsal rhizotomy, children who had SDR surgery were excluded from analyses of neurophysiological criteria.

Comfortable walking speed (Table 6.1) was determined at the start of the session for each participant individually by gradually increasing the belt speed until comfortable as indicated by child and parents. Participants walked for at least six minutes to habituate to the set-up.[38,39] To familiarize to the protocol, participants received three perturbations of each intensity during the last habituation minute.

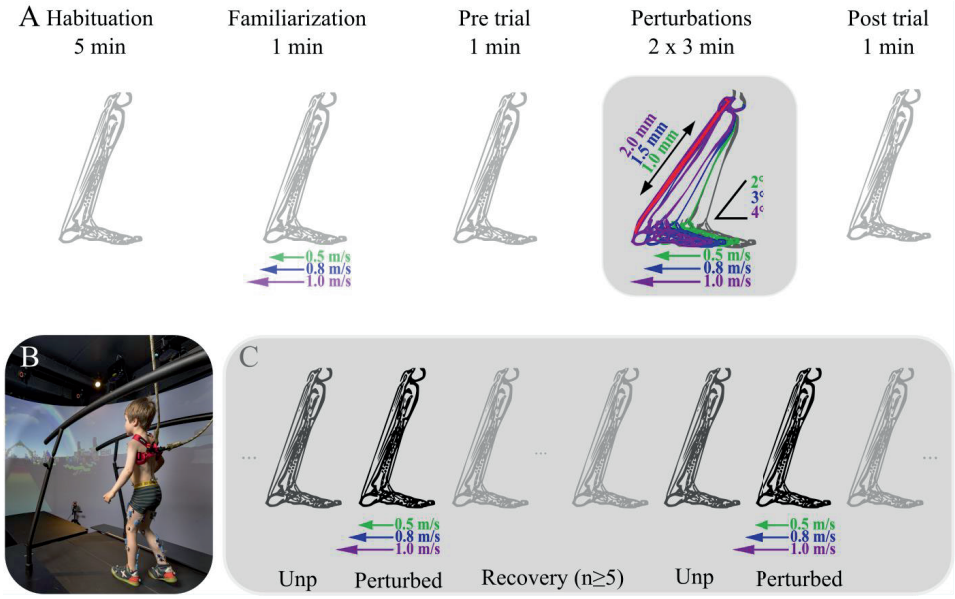

Figure 6.1. Perturbation protocol. (A) Schematic representation of the perturbation protocol. Perturbations of three different intensities were applied over two perturbation trials. (B) Measurement setup. (C) Schematic representation of the perturbation trial: At least five recovery strides occurred between perturbations. Each step before the perturbed trial was used for the average unperturbed (Unp) baseline measures.

Measurements started with a trial of one minute of unperturbed walking (Pre; Fig. 6.1), during which the absence of perturbations was explicitly mentioned to prevent cautious gait. Next, perturbations were applied to the leg with the most spastic calf muscles (cerebral palsy) or the right leg (typically developing). Perturbations were applied over two trials of three minutes each, with a short break in between to limit fatigue. The protocol ended with another explicit unperturbed walking trial of one minute (Post). All participants reported that they were able to feel at least the two most intense perturbations. Therefore, we assessed if anticipation of perturbations influenced their gait pattern, by asking two questions on subjective walking experience (see Fig. 6.2A) after the Pre and after the perturbation trials.

The treadmill perturbations consisted of a short acceleration of the belt during the first period of stance (Fig. 6.1, Fig. 6.3, Table 6.2), pulling the foot backwards and stretching the calf muscles. Three perturbation intensities were applied to evaluate how muscle activity response scales with muscle lengthening velocity. The intensities corresponded to an increased treadmill speed of 0.5 ms^{-1}, 0.8 ms^{-1} and 1.0 ms^{-1}. During each perturbation trial, four to six repetitions of each perturbation intensity were applied to random strides. The number of recovery strides was randomized but at least five strides before the next perturbation were applied. If feet stepped on both treadmill belts, no perturbations were applied to prevent falls.

Data processing

3D joint angles for the hip, knee and ankle, as well as musculo-tendon lengths (MTL) of the Gastrocnemius Medialis (GM), Soleus (SO) and Tibialis Anterior (TA) muscles were calculated using the generic gait model (GAIT2392) in musculoskeletal modeling software (OpenSim).[40] In this software, the model was scaled to fit the individual participants and the inverse kinematics tool was used to fit the individual participant's kinematics.[26] Further calculations were performed using custom made code in Matlab (The Mathworks Inc., Natick MA; version 2019a). MTL was differentiated to obtain musculo-tendon stretch velocity (MTV). Kinematics, MTL and MTV were low-pass filtered (bi-directional 4th order Butterworth at 20Hz) and non-dimensionalized by dividing MTL by the anatomical reference length with all joint angles set at zero (l_{ref}) and MTV by $\sqrt{(g \cdot l_{ref})}$.[27,41]

EMG signals were high-pass filtered (bidirectional 4th order Butterworth at 20Hz), rectified and low-pass filtered (at 50Hz). All EMG signals were normalized to the peak during each muscles' region of interest – defined as swing for TA and push-off for GM and SO – obtained from the further smoothed (8Hz low-pass) Pre-trial signals. Push-off was defined as the moment of zero crossing of anterior-posterior force to toe-off. Initial foot contact and toe-off values were determined using the horizontal position of the heel, toe and pelvic markers, conform the method of Zeni et al.[42], both online to predict initial contact for treadmill perturbation onset, as well as offline to determine actual initial contact. The latter were used to time-normalize belt speed, kinematics, MTL, MTV and EMG signals.

Table 6.2

Perturbation characteristics

	I1	I2	I3	ANOVA	Posthoc
Peak Δ v (ms^{-1})	0.53±0.01	0.80±0.01	1.03±0.01	**<0.001**	**All**
Peak Δ vrel (%)	74.49±31.32	111.60±47.31	144.15±60.99	**<0.001**	**All**
Peak Δ a (ms^{-2})	18.08±1.01	18.41±1.37	18.34±1.30	0.505	**All**
Time to peak v (ms)	72.67±11.04	92.81±15.32	103.75±15.34	**<0.001**	**All**
Time to peak vrel (%)	10.32±0.75	13.24±0.88	15.56±1.04	**<0.001**	**All**
Duration (ms)	143.71±22.07	181.27±29.40	206.69±30.60	**<0.001**	**All**
Duration rel (%)	20.37±1.44	25.93±1.78	30.95±2.03	**<0.001**	**All**
Start (ms)	95.81±51.93	80.34±18.40	77.92±18.63	0.063	-
Start rel (%)	11.64±2.35	11.88±2.72	11.94±2.58	0.877	-
Start rel TD (%)	12.02±1.48	12.11±1.57	11.88±1.53	0.661	-
Start rel CP (%)	11.38±3.61	11.73±3.98	11.99±3.32	0.626	-
Peak Δ Ankle angle (°)	1.99±0.69	3.60±0.94	4.89±1.49	**<0.001**	**All**
Peak Δ Ankle ω ($°s^{-1}$)	45.65±14.65	62.21±16.61	77.83±24.00	**<0.001**	**All**

Spatiotemporal parameters (Time to peak v, perturbation duration and perturbation start) are expressed in absolute values as well as relative to the gait cycle (rel). Mean ± standard deviations are presented. Δ v, difference in treadmill velocity; Δ vrel, difference in treadmill velocity relative to participant's belt velocity; a, treadmill acceleration; ω, angular velocity. Significant values are expressed in bold. All posthoc testing revealed significant differences between all conditions (p<0.05).

Data analysis and statistics

Protocol feasibility

As part of the protocol feasibility, we evaluated any anticipatory changes in children's gait pattern; if the number of steps in between perturbations was sufficient to return to normal after a perturbation; and if timing of the perturbations was consistent. All feasibility analyses were performed for the cerebral palsy and typically developing group separately.

We first evaluated how many children could complete the entire protocol and reported any adverse events such as falls, failures or discomfort. Additionally, we evaluated whether children were able to maintain their walking pattern despite perturbations. We did this by comparing the subjective rating of their gait before and after the perturbation trial (Fig. 6.2A) using Wilcoxon Signed Ranks Test. Likewise, we quantitatively examined any changes in walking pattern by comparing the unperturbed strides directly preceding the perturbed strides with walking when no perturbations were anticipated, i.e., the last ten strides during both Pre and Post trials. Both Pre and Post were included to discriminate between fatigue and perturbation effects.[43] One cerebral palsy and one typically developing child were excluded from this sub-analysis due to missing data for the Post trial. We compared several outcome parameters that are known to be affected by anxiety; i.e. we checked for shorter step length and duration, longer stance phase, smaller step width, increased knee and ankle flexion, as well as reduced peak muscle activation in the GM and SO or increased co-contraction of these muscles with the TA.[43] Co-contraction was measured using the co-contraction index according to the following equation:

$$co-contraction\ index = 1 - \frac{|EMG_{ag} - EMG_{ant}|}{EMG_{ag} + EMG_{ant}} \quad (1)$$

with EMG_{ag} the agonist (GM and SO) and EMG_{ant} the antagonist muscle (TA), with 1 full and 0 absence of co-contraction.[44] A repeated measures ANOVA was performed to compare the Pre, unperturbed and Post strides, and in case of significance post hoc paired sample t-tests were performed.

Protocol reliability

For reliability of our protocol we first assessed if the number of recovery strides was sufficient to return to unperturbed walking, by comparing step width, stride time and stance phase between unperturbed versus perturbed and the five recovery strides using paired t-tests without correction for multiple comparison as to not underestimate any differences.[26] We also evaluated the timing of the perturbations, as the more variable gait pattern of children with cerebral palsy[45] might affect the online predicted initial foot contact and thus the repeatability of perturbations. We reported the perturbation onsets and compared the standard deviation from the cerebral palsy group with the typically developing group (one-tailed independent samples t-test). We furthermore analyzed if perturbation velocity, acceleration and duration and resulting ankle dorsiflexion increased with intensity, using repeated measures ANOVAs with linear polynomial contrast and post hoc independent t-tests.

Next, the within session reliability of the perturbation protocol was assessed as the consistency of response to repeated perturbations, to evaluate if there was habituation to the perturbations reducing the effectiveness of the protocol. We assessed if we could reliably estimate increases in GM and SO EMG (as defined in the validity section) for the highest intensity perturbations. Changes in muscle response size over time were evaluated for both muscles using a repeated measure ANOVA with a polynomial contrast per participant group. We furthermore assessed the required number of repetitions using a bootstrap procedure: for every participant, randomly selected perturbation strides were drawn from the available perturbations and this was repeated 1.000 times. The coefficient of variation, defined as the standard deviation over the 1.000 samples normalized to the mean, was established for every number of perturbations (ranging from 2-8 perturbations) and averaged over participants. When the coefficient of variation values reached a plateau, it was assumed that sufficient numbers of repetitions were included.

Construct validity of stretch reflexes

To assess the construct validity of the perturbation protocol, we first evaluated evoked muscle responses against three neurophysiological criteria, similar to our previous work.[26] All neurophysiological criteria are based on the commonly presumed velocity dependent character of stretch reflexes:[2]

1) Mechanical response: increasing perturbation intensity should result in an increase in MTL and MTV;
2) Electrophysiological response: increasing perturbation intensity should evoke an increasing burst of muscle activity in the stretched muscle;
3) The burst in muscle activity should not (solely) be related to co-contraction with the antagonist muscle.

To evaluate criterium 1, the peak values of the ankle and knee angles as well as GM and SO MTL and MTV were calculated by subtracting each individual perturbed stride from unperturbed walking, that is the average of all strides directly before a perturbed stride (see Fig. 6.1). These values are further referred to as Δankle dorsiflexion, Δknee flexion, ΔMTL and ΔMTV. We additionally analyzed the percentage of trials with a successful mechanical response, defined as a ΔMTV above one standard deviation of unperturbed MTV. Participants without a mechanical response were reported, and neglected for further analysis.

Criterium 2, the GM and SO electrophysiological response (see Fig. 6.3), was calculated as the maximum difference in EMG (ΔEMG) between individual perturbed and average unperturbed strides during the reflex time window. This window was set as 35ms after start of the perturbation until 120ms after the maximum of the perturbation, thus based on the minimal and longest expected stretch reflex delay in calf muscles.[17] Furthermore, we analyzed the percentage of trials with occurrence of a burst. According to our previous work,[26] a burst was defined as an increase in EMG activity exceeding five times the standard deviation of unperturbed EMG activity for at least 8ms during the reflex window (Fig. 6.3). To evaluate criterium 3, the amount of co-contraction was calculated within the reflex time window using equation 1.

Furthermore, we assessed the construct validity of the perturbation protocol from a clinical perspective. This was performed by assessing whether the protocol could distinguish children with cerebral palsy from typically developing children. We furthermore contrasted this with preliminary outcomes of three children who underwent SDR. We calculated the muscular response strength, defined as ΔEMG divided by ΔMTV.26 As children with cerebral palsy often have decreased post-activation depression or a continued increased activation,46 we also examined the duration of the muscular response. GM and SO ΔEMG were averaged per 20ms bins starting from perturbation onset to the end of stance. Response duration was defined as the longest continuous period of positive bins during stance.

Statistical analysis

Numerical data was tested for normality before performing parametric testing. All statistical tests were two-tailed and $p<0.05$ was considered statistically significant, unless indicated otherwise, and analyses were performed in IBM SPSS Statistics version 26 (Armonk, NY, USA). Statistical analyses for feasibility and reliability measures are described within their sections. Neurophysiological criteria for construct validity were statistically analyzed using a repeated measures ANOVA with the three criteria as dependent factors and cerebral palsy/typically developing as grouping factor. Children who underwent SDR surgery were excluded from this analysis, as no enhanced reflexes were expected in this group. Contrasts were used to examine whether the parameters differed between unperturbed and perturbed strides (Helmert contrast; P_{pert}), whether this increased with perturbation intensity (linear polynomial contrast; $P_{intensity}$), and in particular if this increase had an interaction effect with group (cerebral palsy/typically developing, using linear polynomial contrast; P_{inter}). The intensity contrast was additionally assessed for cerebral palsy (P_{CP}) and typically developing (P_{TD}) separately. The clinical criteria, muscular response strength and duration, were compared between participant groups using a repeated measures ANOVA (linear polynomial contrast; P_{group}). Due to the small sample size, SDR muscular response strength and duration were only visually compared.

6.3 Results

Protocol feasibility

All but three children with cerebral palsy finished the protocol, two of which (aged 9 and 12 years) terminated early due to fatigue complaints and one participant (aged 9 years) did not want to continue after the perturbation habituation trial without an explicit reason. No falls occurred and none of the other participants reported discomfort or pain. Two participants reported anxiety for the perturbations but finished the protocol nonetheless. Further analysis was continued with the 21 children with cerebral palsy that finished the protocol.

The gait of both participant groups was not considerably altered during the perturbation trials compared with walking during the Pre trial. Generally, the children rated their gait pattern as normal and relaxed (Fig. 6.2A), with typically developing feeling slightly more disturbed during the perturbation trial (z = -2.16, P=0.03) but as relaxed as normal (z = -1.03, P=0.31); while cerebral palsy did not feel more disturbed (z = -1.24, P=0.22) but showed a trend of feeling slightly less relaxed (z = -1.85, P=0.06). This aligns with the quantitative analysis of the walking pattern, and specifically the potential effect on spatiotemporal parameters, ankle and knee flexion and peak muscle activation. Ankle angles were very similar between Pre, Post and unperturbed strides (Fig. 6.2B). For typically developing, the only difference was a decrease in GM (P_{ANOVA}=0.04) and SO (P_{ANOVA}=0.003) muscle activation between Pre and Post trials (Fig. 6.2C), with no significant differences with the unperturbed strides (P=0.19 with Pre and P=0.20 with Post). In the cerebral palsy group, only stance phase duration was reduced by 2.0% (P_{ANOVA}=0.03) during perturbed versus Pre trial (Fig. 6.2C).

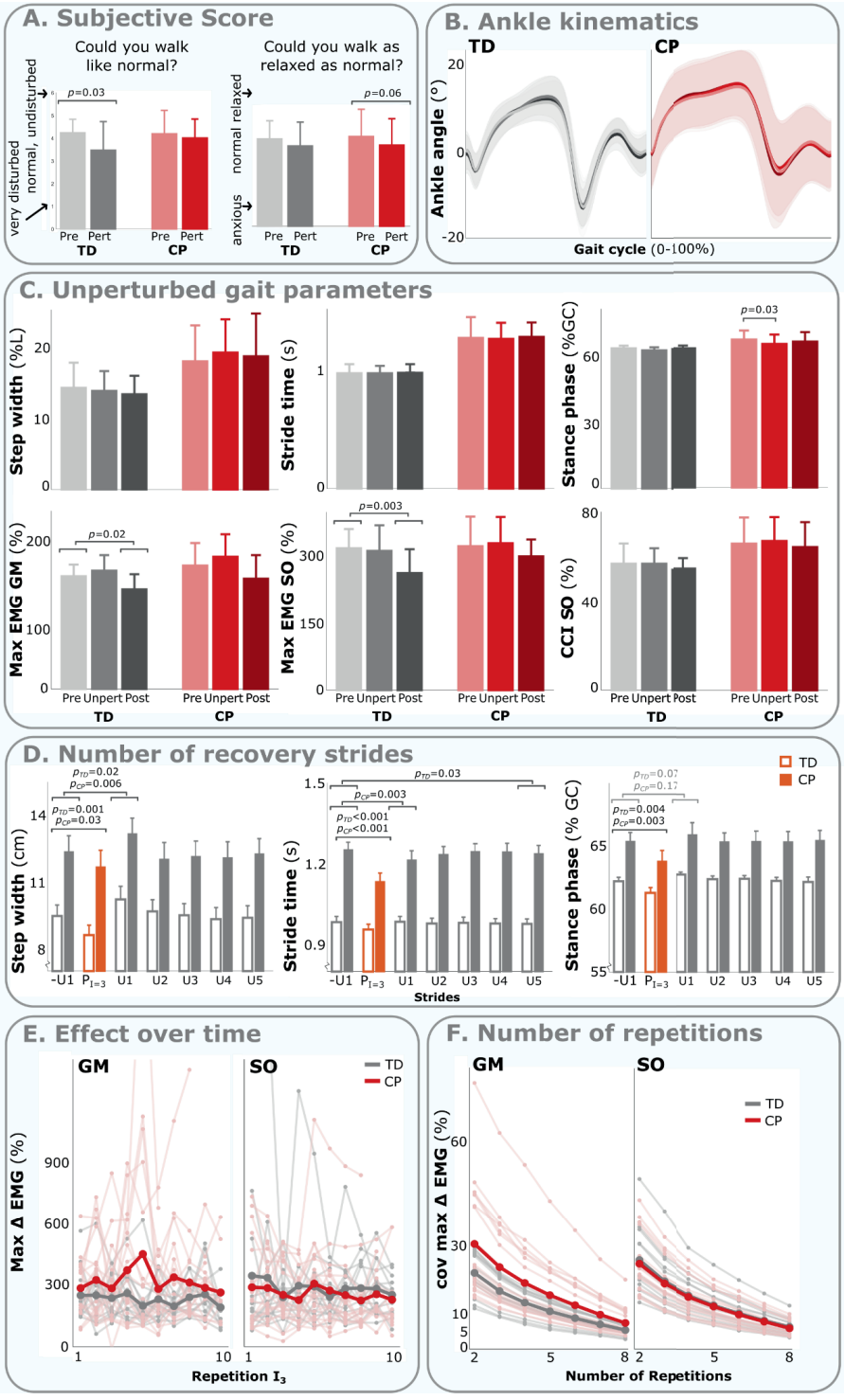
A. Subjective Score
Could you walk like normal?
Could you walk as relaxed as normal?
very disturbed
normal, undisturbed
normal relaxed
anxious
p=0.03
p=0.06
Pre Pert
TD
CP
B. Ankle kinematics
TD
CP
Ankle angle (°)
Gait cycle (0-100%)
C. Unperturbed gait parameters
Step width (%L)
Stride time (s)
Stance phase (%GC)
Max EMG GM (%)
Max EMG SO (%)
CCI SO (%)
p=0.02
p=0.003
p=0.03
Pre Unpert Post
D. Number of recovery strides
Step width (cm)
Stride time (s)
Stance phase (% GC)
TD
CP
Strides
E. Effect over time
GM
SO
Max Δ EMG (%)
Repetition I3
F. Number of repetitions
cov max Δ EMG (%)
Number of Repetitions

Figure 6.2. The feasibility (A-D) and reliability (E-F) of the perturbation protocol. P-values are indicated for the different variables if significant differences were found. Bar graphs indicate mean and standard deviation values. **(A)** Subjective rating of how relaxed and disturbed children with cerebral palsy (CP) and typically developing children (TD) felt before and after the perturbation trials. **(B)** Stride-cycle normalized ankle angle for both typically developing and cerebral palsy group during Pre and Post trials and unperturbed (unp)strides during perturbed trial. Color coding corresponds with that as indicated in the next sub-figure. **(C)** Comparison of parameters calculated for the Pre, Post and unperturbed strides for both participant groups. **(D)** Comparison between the unperturbed and perturbed strides and the five recovery strides for both groups using t-tests (group indicated at the specific p-value). **(E)** Evaluation of the GM and SO muscle response to the highest intensity perturbation over time (i.e., repetitions) for both groups in bold as well as the individual values in lighter colors. EMG is normalized to the average maximum of each participant during push-off of the unperturbed strides. Note that the perturbations were applied during two different trials with a short break in between, which did not visibly affect the results. **(F)** Coefficient of variation (COV) between the 1000 bootstrap repetitions per subset of n (2-8) random selection of strides from the available perturbed strides per participant and the average over participants in bold.

Protocol reliability

The number of recovery strides were sufficient for the children to return to normal gait, with no significant differences from the second recovery stride onwards (Fig. 6.2D). Perturbations started on average around 12±3 % of the gait cycle and were all applied during the stance phase of gait (Table 6.2). Onsets did not differ across the different intensities (P=0.877), although the standard deviation of the onset was significantly higher for cerebral palsy (P=0.028). Perturbation intensity increased with higher intensities (P<0.001; Table 6.2).

The muscular response strength to the perturbations did not change systematically with repeated perturbations (Fig. 6.2E; cerebral palsy GM: P=0.28, SO: P=0.21; typically developing GM: P=0.29, SO: P=0.54). The lack of habituation to the perturbations allowed us to average over multiple repetitions to reduce the coefficient of variation. Including up to 8 repetitions reduced the coefficient of variation to below 7% for typically developing and 8% for cerebral palsy and almost reached a plateau (Fig. 6.2F) with a reduction of around 2% for the latest perturbation.

Construct validity

All children but one had successful mechanical responses to the perturbations (i.e., ΔMTV above one standard deviation of unperturbed MTV; Table 6.4) in at least the highest intensity, fulfilling criterium 1. The exception was one child with cerebral palsy that exhibited considerable reduced ankle range of motion throughout the gait cycle, and as this anatomical constraint prohibited evoking a stretch reflex, this participant was excluded from further analysis. In the other participants, ankle dorsiflexion increased with on average 5.4°±1.3 for typically developing and 3.8°±1.4 for cerebral palsy for the highest intensity perturbations (See Table S6.1, Supplementary Materials 6.1). The increased ankle dorsiflexion resulted in increased GM and SO ΔMTL and ΔMTV ($P_{intensity}$<0.001; Table 6.4; Fig. 6.3, Fig. 6.4). The mechanical response increased with increasing perturbation intensity for ankle dorsiflexion, MTL and MTV ($P_{intensity}$ <0.001; Table 6.4; Fig. 6.3, Fig. 6.4). This increase in response was stronger in typically developing children compared to children with cerebral palsy (P_{inter}= 0.01 – 0.038), with the exception of GM ΔMTV (P_{inter}=0.178). Knee flexion also increased due to perturbations, both in cerebral palsy (P_{CP} =0.002) and typically developing (P_{TD} =0.001; Table S6.1; Supplementary Materials 6.1, Fig. 6.3, Fig. 6.4).

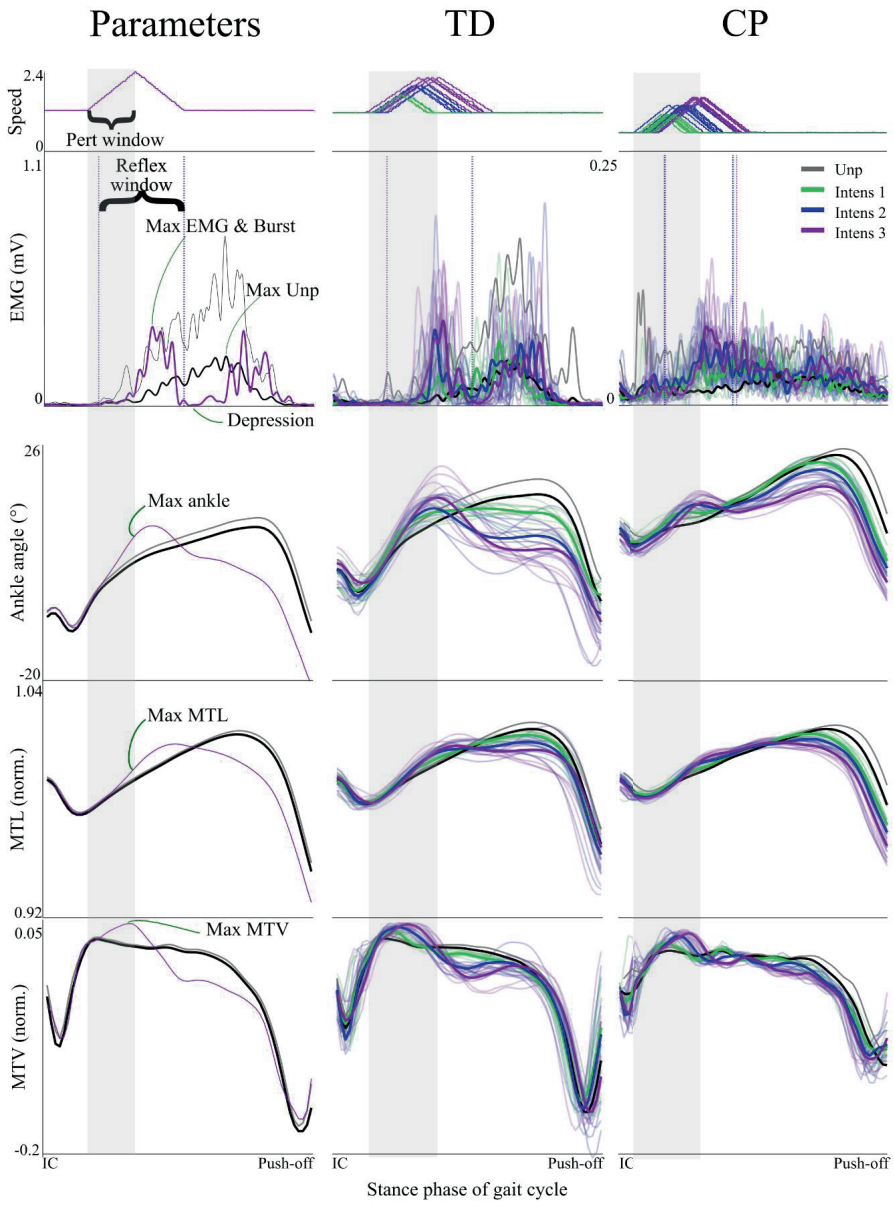

Fig. 6.3. Muscle parameters and typical examples. Treadmill belt perturbations (speed) and the responses for the gastrocnemius medialis muscle are visualized for the different parameters (column 1) and for a typically developing child (TD; column 2) and a child with cerebral palsy (CP; column 3). Values are plotted from initial contact (IC) to push-off. Black represents average unperturbed walking, with grey indicating n x standard error (5 x for EMG and 1 x for ankle angles, MTL and MTV). Light shaded lines represent individual perturbed strides and bold darker colored lines represent average of perturbed strides for the three different intensities. Abbreviations: MTL, muscle tendon lengthening; MTV, muscle tendon velocity; mV millivolt; norm, normalized; Unp, unperturbed.

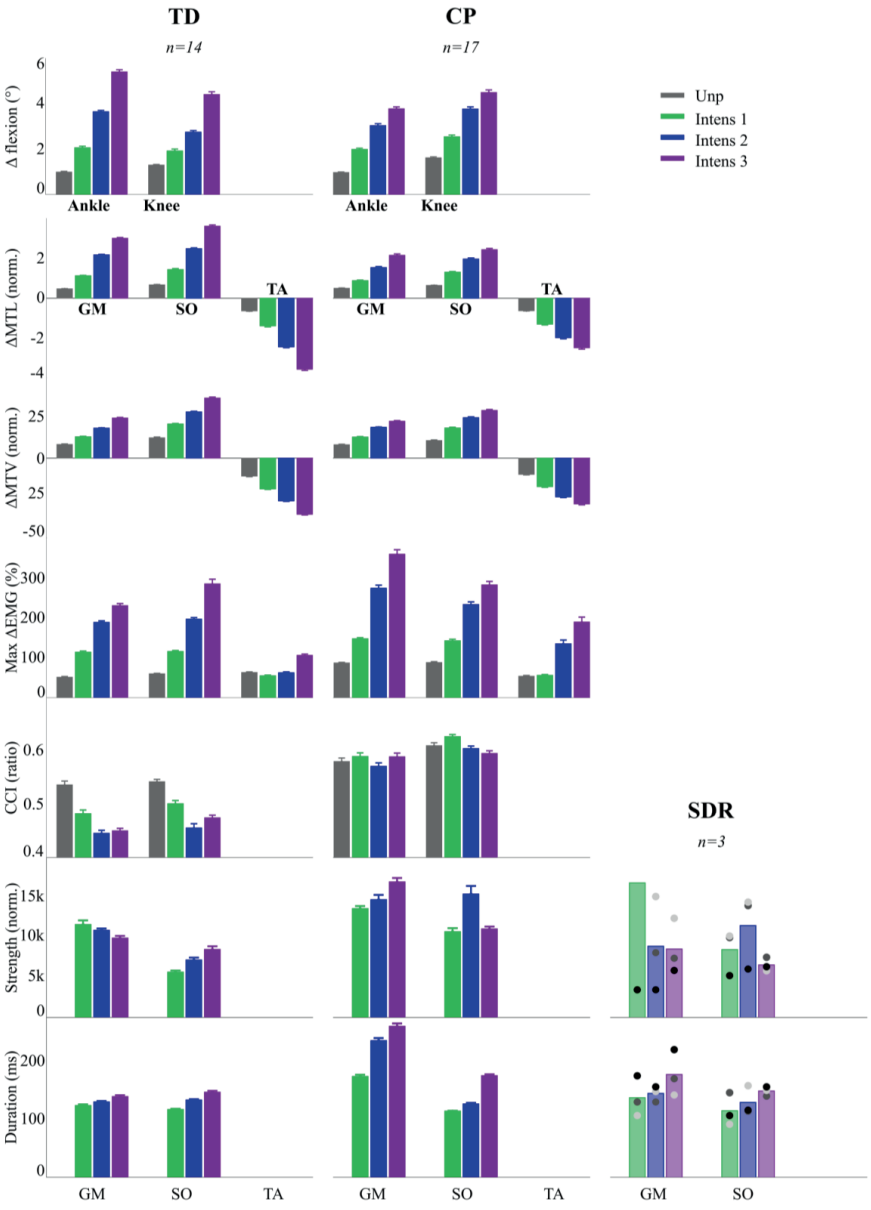

Figure 6.4. Construct validity of stretch reflexes: The three neurophysiological criteria for stretch reflexes are shown (row 1-5), as well as the clinical criteria (row 6-7). 1) The mechanical response for the ankle and knee joint; and 2-3) of the gastrocnemius medialis (GM), soleus (SO) and tibialis anterior (TA) muscle; 4) the electrophysiological response for the GM, SO and TA; 5) the amount of co-contraction; 6) muscular response strength; and 7) response duration. Bar graphs indicate mean and standard error of the mean values. Values are presented for unperturbed (unp) and perturbation intensity (Intens) 1-3. For the clinical criteria, only I1-3 are shown. Values for the selective dorsal rhizotomy (SDR) group are only presented for clinical criteria. Abbreviations: MTL, muscle tendon lengthening; MTV, muscle tendon velocity; CCI, co-contraction index; norm, normalized.

Table 6.3

Mechanical and electrophysiological responses

	Mechanical			Electrophysiological		
	Intensity 1	**Intensity 2**	**Intensity 3**	**Intensity 1**	**Intensity 2**	**Intensity 3**
TD						
GM	93.9%	99.4%	98.2%	30.5%	66.3%	78.4%
SO	83.9%	96.5%	98.8%	55.3%	91.4%	97.9%
CP						
GM	91.1%	97.2%	98.7%	22.8%	55.1%	69.6%
SO	80.3%	92.6%	98.1%	50.5%	84.7%	96.2%

Percentage of perturbations with a successful mechanical (increase in ΔMTV above one standard deviation of unperturbed MTV) and electrophysiological (increase in EMG activity above five times the standard deviation of unperturbed EMG) response for the three different intensities.

Table 6.4

Statistical outcomes for the construct validity evaluation.

Parameters	**TD**	**CP**	P_{pert}	$P_{intensity}$	P_{group}	P_{inter}
Max Δangle (°)						
Ankle	5.40 ± 1.34	3.79 ± 1.40	**<0.001**	**<0.001**	**0.028**	**0.002**
Knee	4.41 ± 1.64	4.50 ± 1.90	**<0.001**	**<0.001**	0.153	0.430
Max ΔMTL (norm.)						
GM	2.99 ± 0.52	2.15 ± 1.05	**<0.001**	**<0.001**	**0.006**	**0.038**
SO	3.60 ± 0.89	2.42 ± 1.02	**<0.001**	**<0.001**	**0.016**	**0.001**
TA	-3.56 ± 0.81	-2.49 ± 1.05	**<0.001**	**<0.001**	**0.028**	**0.002**
Max ΔMTV (norm.)						
GM	25.04 ± 4.04	23.05 ± 5.57	**<0.001**	**<0.001**	0.682	0.178
SO	37.51 ± 7.40	29.76 ± 9.78	**<0.001**	**<0.001**	0.057	**0.011**
TA	-35.14 ± 5.36	-28.72 ± 8.53	**<0.001**	**<0.001**	0.085	**0.006**
Max ΔEMG (%)						
GM	230.92 ± 67.91	359.53 ± 189.63	**<0.001**	**<0.001**	**0.018**	0.051
SO	285.10 ± 160.90	282.69 ± 149.86	**<0.001**	**<0.001**	0.540	0.537
TA	106.47 ± 39.83	189.88 ± 204.63	**0.003**	**0.001**	0.131	0.126
CCI difference						
GM	-0.09 ± 0.06	0.01 ± 0.06	**<0.001**	0.069	**0.001**	0.076
SO	-0.07 ± 0.05	-0.01 ± 0.05	**<0.001**	**0.001**	**<0.001**	0.742
Muscular response strength (norm.)						
GM	9.95 ± 3.74	16.96 ± 8.81	0.658	0.501	0.064	**0.045**
SO	8.52 ± 5.65	11.10 ± 5.05	0.521	0.237	**0.005**	0.351
Muscular response duration (ms)						
GM	102.95 ± 7.47	124.57 ± 15.39	**<0.001**	**<0.001**	**<0.001**	**0.001**
SO	93.88 ± 8.65	114.07 ± 16.70	**<0.001**	**<0.001**	**0.004**	**0.001**

Values for the highest intensity are presented for all construct validity parameters. Values for unperturbed and the three intensities are presented in Table S6.1. Abbreviations: with TD the typically developing group; CP the cerebral palsy group; P_{pert} the effect of perturbations (Helmert contrast); $P_{intensity}$ the effect of intensities (linear polynomial contrast,); P_{group} the difference between CP and TD; P_{inter} the interaction between CP and TD; MTL, musculo-tendon length; GM, gastrocnemius medialis muscle; SO, soleus muscle; TA, tibialis anterior muscle; MTV, musculo-tendon stretch velocity; CCI diff, difference in co-contraction index between unperturbed and highest intensity perturbations. Mean ± standard deviations are presented. Significant p-values are expressed in bold.

The mechanical responses resulted in an electrophysiological response (i.e., a burst in EMG) in all children at least in the highest intensity (Table 6.3), fulfilling criterium 2. Likewise, increased muscle stretch of both muscles resulted in increased muscle activity ($P_{intensity}$<0.001; Table 6.4; Fig. 6.3, Fig. 6.4). The electrophysiological response was overall higher for cerebral palsy than typically developing (P_{group}=0.018) for the GM, but unlike the mechanical response, no interaction effect was present. For the SO no difference was found between cerebral palsy and typically developing (P_{group} = 0.540; P_{inter}=0.537). Co-contraction did not increase due to perturbations, fulfilling criterium 3. In contrast, it decreased for the SO ($P_{intensity}$=0.001), with a trend for GM ($P_{intensity}$=0.069; Table 6.4; Fig. 6.3, Fig. 6.4), especially in typically developing children (P_{TD}=0.008 for GM and P_{TD}=0.006 for SO; see Table S6.1, Supplementary Materials 6.1).

As hypothesized, children with cerebral palsy not only had a higher muscular response strength (ΔEMG/ΔMTV), but the response also continued longer, thereby fulfilling the clinical criteria. The GM response was 48.7% higher (P_{group}=0.017) and lasted 96.4% longer (P_{group}<0.001) on average for cerebral palsy compared to typically developing (Table 6.4; Fig. 6.4). Similarly, the SO response showed a trend of 31.3% increase (P_{group}=0.064), with large interindividual differences, and lasted 28% longer on average (P_{group}<0.001) for cerebral palsy. The children who underwent SDR to reduce their reflex responses showed lower muscular response strength than children with cerebral palsy who did not underwent SDR surgery, despite similar mechanical responses.

6.4 Discussion

This study is, to our knowledge, the first to investigate a perturbation protocol to evoke stretch reflexes in the calf muscles during gait in children with cerebral palsy and typically developing controls. The feasibility of the protocol was reflected by the low number of drop-outs and absence of noticeable gait adaptations in anticipation of the perturbations. We also showed an absence of habituation to the perturbations, allowing to reliably estimate the muscle response by averaging over repetitions. The construct validity of the protocol was based on the evoked muscle responses, showing muscle-lengthening velocity dependency conform the neurophysiological criteria of stretch reflexes. Furthermore, results complied with clinical criteria, as the protocol could distinguish between the group of children with spastic cerebral palsy and typically developing children.

Protocol feasibility and reliability

Feasibility of the protocol in children as well as reliable responses are a prerequisite for clinical implementation. While three children did not finish the protocol, this was due to fatigue of this comprehensive protocol implementation and not the perturbations themselves. The children that did finish the protocol generally felt relaxed and although subjective scores showed that typically developing children were slightly more anxious during the perturbation trials, this was not reflected in their walking pattern. They did show significantly reduced maximum EMG between Pre and Post trials, but this was not visible in the perturbation trial and therefore more likely attributed to habituation or fatigue. Children with cerebral palsy did reduce their stance time during the perturbed trial, but this change was, if anything, not indicative of a more cautious walking pattern.[47] Furthermore, their perceived gait did not change due to the perturbations. There were no further indications for an adaptive gait pattern during the perturbation trials, hence we confirmed that the children maintained their gait pattern despite the perturbations. Overall, the protocol appears feasible, except maybe for

children with severely reduced ankle range of motion, as it was not possible to evoke stretch reflexes in one such subject with the current perturbation settings.

Although the treadmill perturbations are designed to impose ankle dorsiflexion, any resulting knee flexion could interfere with stretching the bi-articular GM muscle. This can possibly explain differences between two previous studies applying perturbations of similar intensity around the ankle joint of healthy adults: Sinkjaer et al.[22] found short- and long-latency muscle responses to orthotic ankle perturbations, while Dietz et al.[30] only found long-latency muscle responses to perturbations applied using treadmill accelerations. Knee flexion angles were not reported in either study, but were likely increased in the latter study, as this was also the case in a similar study on healthy adults by Sloot et al.[26] Even though we noted a large increase in knee flexion in our patients, we did elicit stretch in the GM muscle, resulting in reflex responses which even appeared higher than the SO muscle response. This is of particular interest because bi-articular muscles are more often targeted in stretch hyperreflexia treatment.

Our protocol distinguishes itself from other perturbation protocols[22,25,30] in that one uniform device is needed for all participants. Our perturbations require a treadmill with possibility for real-time perturbations, which are becoming increasingly popular in gait labs that treat more severely impaired patients with motor disorders, also driven by the increased evidence for perturbation-based training.[48–53] Furthermore, perturbation treadmill requirements are lower compared to those for previous treadmill perturbation studies[29,31] with relatively low perturbation intensities (e.g. increased belt speed of 1 m/s compared to 6 m/s[31]).We previously applied perturbations of even lower intensity in able-bodied adults (increased belt speed of 0.5 m/s[26]), but shortened the duration of the perturbations in this protocol, as children have shorter stance duration. This resulted in more ankle flexion (2 and 1.3 times as large as in able-bodied adults[26] for typically developing and cerebral palsy respectively), but mechanical responses remained smaller compared to the treadmill perturbations from Berger et al.[31] (78 °/s versus 250-300 °/s ankle angular velocity, respectively) and orthotic perturbations[54] (5° versus 8° ankle dorsiflexion, respectively). Such more intense perturbations are needed to further elucidate the exact character of the muscle response, for instance to distinguish between short and long latency stretch reflexes.[31,54] Besides the fact that such distinctions are difficult, even with more intense perturbations,[55] these high intensity perturbations will be challenging in children with cerebral palsy and might cause instability. Furthermore, although a distinction is very interesting from a research perspective, this is not necessarily required for clinical purposes, nor assessed in current clinical stretch hyperreflexia measures. Given that our current velocities appear high enough to elicit muscle responses, we therefore recommend similar perturbations for clinical implementation.

The feasibility can be further improved through shortening the protocol, by removing baseline assessments and reducing the number of recovery strides, as children were generally stable after the second recovery stride. Furthermore, habituation might not be necessary for the stretch hyperreflexia assessment, as long as people feel comfortable, especially for participants already familiar with treadmill walking. Additionally, repetitions could be reduced as we showed stable responses and acceptable coefficient of variation after eight repetitions. Thus, we recommend a perturbation protocol of similar intensities with eight repetitions and three recovery strides for implementation.

Future research could focus on the need for three different intensities or alternatively a range of intensities that can be online adjusted based on the mechanical response.

Validity of stretch reflexes

The type of evoked muscle response, and whether or not these are due to stretch reflexes, is important to establish. Stretch reflexes are generally accepted to be velocity dependent. Clinical tests utilize this dependency by comparing perceived resistance between fast and slowly applied rotations around joints to discriminate between presumed stretch reflexes and other effects. However, part of the velocity dependent resistance can be caused by the viscoelastic component of the muscle-tendon complex,[56] therefore stretch hyperreflexia measures should include muscle responses to discriminate between passive and reflexive resistance. The muscle responses to our perturbations clearly increased with increasing perturbation intensity and hence with musculo-tendon lengthening velocity, without being caused by increasing co-contraction as part of for instance a stabilization strategy. Therefore, responses comply with neurophysiological criteria for reflexes. However, we assessed muscle activity in a relatively long response time window (190-220 ms), based on the perturbation duration (70-100 ms to maximum velocity), to ensure that all reflexive activity was included. Given the long window, we cannot rule out that responses are partly caused by voluntary activity and other trans-cortical contributions.[55] Nevertheless, two findings from a clinical perspective further support the reflexive nature of the responses. First, responses were higher in children with cerebral palsy, who are known to have increased reflexes[57] and decreased voluntary activity.[58] In line with this are the low muscular responses found in the SDR group, who are expected to have considerably decreased reflexes. Secondly, calf muscles remained increasingly active for a longer duration in children with cerebral palsy. This finding can be explained by decreased post-activation depression, which has been related to stretch hyperreflexia.[59,46] Accordingly, visual inspection of the data showed that only some of the children with cerebral palsy (4/23) showed a clear depression after peak activity, whereas this was visible in most typically developing children (10/14). All findings combined suggest that the protocol indeed appeared to evoke stretch reflex activity.

To be useful in the clinic, the perturbation protocol should be able to identify abnormal stretch reflex activity in patients. In this study, we established the first step: the protocol was able to discriminate between typically developing children and children with cerebral palsy at group level. We additionally measured visibly lower muscular response strength in the pilot SDR group compared to the cerebral palsy group, reflecting the reduction in feedback activity due to the surgery. The group differences might have even been underestimated, for several reasons. First, the perturbation protocol used modeled MTU lengthening, instead of fascicle lengthening which is more directly related to stretch reflex responses.[60] Children with spastic cerebral palsy can experience limited fascicle lengthening – for example due to compliant tendons or increased baseline fascicle length prior to perturbations – and thereby low reflexive responses, despite high ΔMTV.[60,61] This difference can arise due to increased activation in children with cerebral palsy,[27] but also due to differences in tendon compliance.[61,62] The error introduced by this factor can be analyzed by measuring muscle fascicle lengthening velocity (e.g., using ultrasound) instead of modeled MTV. Second, walking at an increased speed has been shown to result in increased reflex activity.[22,27,63] Children with cerebral palsy walked almost twice as slow in our study, but despite this had increased reflexes, as was also found in previous research.[27] Having children walk at similar speed might enlarge differences between groups,[27] but it would be less feasible to increase walking speed for children with cerebral palsy and a less ecologically valid comparison to make

typically developing children walk slower then their preferred walking speed.[27,28,64] Furthermore, we did not find any relation between walking speed and stretch hyperreflexia, as is explained in detail in Supplementary Materials 6.2. The final factor affecting differences between groups is that pathological gait patterns in cerebral palsy can change the mechanical responses. Different gait patterns can result in differences in initial ankle angles and relative fascicle length, which can influence the stimulation strength. We indeed found less ankle dorsiflexion in general in children with cerebral palsy, but this was corrected for in the muscular response strength. There were no apparent differences between outcomes caused by different gait patterns within the cerebral palsy group, as is presented in detail in the Supplementary Materials 6.3. However, these differences might appear when looking at more severely impaired gait. Further studies should look into the effect of more detailed but time-consuming analysis that includes correcting for initial fascicle length and relative ankle movement compared to the range of motion on the identification of individual's identified muscle hyperreflexia. Despite these factors that could reduce the group effect, the protocol was still able to distinguish patients from typically developing children at group level and hence could be used in clinical settings to test treatment effects at group level.

The next important step towards clinical implementation is to validate the protocol for use in individual patients. While our experimental set-up was not aimed at this type of analysis, we did visualize the individual muscle responses of children with cerebral palsy. Fig. 6.5 shows the large between-patient variability for the GM, as would be expected in such a heterogenous patient population. This variability cannot be explained by subject characteristics nor by perturbation characteristics - such as age, walking speed, musculo-tendon lengthening and relative increase in treadmill velocity - as is explained in more detail in Supplementary Materials 6.2. Although children with cerebral palsy had a higher muscular response strength on group level, some of these children had similar or even lower muscle response compared to typically developing children. This aligns with the recent notion that stretch hyperreflexia during passive movements is not strongly related to stretch hyperreflexia during active movements.[17,18] Not necessarily all children with spastic cerebral palsy experience stretch hyperreflexia during gait, for instance due to a protective function of increased co-contraction[65,66] or increased muscle stiffness.[60] Some researchers even suggest that stretch hyperreflexia does not negatively affect gait for patients with clinically diagnosed stretch hyperreflexia,[66] although these findings are debated by other researchers.[16,28] Our perturbation protocol can help provide insight into the contribution of stretch hyperreflexia to impaired gait. Our heterogenous results furthermore amplify the necessity of individual assessment of stretch hyperreflexia during functional movements. Treadmill perturbations can be a tool to evoke the stretch hyperreflexia and thereby explore if this is the primary cause of gait deviations on an individual basis.

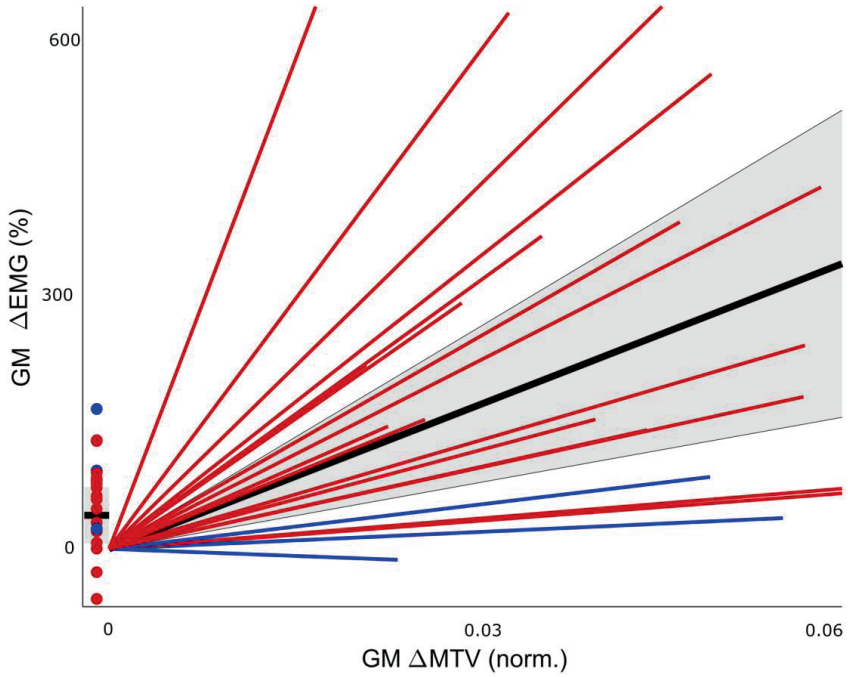

Figure 6.5. Between-participant variability in the muscular response for the GM. Lines represent the gain of the linear regression between musculo-tendon lengthening velocity and muscular responses, calculated for each participant individually from all measured strides from the perturbation trials (i.e., unperturbed and I1-I3). Dots represent the offset of the linear regression. Data is presented for typically developing children (bold black line represents mean, grey area represents mean ± standard deviation), children with cerebral palsy (red) and children who underwent SDR (blue). The offset of the linear regression is presented in a bar (typically developing) and circles (cerebral palsy and SDR), with higher values indicating high peak muscle activation levels at low levels of muscle lengthening velocity. The linear relation coefficient is presented as line steepness, with steeper lines indicating higher values of stretch hyperreflexia.

Recommendations

The applicability of this method for individual assessment should be further studied. Clinically relevant differences can be assessed by comparing stretch hyperreflexia evoked with perturbations pre- and post-treatments. Our results already indicate an effect of SDR surgery, which directly targets stretch hyperreflexia. This should be further studied by assessing more patients and including pre-SDR comparisons. The effect of other treatments, such as botulinum toxin injections, considered to affect spasticity, can also be studied using treadmill perturbations. This will add to the current clinical decision making and treatment evaluation, which mostly has to rely on less specific ordinal measures such as the Modified Ashworth Scale, and indirect functional measures such as passive ankle range of motion, walking speed, or - in more exceptional rehabilitation centers - the total muscle activity patterns during normal walking.[67–69] Larger sample sizes with more homogenous groups (e.g. only toe- or crouch walking) are needed to perform a generalizability study[70] to assess the smallest detectable differences of stretch hyperreflexia within and between patients.

This study assessed triceps surae muscles during gait, but the protocol could theoretically be redesigned to assess other muscles during functional activities, such as acceleration perturbations applied during late stance to assess rectus femoris hyperreflexia in early swing, or even during other activities such as hand biking to assess biceps hyperreflexia. It would be interesting to explore if stretch hyperreflexia expresses itself similarly in different functional activities, but we speculate a more similar expression during dynamic as opposed to passive tasks. While we show the feasibility of the protocol in more functional children, the applicability to more severe patients should be further explored. With some protocol adjustments, such as the usage of handrails on the treadmill to function as a surrogate hand-held mobility device and virtual feedback to reduce belt-cross stepping, the protocol might be well applicable across patients. Lastly, although this study focused on children with cerebral palsy, the protocol may well be applicable for use in patients with other central neurological system disorders, such as stroke and spinal cord injury, which should be further studied.

6.5 Conclusions

In summary, we present a treadmill perturbation protocol to functionally assess stretch hyperreflexia in children with cerebral palsy. This study provides evidence supporting the feasibility, reliability and validity of the protocol. We provide a framework for future studies to analyze stretch hyperreflexia in patients with central nervous system disorders at an individual level for personalized interventions.

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References

1. Bax M, O F, C T. From Syndrome Towards Disease: The Definition and Classification of Cerebral Palsy. *Dev Med Child Neurol F* 2007; 109: 39–41.
2. van den Noort JC, Bar-On L, Aertbeliën E, et al. European consensus on the concepts and measurement of the pathophysiological neuromuscular responses to passive muscle stretch. *Eur J Neurol* 2017; 24: 981-e38.
3. Sheean G. Neurophysiology of spasticity. *Up Mot neurone Syndr spasticity Clin Manag Neurophysiol* 2001; : 12–78.
4. Hodapp M, Klisch C, Berger W, Mall V, Faist M. Modulation of soleus H-reflexes during gait in healthy children. *Exp Brain Res* 2007; 178: 252–60.
5. van den Noort JC, Scholtes VA, Harlaar J. Evaluation of clinical spasticity assessment in cerebral palsy using inertial sensors. *Gait Posture* 2009; 30: 138–43.
6. Bax MCO, Brown JK. Contractures and their therapy. *Dev Med Child Neurol* 2008; 27: 423–4.
7. Holly RG, Barnett JG, Ashmore CR, Taylor RG, Mole PA. Stretch-induced growth in chicken wing muscles: a new model of stretch hypertrophy. *Am J Physiol - Cell Physiol* 1980; 238.
8. Smith LR, Lee KS, Ward SR, Chambers HG, Lieber RL. Hamstring contractures in children with spastic cerebral palsy result from a stiffer extracellular matrix and increased *in vivo* sarcomere length. *J Physiol* 2011; 589: 2625–39.
9. Rosales RL, Chua-Yap AS. Evidence-based systematic review on the efficacy and safety of botulinum toxin-A therapy in post-stroke spasticity. *J Neural Transm* 2008; 115: 617–23.
10. Novak I, Mcintyre S, Morgan C, et al. A systematic review of interventions for children with cerebral palsy: state of the evidence. *Dev Med Child Neurol* 2013; 55: 885–910.
11. Steinbok P. Outcomes after selective dorsal rhizotomy for spastic cerebral palsy. *Child's Nerv Syst* 2001; 17: 1–18.
12. Brouwer B, Wheeldon RK, Stradiotto-Parker N. Reflex excitability and isometric force production in cerebral palsy: the effect of serial casting. *Dev Med Child Neurol* 2008; 40: 168–75.
13. Scholtes VAB, Dallmeijer AJ, Becher JG. The Spasticity Test: a clinical instrument to measure spasticity in children with cerebral palsy. In: The Effectiveness of Multilevel Botulinum Toxin Type A and Comprehensive Rehabilitation in Children with Cerebral Palsy. Citeseer, 2007: 29–64.
14. Pandyan A. A review of the properties and limitations of the Ashworth and modified Ashworth Scales as measures of spasticity. *Clin Rehabil* 1999; 13: 373–83.
15. Haugh AB, Pandyan AD, Johnson GR. A systematic review of the Tardieu Scale for the measurement of spasticity. *Disabil Rehabil* 2006; 28: 899–907.
16. Bar-On L, Molenaers G, Aertbeliën E, et al. Spasticity and its contribution to hypertonia in cerebral palsy. Biomed Res. Int. 2015; 2015. DOI:10.1155/2015/317047.
17. Dietz V, Sinkjaer T. Spastic movement disorder: impaired reflex function and altered muscle mechanics. *Lancet Neurol* 2007; 6: 725–33.
18. Schindler-Ivens S, Brown DA, Lewis GN, Nielsen JB, Ondishko KL, Wieser J. Soleus H-reflex excitability during pedaling post-stroke. *Exp Brain Res* 2008; 188: 465–74.
19. Kawashima N, Sekiguchi H, Miyoshi T, Nakazawa K, Akai M. Inhibition of the human soleus Hoffman reflex during standing without descending commands. *Neurosci Lett* 2003; 345: 41–4.
20. Capaday C, Stein RB. Amplitude modulation of the soleus H-reflex in the human during walking and standing. *J Neurosci* 1986; 6: 1308–13.
21. Stein RB, Capaday C. The modulation of human reflexes during functional motor tasks. Trends Neurosci. 1988; 11: 328–32.
22. Sinkjaer T, Andersen JB, Larsen B. Soleus stretch reflex modulation during gait in humans. *J Neurophysiol* 1996; 76: 1112–20.
23. Hodapp M, Klisch C, Mall V, Vry J, Berger W, Faist M. Modulation of Soleus H-Reflexes During Gait in Children With Cerebral Palsy. *J Neurophysiol* 2007; 98: 3263–8.
24. Berger W, Dietz V, Quintern J. Corrective Reactions to Stumbling in Man - Neuronal Coordination of Bilateral Leg Muscle-Activity During Gait. *J Physiol* 1984; 357: 109–25.
25. Andersen JBJ, Sinkjaer T. An Actuator System for Investigating Electrophysiological and Biomechanical Feature Around the Human Ankle Joint During Gait. *IEEE Trans Rehabil Eng* 1995; 3: 299–306.
26. Sloot LH, Van Den Noort JJC, van der Krogt MMM, Bruijn SMS, Harlaar J. Can treadmill perturbations evoke stretch reflexes in the calf muscles? *PLoS One* 2015; 10. DOI:10.1371/journal.pone.0144815.
27. Van der Krogt MM, Doorenbosch CAM, Becher JG, Harlaar J. Dynamic spasticity of plantar flexor

muscles in cerebral palsy gait. *J Rehabil Med J Rehabil Med J Rehabil Med* 2010; 42: 656–63.
28. Bar-On L, Molenaers G, Aertbeliën E, Monari D, Feys H, Desloovere K. The relation between spasticity and muscle behavior during the swing phase of gait in children with cerebral palsy. *Res Dev Disabil* 2014; 35: 3354–64.
29. Engel T, Mueller J, Kopinski S, Reschke A, Mueller S, Mayer F. Unexpected walking perturbations: Reliability and validity of a new treadmill protocol to provoke muscular reflex activities at lower extremities and the trunk. *J Biomech* 2017; 55: 152–5.
30. Dietz V, Quintern J, Sillem M. Stumbling reactions in man: significance of proprioceptive and pre-programmed mechanisms. *J Physiol* 1987; 386: 149–63.
31. Berger W, Horstmann G, Dietz V. Tension Development and Muscle Activation in the Leg During Gait in Spastic Hemiparesis - Independence of Muscle Hypertonia and Exaggerated Stretch Reflexes. *J Neurol Neurosurg Psychiatry* 1984; 47: 1029–33.
32. Kurz MJ, Arpin DJ, Corr B. Differences in the dynamic gait stability of children with cerebral palsy and typically developing children. *Gait Posture* 2012; 36: 600–4.
33. Olney SJ, MacPhail HA, Hedden DM, Boyce WF. Work and Power in Hemiplegic Cerebral Palsy Gait. *Phys Ther* 1990; 70: 431–9.
34. Palisano R, Rosenbaum P, Bartlett D, et al. Gross Motor Function Classification System. *Dev Med Child Neurol* 1997; 39: 214–23.
35. Flux E, van der Krogt MM, Cappa P, Petrarca M, Desloovere K, Harlaar J. The Human Body Model versus conventional gait models for kinematic gait analysis in children with cerebral palsy. *Hum Mov Sci* 2020; 70: 102585.
36. van den Bogert AJ, Geijtenbeek T, Even-Zohar O, Steenbrink F, Hardin EC. A real-time system for biomechanical analysis of human movement and muscle function. *Med Biol Eng Comput* 2013; 51: 1069–77.
37. Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr Kinesiol* 2000; 10: 361–74.
38. Matsas A, Taylor N, Mcburney H. Knee joint kinematics from familiarised treadmill walking can be generalised to overground walking in young unimpaired subjects. *Gait Posture* 2000; 11: 46–53.
39. Zeni JA, Higginson JS. Gait parameters and stride-to-stride variability during familiarization to walking on a split-belt treadmill. *Clin Biomech* 2010; 25: 383–6.
40. Delp SL, Anderson FC, Arnold AS, et al. OpenSim: open-source software to create and analyze dynamic simulations of movement. *IEEE Trans Biomed Eng* 2007; 54: 1940–50.
41. Hof AL. Scaling gait data to body size. *Gait Posture* 1996; 4: 222–3.
42. Zeni JA, Richards JG, Higginson JS. Two simple methods for determining gait events during treadmill and overground walking using kinematic data. *Gait Posture* 2008; 27: 710–4.
43. Pijnappels M, Bobbert MF, van Dieën JH. EMG modulation in anticipation of a possible trip during walking in young and older adults. *J Electromyogr Kinesiol* 2006; 16: 137–43.
44. Doorenbosch CAM, Harlaar J, van Ingen Schenau GJ. Stiffness control for lower leg muscles in directing external forces. *Neurosci Lett* 1995; 202: 61–4.
45. Oudenhoven LM, Booth ATC, Buizer AI, Harlaar J, van der Krogt MM. How normal is normal: Consequences of stride to stride variability, treadmill walking and age when using normative paediatric gait data. *Gait Posture* 2019; 70: 289–97.
46. Achache V, Roche N, Lamy JC, et al. Transmission within several spinal pathways in adults with cerebral palsy. *Brain* 2010; 133: 1470–83.
47. Brown LA, Gage WH, Polych MA, Sleik RJ, Winder TR. Central set influences on gait. *Exp brain Res* 2002; 145: 286–96.
48. Grabiner MD, Crenshaw JR, Hurt CP, Rosenblatt NJ, Troy KL. Exercise-based fall prevention: Can you be a bit more specific? *Exerc Sport Sci Rev* 2014; 42: 161–8.
49. Kümmel J, Kramer A, Giboin LS, Gruber M. Specificity of Balance Training in Healthy Individuals: A Systematic Review and Meta-Analysis. Sport. Med. 2016; 46: 1261–71.
50. Marigold DS, Misiaszek JE. Reviews: Whole-body responses: Neural control and implications for rehabilitation and fall prevention. Neuroscientist. 2009; 15: 36–46.
51. Hamed A, Bohm S, Mersmann F, Arampatzis A. Follow-up efficacy of physical exercise interventions on fall incidence and fall risk in healthy older adults: a systematic review and meta-analysis. Sport. Med. - Open. 2018; 4. DOI:10.1186/s40798-018-0170-z.
52. Gerards MHG, McCrum C, Mansfield A, Meijer K. Perturbation-based balance training for falls reduction among older adults: Current evidence and implications for clinical practice. Geriatr. Gerontol. Int. 2017;

17: 2294–303.
53. Olson M, Lockhart TE, Lieberman A. Motor learning deficits in Parkinson's disease (PD) and their effect on training response in gait and balance: A narrative review. Front. Neurol. 2019; 10. DOI:10.3389/fneur.2019.00062.
54. Sinkjær T, Andersen JB, Nielsen JF, Hansen HJ. Soleus long-latency stretch reflexes during walking in healthy and spastic humans. *Clin Neurophysiol* 1999; 110: 951–9.
55. Schuurmans J, de Vlugt E, Schouten AC, Meskers CGM, de Groot JH, van der Helm FCT. The monosynaptic Ia afferent pathway can largely explain the stretch duration effect of the long latency M2 response. *Exp brain Res* 2009; 193: 491–500.
56. Singer B, Dunne J, Singer K, Allison G. Velocity dependent passive plantarflexor resistive torque in patients with acquired brain injury. *Clin Biomech (Bristol, Avon)* 2003; 18: 157–65.
57. Crenna P. Spasticity and 'Spastic' Gait in Children with Cerebral Palsy. *Neurosci Biobehav Rev* 1998; 22: 571–8.
58. Stackhouse SK, Binder-Macleod SA, Lee SCK. Voluntary muscle activation, contractile properties, and fatigability in children with and without cerebral palsy. *Muscle Nerve Off J Am Assoc Electrodiagn Med* 2005; 31: 594–601.
59. Grey MJ, Klinge K, Crone C, et al. Post-activation depression of Soleus stretch reflexes in healthy and spastic humans. *Exp Brain Res* 2008; 185: 189–97.
60. Bar-On L, Kalkman BM, Cenni F, et al. The Relationship Between Medial Gastrocnemius Lengthening Properties and Stretch Reflexes in Cerebral Palsy. *Front Pediatr* 2018; 6: 259.
61. Kalkman BM, Bar-On L, Cenni F, et al. Muscle and tendon lengthening behaviour of the medial gastrocnemius during ankle joint rotation in children with cerebral palsy. *Exp Physiol* 2018; 103: 1367–76.
62. Kalsi G, Fry NR, Shortland AP. Gastrocnemius muscle–tendon interaction during walking in typically-developing adults and children, and in children with spastic cerebral palsy. *J Biomech* 2016; 49: 3194–9.
63. Edamura M, Yang JF, Stein RB. Factors that Determine the Magnitude and Time Course of Human H-Reflexes in Locomotion. *J Neurosci* 1991; 17: 420–7.
64. Van Campenhout A, Bar-On L, Aertbeliën E, Huenaerts C, Molenaers G, Desloovere K. Can we unmask features of spasticity during gait in children with cerebral palsy by increasing their walking velocity? *Gait Posture* 2014; 39: 953–7.
65. Lorentzen J, Willerslev-Olsen M, Hü H, Larsen C, Farmer SF, Nielsen JB. Maturation of feedforward toe walking motor program is impaired in children with cerebral palsy. *Brain* 2019; 142: 526–41.
66. Nielsen JB, Christensen MS, Farmer SF, Lorentzen J. Spastic movement disorder: should we forget hyperexcitable stretch reflexes and start talking about inappropriate prediction of sensory consequences of movement? Exp. Brain Res. 2020; 238: 1627–36.
67. Gupta AD, Chu WH, Howell S, et al. A systematic review: efficacy of botulinum toxin in walking and quality of life in post-stroke lower limb spasticity. Syst. Rev. 2018; 7: 1.
68. Blumetti FC, Belloti JC, Tamaoki MJ, Pinto JA. Botulinum toxin type A in the treatment of lower limb spasticity in children with cerebral palsy. *Cochrane Database Syst Rev* 2019; 10. DOI:10.1002/14651858.cd001408.pub2.
69. Yana M, Tutuola F, Westwater-Wood S, Kavlak E. The efficacy of botulinum toxin A lower limb injections in addition to physiotherapy approaches in children with cerebral palsy: A systematic review. NeuroRehabilitation. 2019; 44: 175–89.
70. Roebroeck ME, Harlaar J, Lankhorst GJ. The Application of Generalizability Theory to Reliability Assessment: An Illustration Using Isometric Force Measurements. *Phys Ther* 1993; 73: 386–95.

Supplementary Materials 6.1: Validity outcomes

Table S6.1.

Parameters	I1 TD			I2 TD			I3 TD			Effect I TD	I1 CP			I2 CP			I3 CP			Effect I CP
Kinematics																				
Max Δangle (°)																				
Ankle	2,07	±	0,85	3,65	±	0,69	5,40	±	1,34	**<0.001**	2,00	±	0,83	3,05	±	1,34	3,79	±	1,40	**<0.001**
Knee	1,93	±	1,08	2,75	±	0,90	4,41	±	1,64	**<0.001**	2,56	±	1,17	3,78	±	1,53	4,50	±	1,90	**0.001**
Max ΔMTL (norm.)																				
GM	1,12	±	0,25	2,17	±	0,31	2,99	±	0,52	**<0.001**	0,87	±	0,44	1,53	±	0,73	2,15	±	1,05	**<0.001**
SO	1,43	±	0,59	2,47	±	0,53	3,60	±	0,89	**<0.001**	1,30	±	0,56	1,95	±	0,87	2,42	±	1,02	**<0.001**
TA	-1,39	±	0,56	-2,44	±	0,51	-3,56	±	0,81	**<0.001**	-1,30	±	0,56	-1,98	±	0,86	-2,49	±	1,05	**<0.001**
Max ΔMTV (norm.)																				
GM	13,30	±	3,09	18,67	±	2,68	25,04	±	4,04	**<0.001**	13,12	±	4,06	19,24	±	3,68	23,05	±	5,57	**<0.001**
SO	21,27	±	4,44	28,91	±	4,83	37,51	±	7,40	**<0.001**	18,82	±	7,03	25,31	±	6,67	29,76	±	9,78	**<0.001**
TA	-19,31	±	3,59	-26,78	±	3,65	-35,14	±	5,36	**<0.001**	-17,84	±	6,20	-24,27	±	6,08	-28,72	±	8,53	**<0.001**
Max ΔEMG (%)																				
GM	114,35	±	34,38	189,03	±	48,60	230,92	±	67,91	**<0.001**	147,88	±	42,68	274,40	±	131,02	359,53	±	189,63	**<0.001**
SO	116,21	±	32,12	196,90	±	53,71	285,10	±	160,90	**0.001**	142,93	±	59,97	233,60	±	109,65	282,69	±	149,86	**<0.001**
TA	55,48	±	16,72	62,98	±	28,57	106,47	±	39,83	**<0.001**	56,45	±	32,49	135,43	±	155,15	189,88	±	204,63	**0.012**
Duration (ms)																				
GM	123,86	±	29,27	130,12	±	21,32	139,29	±	30,20	**0.026**	173,90	±	50,86	235,61	±	71,26	260,12	±	85,92	**<0.001**
SO	117,00	±	20,84	133,45	±	16,72	146,71	±	34,24	**0.001**	126,55	±	33,69	175,03	±	45,70	212,82	±	60,79	**<0.001**
Strength (norm.)																				
GM	11,65	±	7,33	10,92	±	2,97	9,95	±	3,74	0.486	13,63	±	5,66	14,75	±	10,20	16,96	±	8,81	**0.033**
SO	5,70	±	2,84	7,22	±	4,23	8,52	±	5,65	0.062	10,76	±	7,48	15,47	±	17,37	11,10	±	5,05	0.295
TA	-3,86	±	3,63	-2,59	±	1,18	-3,51	±	1,64	0.622	-3,96	±	4,25	-8,19	±	8,65	-7,84	±	7,56	0.051
Co-contraction index																				
GM	0,48	±	0,09	0,45	±	0,08	0,45	±	0,07	**0.008**	0,59	±	0,12	0,57	±	0,10	0,59	±	0,12	0.975
SO	0,50	±	0,08	0,46	±	0,11	0,47	±	0,07	**0.006**	0,63	±	0,07	0,61	±	0,08	0,60	±	0,08	**0.018**

Values for the three different intensities (I1-3) are presented for all construct validity parameters. Abbreviations: with TD the typically developing group; CP the cerebral palsy group; P_{pert} the effect of perturbations (Helmert contrast); $P_{intensity}$ the effect of intensities (linear polynomial contrast,); P_{group} the difference between CP and TD; P_{inter} the interaction between CP and TD; MTL, musculo-tendon length; GM, gastrocnemius medialis muscle; SO, soleus muscle; TA, tibialis anterior muscle; MTV, musculo-tendon stretch velocity; CCI diff, difference in co-contraction index between unperturbed and highest intensity perturbations; Strength, muscular response strength. Mean ± standard deviations are presented. Significant p-values are expressed in bold.

Supplementary Materials 6.2: Correlation analyses

Given the heterogeneity in the group of participants with cerebral palsy, we explored the relationship between the reflex response and several parameters that might influence the response size. The reflex response was calculated similar to the gain in figure 5, as the linear relation coefficient between musculo-tendon lengthening velocity and muscular response, with higher values representing higher levels of stretch hyperreflexia.

First, we looked at subject characteristics including age, level of passive hyperreflexia as measured with the SPAT, gross motor function classification system (GMFCS) level, and baseline walking speed. Furthermore, the children with cerebral palsy had various different gait patterns, which can influence the effect of the perturbations. Therefore, we also assessed the relationship between reflex response and thee perturbation characteristics, being the maximum relative change in treadmill velocity (Peak Δv_{rel}) induced by the perturbation and the resulting increase in ankle dorsiflexion (Peak ΔAnkle angle) and maximum increase in musculo-tendon lengthening (Max ΔMTL) relative to baseline walking.

Scatterplots for all parameters are presented in Fig. S6.1. Statistical analysis was performed for the cerebral palsy group and the typically developing group separately. The selective dorsal rhizotomy (SDR) group was left out of analyses, as the SDR surgery intervenes with normal and CP-pathological reflex responses. The relation between reflex response and age, walking speed, relative increases in treadmill velocity, induced ankle dorsiflexion, and induced musculo-tendon lengthening were explored using a Pearson correlation analysis. Outcomes of the Pearson correlation were squared to determine the explained variability on the hyperreflexia measure. Correlations for SPAT and GMFCS were calculated using Spearman's rho and for gender using partial eta squared.

Table S6.2

Correlations with stretch hyperreflexia

Parameters	Cerebral Palsy		Typically developing	
	r	p-values	r	p-values
Age	0.100	0.356	0.291	0.156
SPAT*	0.305*	0.250	-	-
GMFCS*	0.027*	0.921	-	-
Gender**	0.012	0.680	0.009	0.743
Walking speed	0.009	0.487	0.443	0.056
Peak Δv_{rel}	-0.081	0.383	-0.239	0.205
Peak ΔAnkle angle	0.074	0.392	-0.425	0.065
Max ΔMTL	0.006	0.491	-0.009	0.487

**A spearman's rho was calculated for these variables, given the ordinal nature of the parameters. ** Similarly, partial eta squared was calculated for gender, given the nominal nature of this parameter. Abbreviations: r = Pearson correlation coefficient; SPAT = passive spasticity assessment; GMFCS = gross motor function classification system; Δvrel = peak difference in treadmill velocity during the perturbation relative to participant's baseline belt velocity; ΔAnkle angle = increase in ankle dorsiflexion due to the perturbations relative to the baseline pattern; ΔMTL = difference in musculo-tendon length due to the perturbations relative to the baseline pattern.*

None of the parameters were significantly related to the reflex response (See Table S6.1). For the typically developing children, there appeared to be a trend for walking speed (p=0.056) and induced ankle dorsiflexion (p=0.065), but both correlations were weak (r^2<0.2 for both variables) and therefore cannot explain large parts of the variability in stretch hyperreflexia measures. Furthermore, this correlation was absent in the children with cerebral palsy (p=0.487 and p=0.392).

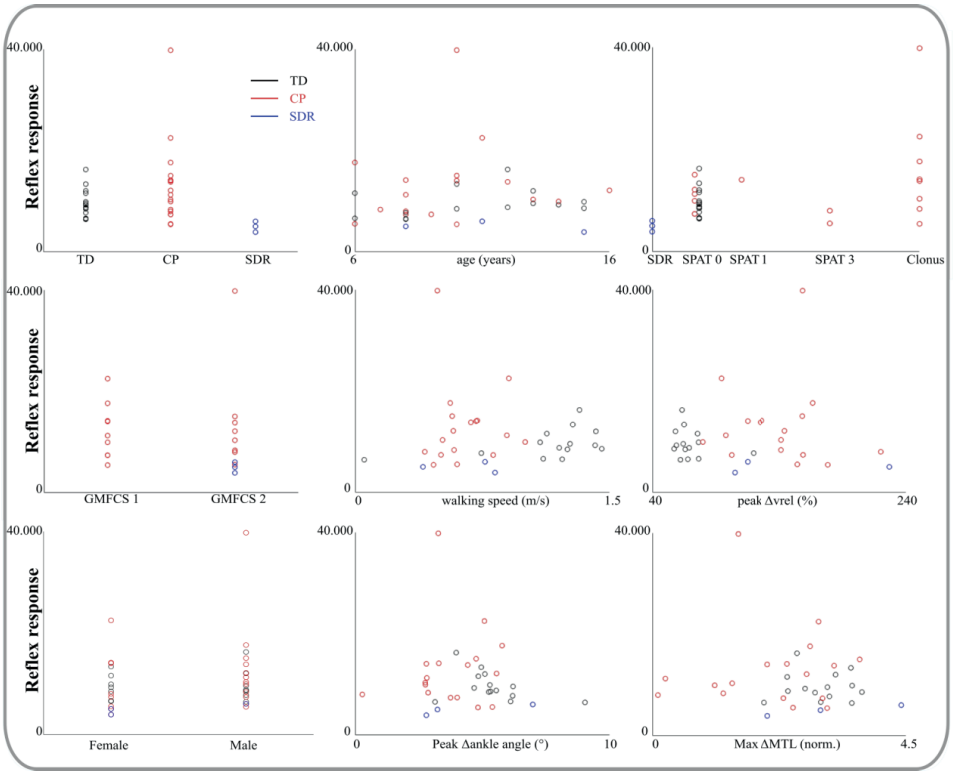


Figure S6.1. Scatter plots for hyperreflexia versus several parameters that potentially influence the hyperreflexia assessment. Reflex response reflects the increase in muscle activity relative to the increase in musculo-tendon lengthening velocity. Abbreviations: TD: typically developing children; CP = children with cerebral palsy; SDR = children with cerebral palsy who underwent selective dorsal rhizotomy surgery; SPAT = passive spasticity assessment. GMFCS = gross motor function classification system; Δvrel = peak difference in treadmill velocity during the perturbation relative to participant's baseline belt velocity; ΔAnkle angle = increase in ankle dorsiflexion due to the perturbations relative to the baseline pattern; ΔMTL = difference in musculo-tendon length due to the perturbations relative to the baseline pattern. Note that the SDR group was left out of the correlation analyses and only presented here for visual comparison.

Supplementary Materials 6.3: Deviating gait patterns

As more impaired gait patterns might affect the ability of treadmill perturbations to evoke ankle dorsiflexion, and thereby muscle lengthening and muscle responses, we more closely examined a selected group of cerebral palsy patients with most extreme deviations in the gait pattern by visual inspection of ankle and knee angles (see Fig. S1). Selecting the participants with distinct excessive flexion during the stance phase resulted in three gait deviation groups: excessive knee flexion angle during stance (*n*=5), excessive ankle dorsiflexion during stance (*n*=5) and excessive plantar flexion during early stance (toe walking, *n*=2). We compared mechanical (peak Δankle dorsiflexion, Δknee flexion, and ΔMTV) and electrophysiological (ΔEMG for GM and SO) responses between these three subgroups and the remaining children with cerebral palsy (n=13). Statistical comparison was performed using unpaired rank-sum tests given the small group sizes.

The perturbation protocol seemed effective even in the more severely impaired gait patterns, as the mechanical and electrophysiological responses generally looked similar compared with the children with less abnormal gait patterns (Fig. S6.1 D-I). Walking with excessive knee flexion did reduce the mechanical response in terms of peak ankle dorsiflexion (p=0.04), possibly resulting in less SO MTV(p=0.16).

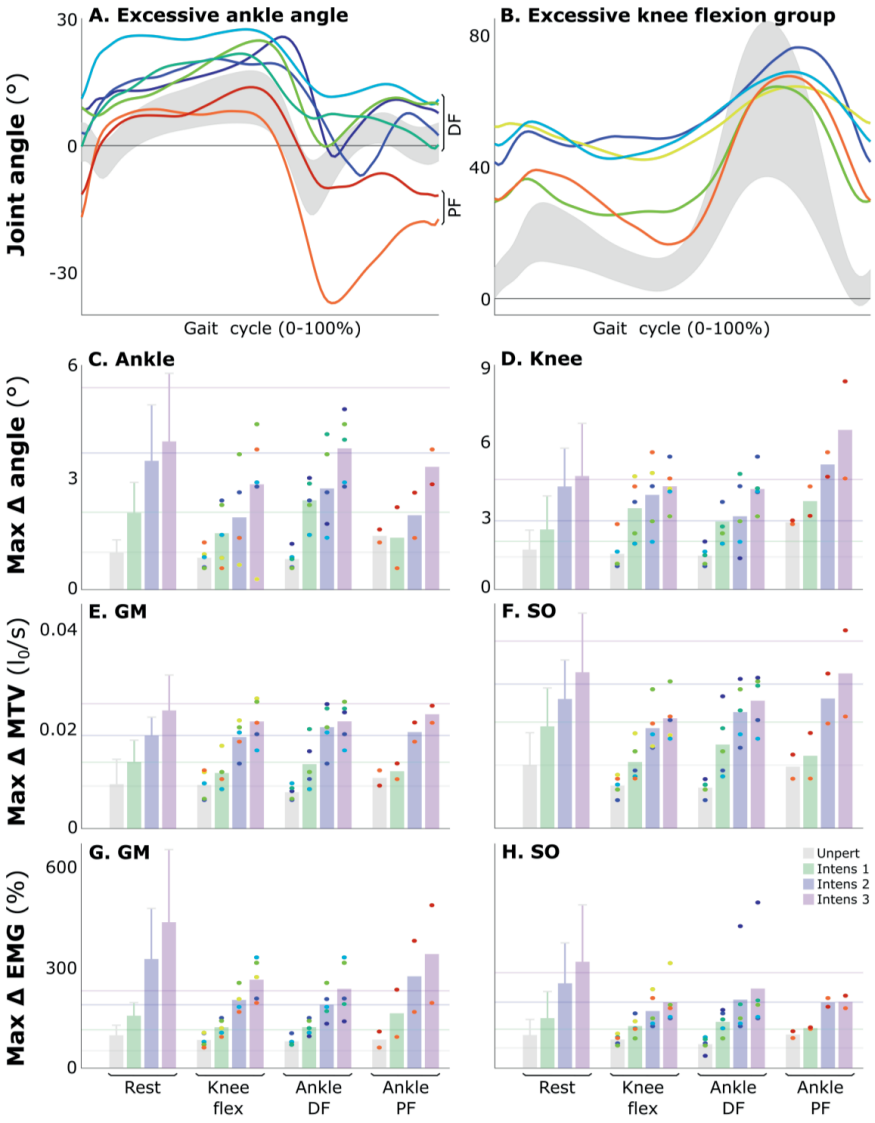
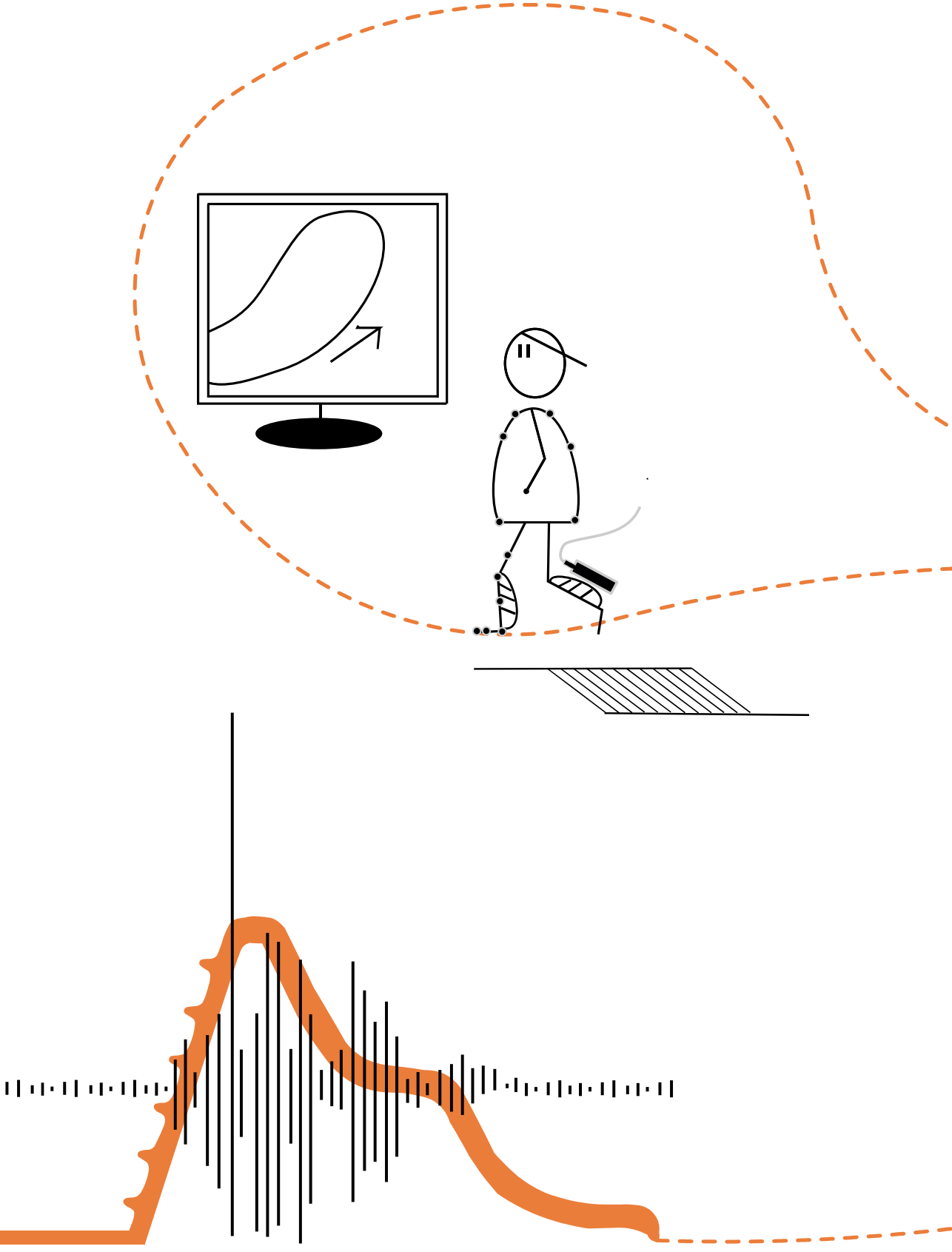


Figure S6.2. Effect of different gait patterns. **(A,B)** The time-normalized ankle and knee angle patterns for the participants of the different gait type groups: excessive ankle dorsiflexion (**B**; n=5), plantarflexion **(B)**; n=2) and knee flexion (**C**; n=5). Note that each participant has a unique color which is used throughout the figure, and three participants had both excessive ankle and knee angles. D-I) Bar graphs indicate mean and standard deviation for the average group (Rest) and individual average values are indicated for the deviating gait groups instead of error bars, due to the small sample sizes. Values are depicted for unperturbed steps (Unpert) and the three different intensities (Intens).



7

RELATION BETWEEN GASTROCNEMIUS MEDIALIS MUSCLE-TENDON STRETCH AND MUSCLE ACTIVATION DURING GAIT IN CHILDREN WITH CEREBRAL PALSY

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Abstract

Background. Stretch hyperreflexia is presumed to affect gait in children with spastic cerebral palsy (CP), and is a treatment target.

Aims: This study investigates the relation between gastrocnemius medialis muscle-tendon stretch and muscle activation during gait in children with CP and typically developing (TD) children.

Methods. 3D gait analysis including electromyography (EMG) was performed on a treadmill. Stretch of gastrocnemius medialis fascicle, belly, and tendon during treadmill walking were measured using dynamic ultrasound, and musculotendon-unit stretch was estimated using OpenSim. Ratios of EMG/peak lengthening velocities and accelerations were compared between CP and TD. Velocity and acceleration peaks prior to EMG peaks were qualitatively assessed.

Outcomes. EMG/velocity and EMG/acceleration ratios were up to 500% higher for CP (n=14) than TD (n=15) for most structures. Increased late swing muscle activation in CP was often preceded by fascicle and musculotendon-unit peak lengthening velocity, and early stance muscle activation by peaks in multiple structures.

Conclusions and Implications. Increased muscle activation in CP is associated with muscle-tendon stretch during gait. Late swing muscle activation in CP appears velocity-dependent, whereas early stance activation can be velocity- and acceleration-dependent. These insights into stretch reflex mechanisms during gait can assist development of targeted interventions.

7.1 Introduction

Cerebral palsy (CP) is associated with lesions in the developing brain.[1] Children with spastic CP experience excessive muscle activation responses to muscle stretch, also known as spasticity or velocity-dependent stretch hyperreflexia.[2] Lower-limb stretch hyperreflexia is assumed to affect gait, and is often a target of treatment with the goal of improving walking performance. Stretch hyperreflexia is measured clinically by passively rotating the joint and grading the perceived resistance, most commonly with the Modified Ashworth Scale or the Modified Tardieu Scale.[3] However, stretch hyperreflexia is known to present itself differently during active conditions such as walking,[4,5] leading to a poor relationship between passive measures of stretch hyperreflexia and gait parameters.[6]

During gait, the calf muscles, i.e. the soleus and gastrocnemius, of children with CP can be overactive during late swing[7] and/or during early stance.[8,9] Coupled with reduced activation during push-off, this pathological calf muscle activation pattern is thought to lead to reduced walking speed and increased energy cost of walking.[9,10] To what extent these aberrant calf muscle activation patterns are due to stretch hyperreflexia remains a topic of debate. It is important to identify the causes of the pathological activation pattern and subsequent gait deviations seen in children with stretch hyperreflexia for appropriate treatment selection.

Several studies have attempted to quantify stretch hyperreflexia in the calf muscles during gait by assessing the relationship between gastrocnemius medialis musculo-tendon unit (MTU) lengthening velocity and muscle activation.[5,7,11–18] Our group previously assessed dynamic stretch hyperreflexia using treadmill perturbations and measured increased stretch reflexes in the early stance phase of gait in children with CP compared to typically developing (TD) children.[12] De Niet et al.[14], however, did not find a relation between calf muscle activation and MTU lengthening velocity during the stance phase of unperturbed gait in children with stretch hyperreflexia due to hereditary spastic paresis, and concluded that the participants did not experience dynamic short-latency stretch reflexes. Van der Krogt et al.[7] only assessed reflexes during the swing phase and found increased dynamic stretch reflexes for children with CP, quantified as the ratio between muscle activation and MTU lengthening velocity. Crenna[5] found indications for the occurrence of dynamic stretch hyperreflexia during the early stance phase of gait of children with CP, measured as an increased coupling between MTU lengthening velocity and muscle activation. Thus, quantification methods of dynamic stretch hyperreflexia have differed between studies, as well as the interpretation of the outcomes, resulting in variable conclusions.

A major limitation of the previous studies is that they often model MTU stretch as a proxy for muscle spindle stretch.[5,7,11–18] However, it is commonly accepted that stretch reflexes are triggered by muscle spindle stretch instead of MTU stretch. The individual contributions of the muscle-tendon structures - i.e. fascicles, whole muscle belly, and tendon - are not accounted for when assessing MTU stretch.[19] Therefore, the original trigger of increased activation might be missed. Furthermore, children with CP experience altered compliance of the muscle-tendon structures compared to TD children,[20–22] resulting in a different distribution of stretch within the MTU for children with CP compared to TD children. Additionally, the gait pattern in children with CP is heterogeneous,[23–25] which could result in differences in stretch between children with CP. This underlines the importance of separate

assessments of the length changes of the different structures, which can now be achieved with dynamic ultrasonography.[26]

Muscle fascicle stretch, assessed with dynamic ultrasound imaging during gait, might be a more suitable measure than MTU stretch to assess stretch reflexes. Muscle fascicles are the smallest muscle structures that can be visualized using ultrasonography, and are directly related to muscle spindle output.[27] The behavior of separate fascicles can differ within the muscle belly.[26,28] Dynamic ultrasound imaging is constraint to a small region of the muscle belly, therefore limiting acquisition of multiple fascicles simultaneously. Muscle belly stretch can also be assessed with ultrasound imaging and may be more representative for stretch of multiple fascicle.

Most previous studies on dynamic stretch hyperreflexia have limited their analysis to MTU lengthening velocity. However, stretch reflexes may also be acceleration- or force-dependent,[29–32] indicating that multiple pathways and structures likely play a role in hyperactive stretch reflexes.[33] Analyzing fascicle acceleration during gait can possibly improve the understanding of the cause of the aberrant muscle activation patterns. Additional methods are necessary to analyze force within the MTU,[34] but tendon stretch could be considered a proxy of muscle force.
This study aims to explore the relation between elevated calf muscle activation and the stretch of different structures of the gastrocnemius medialis muscle (MTU, fascicle, muscle belly, and tendon) during comfortable gait in children with CP and TD children, with stretch defined as both lengthening velocity and acceleration. For this purpose, we assess the relationship between stretch of the muscle-tendon structures and muscle activation quantitatively, by calculating ratio values between muscle activation and muscle stretch, as well as qualitatively, by assessing the presence of peaks in muscle stretch preceding increased muscle activation. We hypothesize that a stronger relation exists between muscle stretch and increased muscle activation for children with CP compared with TD children, expressed by a higher muscle activation/stretch ratio. Additionally, we hypothesize that increased muscle activation is preceded most frequently by peaks in fascicle lengthening velocity.

7.2 Material and Methods

Participants

Eighteen children diagnosed with uni- or bilateral spastic CP or related forms of spastic paresis (further referred to as ‘CP’ for the whole group), and seventeen age-matched TD children participated in this cross-sectional study. Children aged six to seventeen were included when able to follow simple instructions and walk for approximately half an hour in total with sufficient rest. Children with CP had a level of functioning equal to gross motor function classification system[35] level I or II, and experienced stretch hyperreflexia in at least one calf muscle, as measured using a clinical spasticity assessment (SPAT).[36] Children were excluded when they had received orthopedic surgery on the legs (<12 months), lower limb botulinum toxin-A injections (<6 months), selective dorsal rhizotomy surgery, frequent epilepsy, behavioral problems, or comorbidities affecting gait. Specific exclusion criteria for the TD group consisted of a history of neurological or orthopedic diseases. The study protocol was approved by the local medical ethics committee of the VU University Medical Center (NL65846.029.18) and conformed to the Declaration of Helsinki guidelines. All participants aged twelve years and older provided written informed consent, as well as the parents of participants under the age of sixteen.

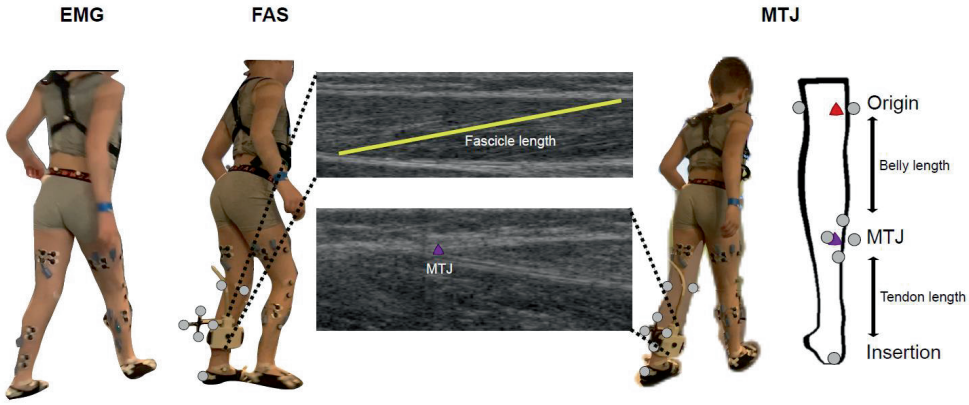

Figure 7.1. Experimental protocol. Three different trials were performed, first with electrodes on the gastrocnemius medialis muscle to capture the electromyographic activity of the gastrocnemius medialis muscle (*EMG trial*), then with the ultrasound probe on the mid-muscle to capture the fascicles (*FAS trial*) and finally on the most distal muscle-tendon junction (*MTJ trial*) to capture belly and tendon length. The FAS trial was also used to compute musculo-tendon unit (MTU) length. Both FAS and MTJ trials were captured twice, of which the one with optimal visibility was selected for processing. Muscle origin was set at ¼th between the medial and lateral knee epicondyles and insertion at the height of the heel marker. Muscle belly length and tendon length were calculated as the distance between the origin (red triangle) and MTJ (purple triangle), and the distance between MTJ and insertion, respectively.[40]

Procedures

The protocol started with a clinical exam, in which passive stretch hyperreflexia was assessed through a spasticity test (SPAT).[36] Height, body mass, and lower leg length from the medial knee epicondyle to the medial malleolus were measured. Participants were fitted with light and non-supporting fabric gym shoes to avoid the influence of shoeware. Infrared-reflective markers were placed on anatomical reference points using the Human Body Model marker set to collect 3D kinematics.[37,38] 3D kinematic data were collected at a sampling frequency of 100 Hz with a 10-camera system (Vicon Motion Systems, Oxford, UK). Wireless EMG electrodes (bipolar, Ø 15mm, 24 mm inter-electrode distance) were placed on the gastrocnemius medialis muscle (GM) according to the SENIAM protocol.[39]

EMG data were collected at 1000 Hz via a wireless system (Wave, Cometa, Italy). The Telemed SmartUS system with a 59mm linear probe (Telemed, Lithuania) was used to capture B-mode ultrasound images at a sampling frequency of 60 Hz. A custom-made probe holder (Probefix Dynamic T, USONO, The Netherlands) and Velcro straps were used to attach the probe to the lower leg (Fig. S1). The probe holder was equipped with a four-marker cluster to enable 3D motion tracking.

The experiment was conducted on an instrumented split-belt treadmill in an immersive virtual reality environment (GRAIL, Motek ForceLink BV, The Netherlands). A standing calibration trial was performed in which participants were instructed to stand as upright as possible. All participants wore a safety harness and handrails were present on the treadmill for additional safety. The experiment started with six minutes of habituation walking[41] to determine comfortable walking speed, which was used throughout the experiment. After habituation, three walking trials were performed, during which

kinematics were recorded (Fig. 7.1). In the first trial, GM muscle activation was collected for 30 seconds of walking (*EMG trial*). Simultaneous recording of ultrasound images and muscle activation was impossible due to interference of the EMG electrode positions with ultrasound probe placement. Therefore, fascicle length was determined through ultrasound during a second walking trial (*FAS trial*), by placing the ultrasound probe at the mid-muscle belly aligned with the fascicle plane.[42] In the third walking trial, the ultrasound probe was placed on the distal end of the muscle belly to visualize the muscle-tendon junction (MTJ), to obtain muscle belly length and tendon length (*MTJ trial*).[40] Children were instructed to ignore the ultrasound probe and walk like they regularly do. Our previous study indicates that TD children and children with CP can largely maintain their regular gait pattern throughout the EMG, FAS and MTJ walking trials.[43] For both ultrasound trials, two attempts were recorded of ten strides each. The clearest recordings were used for data analysis.

Data processing

3D marker data were processed with Vicon Nexus (version 2.3, Oxford, UK) and ankle and knee kinematics were computed using the gait off-line analysis tool (GOAT, version 4.2, Motek Medical, The Netherlands). The horizontal positions of the heel, toe, and pelvic markers were used to determine initial contact (IC) and toe-off values.[44] A selection of strides from the EMG trial were chosen that visually matched the FAS and MTJ trial kinematics. Subsequently, ten strides were extracted from this selection that most resembled the stride time of the FAS and MTJ trial and were used for further analysis. Five strides were analyzed from the MTJ trial and three for the FAS trial. Participants were excluded from quantitative and qualitative analyses if the average stride time between the EMG and MTJ/FAS trials exceeded 0.10s, as this might affect relating stretch of the muscle-tendon structures with muscle activation.

MTU length of the GM muscle was calculated with musculoskeletal modeling software (OpenSim).[45] First, a generic gait model (GAIT2392) was scaled to the participant using the standing calibration trial marker data. Second, the inverse kinematic tool was used to track the marker data of the FAS trial with the scaled model.[45] Next, MTU length was calculated using the muscle analysis tool, which estimates MTU length based on muscle attachments and moments arms around the joints.

Fascicle length was measured using the FAS trial. The most visible fascicle was manually tracked every two ultrasound frames using ImageJ software (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation)[46] by placing a line parallel to the fascicle from the superficial to the deep aponeurosis (Fig. 7.1). When the fascicles were too long for the ultrasound field of view, the pennation angle (α) with the deep aponeurosis and muscle width perpendicular to the deep aponeurosis were used to calculate fascicle length by dividing muscle width with the sine of alpha. Fascicle and MTU lengths were determined for three strides, as this appeared to be sufficiently informative based on previous studies[19,20] and given the time-intensive nature of the ultrasound analysis.

The MTJ trial was used to identify the distal MTJ position every five ultrasound frames using the semi-automatic script of Cenni et al.[40] Five strides were assessed to reduce variability as the semi-automatic method for MTJ determination has difficulties tracking hyper-echoic ultrasound images, which are occasionally seen in children with CP,[40] but shows higher reliability compared to tracking of fascicles.[47]

MTJ position was calculated using the MTJ movement as measured with the probe and the movement of the probe as measured using the probe marker tracking.

Muscle belly length was estimated as the distance between the MTJ position and the virtual origin of the GM muscle, placed on ¼ of the distance between medial and lateral femoral condyles in the medial direction[42] (Fig. 7.1). Tendon length was defined as the distance from the distal MTJ to the heel marker as indicator for the insertion (Fig. 7.1).

All kinematics and length profiles of the muscle-tendon structures were low-pass filtered at 8 Hz (2nd order Butterworth). Subsequently, the first and second derivatives were calculated and structure length, velocity and acceleration were nondimensionalized by lower leg length (L_{low}), $\sqrt{(g \cdot L_{low})}$, and g, respectively.[48]

The EMG data was analyzed using both raw and filtered signals, depending on the performed analyses. Filtering was performed with a high-pass filter (bidirectional, 2nd order Butterworth at 20 Hz), rectified and low-pass filtered with a cut-off frequency of 50 Hz to obtain the linear envelope and avoid filtering out the peaks. EMG was normalized to the median of all average values of each stride.[49] This normalization method was chosen over normalization to peak muscle activation, as peak muscle activation might include stretch reflex activity instead of voluntary activity in children with CP, of which the magnitude is strongly dependent on the filtering. EMG, kinematics, and tissue dynamics were time-normalized from initial contact until 50% of the next stride, defined using initial contacts, therefore covering one and a half strides. This was done as our area of interest was around initial contact. Therefore, further analysis was performed on nine EMG, four tendon and muscle belly, and two fascicle and MTU cycles of one and a half strides each.

Quantification of the relation between gastrocnemius medialis muscle-tendon stretch and muscle activation

Muscle activation was compared quantitatively between CP and TD by calculating the RMS over the linear envelope of the average EMG curve for each participant for late swing (80-100%; see Fig. 7.2A) and early stance phase of gait (initial contact - 25% of the stance phase; see Fig. 7.2B). This interval of the stance phase was chosen to include the loading response and exclude the push-off. In addition, maximum velocity and acceleration were calculated for the MTU, fascicle, belly and tendon in two reflex windows, preceding the RMS EMG windows (Fig. 7.2). The reflex windows were chosen to account for a reflex delay of 40ms[50] as the shortest expected reflex delay and 120ms as the longest expected reflex delay.[51] Finally, the ratio between RMS EMG and maximum muscle-tendon lengthening velocity/acceleration was calculated.

Qualitative assessment of the relation between stretch of the muscle-tendon structures and muscle activation

First, three trained clinicians observed the EMG linear envelope and identified participants with increased versus regular muscle activation during both the late swing phase and/or the early stance phase, as commonly applied in clinical decision-making. This was performed for all subjects (TD and CP) on all strides in blinded randomized order. Increased muscle activation had to comply with the following two criteria, being 1) a clear sudden increase in EMG and 2) not part of the push-off. When

increased EMG was identified, the three observers manually determined the onset of this increased muscle activation using the rectified raw EMG signal.

Subsequently, two observers separately detected if MTU, fascicle, belly, and tendon lengthening velocity and acceleration peaks were present in the reflex window 40-120ms preceding the onset of this increased muscle activation. This was performed for each stride separately for the children with CP (Fig. 7.3). In case of contradicting results, the specific case was discussed until mutual agreement was reached. Structures were rated as 0) not showing a peak within the reflex window or one out of four for the belly or tendon structures, 1) showing a peak in one out of two strides (MTU/fascicle) or two out of four strides (belly/tendon) or 2) showing a peak in at least three out of four strides.

Statistics

Data was checked for normality using a Shapirow-Wilk test. CP and TD group characteristics were compared using independent samples t-tests. Increased muscle activation, structure lengthening velocities and accelerations and ratios between activation and stretch were compared between CP and TD using independent samples t-test in case of normally distributed data and a Wilcoxon rank sum test otherwise. Significance level was set at $p<0.05$, with the Holm procedure [52] to correct for multiple testing, resulting in thresholds for significance of 0.05/(N) for the lowest p-value, 0.05/(N-1) for the second, etc. For the qualitative assessment, potential causes of stretch reflexes were visualized in a bar plot.

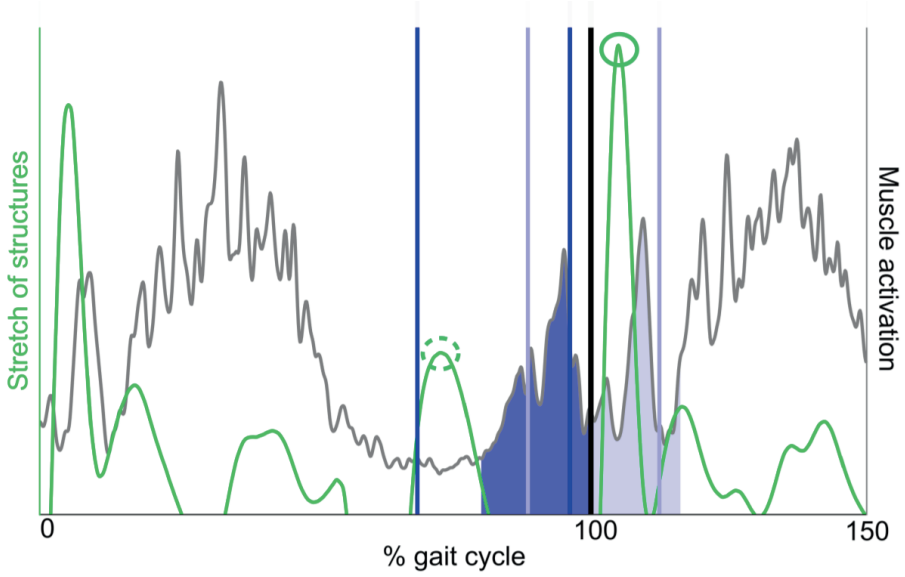

Figure 7.2. Example of the quantitative assessment. Stretch on the gastrocnemius medialis muscle-tendon structures (in green), i.e. lengthening velocity/acceleration, and gastrocnemius medialis electromyographic activation (in grey) are displayed for one and a half gait cycles (150% gait cycle (GC)). A solid black line indicates initial contact. Muscle activation was calculated over late swing (80-100%; dark blue area) and early stance (100-100%GC+25% of the stance phase; light blue area). Maximum stretch velocity/acceleration (green circles) of the involved structures were assessed in a window preceding the muscle activation that accounted for the minimum and maximum reflex delays: 80% GC minus 120ms to 100% GC minus 40ms for late swing (dark blue lines); and 100% GC minus 120ms – 25% of stance minus 40ms for early stance (light blue lines).

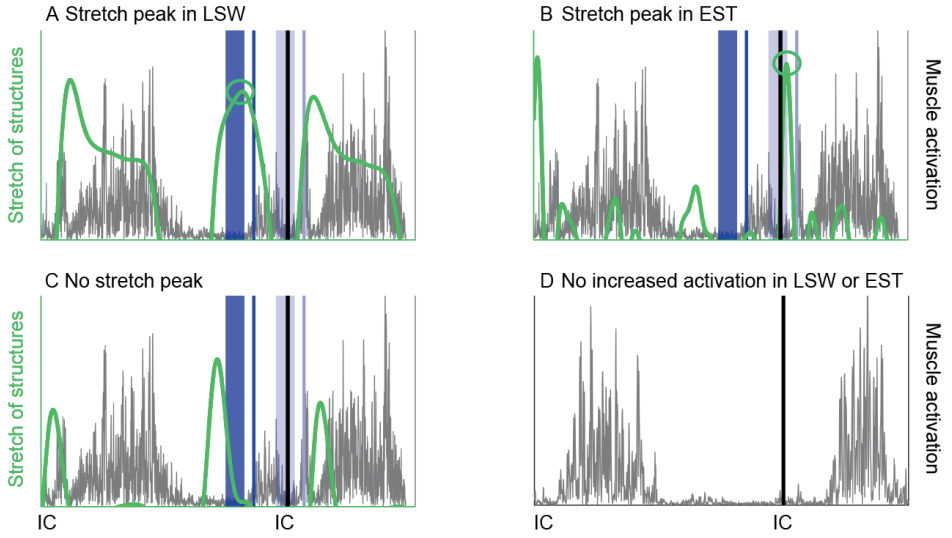

Figure 7.3. Example of the qualitative assessment. Color coding is equal to Fig. 7.2. Dark and light blue lines represent EMG onsets for late swing and early stance activation, respectively. The dark and light blue areas indicate the respective reflex windows determined from the EMG onsets identified by clinicians and therefore slightly deviate from the windows in Fig. 7.2A. A. Example of a qualitatively identified peak in muscle-tendon stretch (green circle) proceeding increased muscle activation during late swing. B. Example of an identified peak (green circle) proceeding increased muscle activation during early stance. C. Example of no identified peak within the reflex windows. D. Example of absent late swing and early stance muscle activation. Note that no reflex window is shown as there was no reflex activity, therefore also no muscle-tendon stretch is displayed. Abbreviations: LSW, late swing; EST, early stance; IC, initial contact.

Table 7.1.
Patient characteristics.

Characteristics	Children with CP (N=14)	TD children (N=15)	p-values
Age (y)	11.6 ± 3.6	11.6 ± 3.0	0.961
Gender (F/M)	6/8	11/4	
Body mass (kg)	40.4 ± 16.0	44.4 ± 17.1	0.527
GMFCS (I/II)	7/7	-	
Distribution (uni/bi)	7/7	-	
Tibia length (mm)	348.1 ± 53.2	358.6 ± 48.4	0.584
Walking speed (m/s)	0.73 ± 0.14	1.02 ± 0.12	**<0.001**

Mean values with standard deviations are displayed for age, body mass, tibia length, and walking speed. P-values indicate outcomes from independent samples t-test, with bold values indicating significant differences. Abbreviations: CP, children with cerebral palsy; TD, typically developing children; GMFCS, gross motor function classification system;[35] uni, unilateral; bi, bilateral.

7.3 Results

Four children with CP and two TD children were excluded due to time differences between walking trials (N=3), missing data (N=2), and insufficient data quality (N=1). Children with CP walked 0.28 m/s slower than TD ($p<0.001$). Characteristics of all included participants are shown in Table 7.1.

Muscle activation was higher in CP than in TD during late swing (135% increase, $p<0.001$; Fig. 7.4A, Supplementary Materials 7.1) and early stance (157% increase, $p<0.001$; Fig.7.4B). The maximum lengthening velocities of the different structures were in general lower for CP, with significantly lower velocities for the fascicles in late swing (43% decrease, $p=0.01$), and MTU during late swing (44% decrease, $p<0.001$) and early stance (30% decrease, $p=<0.001$). Furthermore, trends were found towards a decrease in belly velocity in late swing (36% decrease, $p=0.05$) and fascicle velocity in stance (37% decrease, $p=0.03$). Interestingly, children with CP reached higher tendon lengthening velocities than TD during early stance (45% increase, $p=0.01$). Furthermore, CP showed decreased maximum accelerations compared to TD in the MTU during both gait phases (40% and 44% decrease; $p<0.001$) and a trend towards decreased tendon acceleration during swing (78% decrease, $p=0.03$).
The altered stretch of muscle-tendon structures and muscle activation resulted in a larger EMG/velocity ratio for CP compared to TD, which was significant for all structures during both late swing and early stance activation (83-393%, $p\leq0.01$). Additionally, EMG/acceleration ratio was increased for CP compared to TD (231-504%, $p<0.001$), except for the tendon during late swing ($p=0.62$) and the fascicles ($p=0.03$) and belly during early stance ($p=0.03$).

Qualitative assessments revealed that all children with CP were classified as having increased muscle activation, with 10/14 showing increased late swing activation and 13/14 showing early stance activation. One participant with CP only had late swing activation and nine had both increased late swing and early stance activation. Furthermore, one TD child was classified with increased late swing, one with increased early stance activation and one with both.

Children with CP with increased late swing activation most often showed a peak in the fascicle lengthening velocity (80%) and MTU velocity (70%) (Fig. 7.5A). For all other structures, peak lengthening velocities and accelerations were identified in less than 30% of participants. Most participants (80%) showed a peak in lengthening velocity in at least one of the muscle-tendon structures (CombV), but for peak acceleration (CombA) only in half of the participants. For those with increased early stance muscle activation, fascicle acceleration (83%) and belly acceleration peaks (83%) were most often present within the reflex window (Fig. 7.5B). However, many of these children (5/10 for fascicle acceleration and 4/10 for belly acceleration) only showed peaks in one of two strides. Belly velocity (75%), and tendon velocity (75%) and acceleration (75%) often showed a peak within the reflex window as well. In all cases, there was a peak velocity and acceleration in at least one of the muscle-tendon structures preceding the early stance muscle activation.

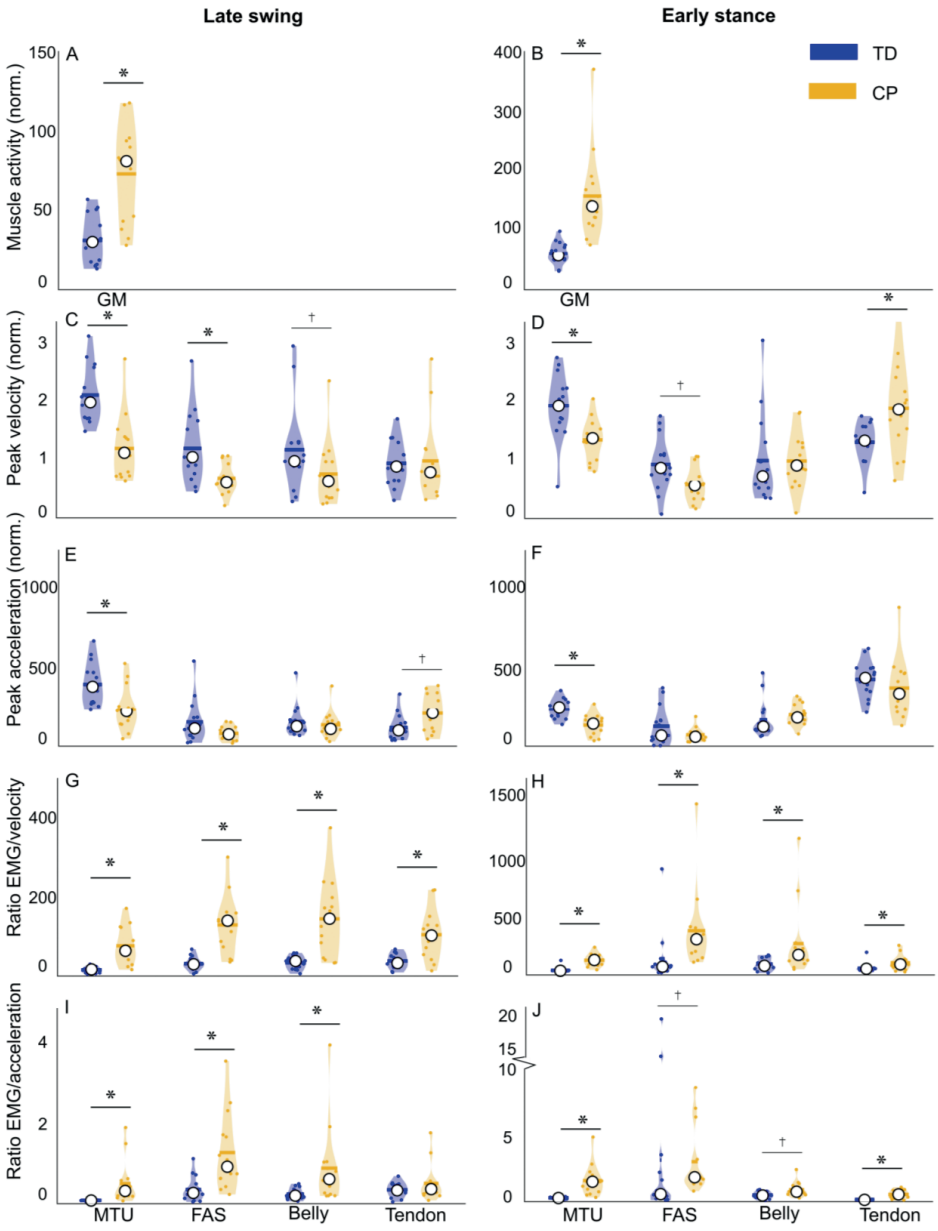

Figure 7.4. **Quantitative assessment of elevated muscle activation, structure lengthening velocity and acceleration, and their ratio.** The median and mean are displayed with a white dot and stripe respectively. All participants are represented with small dots. Stars indicate significant differences between CP and TD and crosses represent trends. Specific values can be found in Table S7.1 in Supplementary Materials 7.1. Abbreviations: GM, gastrocnemius medialis muscle; TD, typically developing children; CP, children with cerebral palsy; MTU, muscle-tendon unit; FAS, fascicle; norm., normalized.

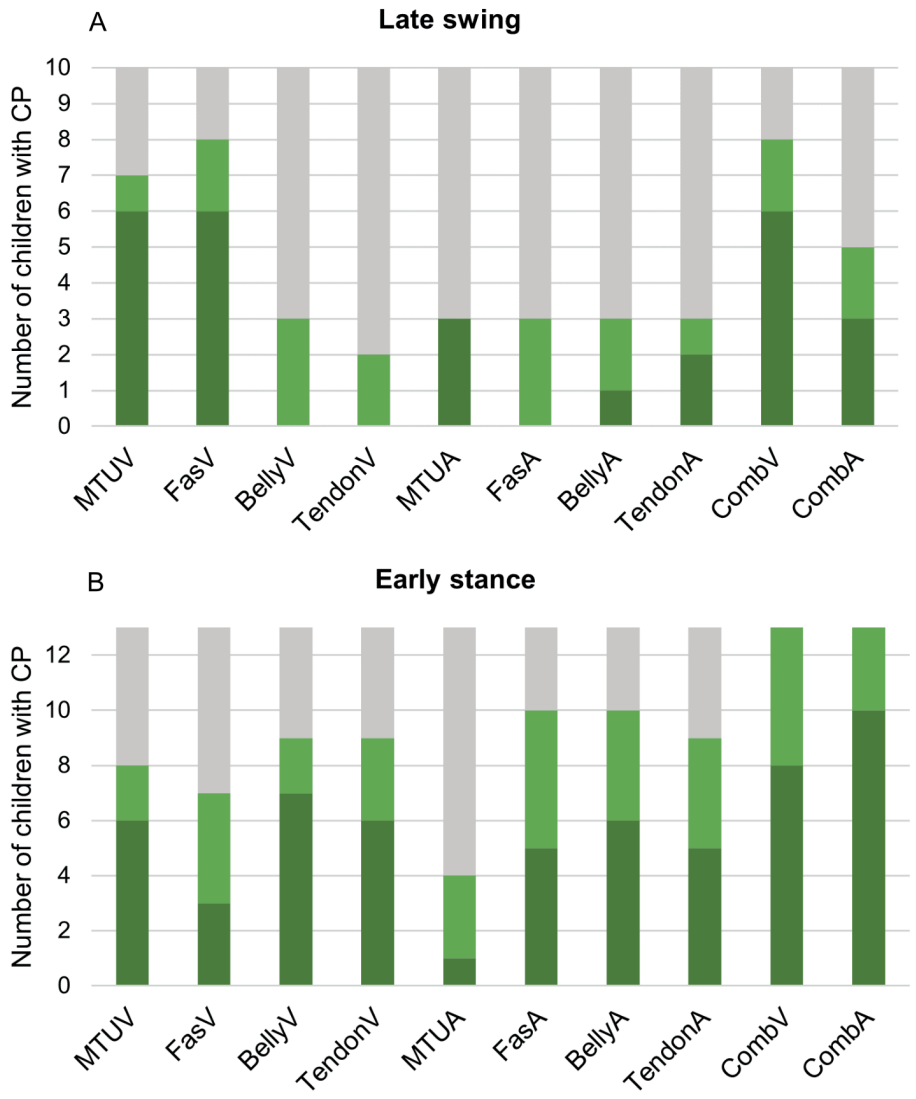

Figure 7.5. Qualitative assessment of stretch hyperreflexia during late swing (A) and early stance phase of gait (B). Colored bars indicate the number of children with CP who showed a peak in the muscle-tendon lengthening velocity (V) or acceleration (A) preceding an increase in EMG. Dark green indicates presence of a peak in most strides (showing a peak in both strides for musculo-tendon unit (MTU)/fascicle or at least three out of four for belly/tendon strides), and light green in part of the strides (showing a peak in one out of two strides for MTU/fascicle or two out of four strides for belly/tendon). The grey bars indicate the number of participants without a peak within the reflex window, or only one out of four for the belly or tendon structures. The final two bars show the number of children who had of a peak in velocity (CombV) or acceleration (CombA) in any of the muscle-tendon structures.

7.4 Discussion

This study assessed the relation between GM MTU, fascicle, belly, and tendon lengthening velocity and acceleration with GM muscle activation during comfortable walking in children with CP and TD children. This is, to our knowledge, the first study assessing the relation between the dynamics of different structures within the GM MTU and muscle activation during gait. As hypothesized, increased ratios between muscle activation and muscle-tendon lengthening velocity were found in children with CP compared to TD children, as well as increased ratios for acceleration of MTU, fascicle and belly during late swing and MTU and tendon during early stance. In late swing, increased muscle activation was preceded by peaks in fascicle velocity in all but two children with CP. Early stance activation was not always proceeded by a peak in the same structure, but all children with CP showed a velocity and acceleration peak in at least one of the muscle-tendon structures.

These findings add to the available evidence of the presence of stretch hyperreflexia in the late swing and early stance phase of gait in children with CP. In late swing, similar to the findings of Van der Krogt et al.[7], MTU velocity was lower and muscle activation higher for children with CP compared to TD children, resulting in an increased ratio between muscle activation and peak lengthening velocity. This indicates a reduced threshold for reflex activity, and therefore increased reflex loop sensitivity. This was also confirmed by the qualitative analysis revealing that peaks in the late swing phase of the EMG signal were almost always preceded by peaks in MTU lengthening velocity within the reflex window. MTU and fascicles displayed similar results in swing with regard to both the quantitative and qualitative measures. Grey et al.[53] showed a relation between Golgi tendon organ feedback and the amount of muscle-tendon stretch and loading. The loading on the muscle is small in swing due to absent external forces. Therefore, differences between MTU and fascicle stretch are probably small in the swing phase, hence the minor length changes in the tendon. Correspondingly, Lichtwark and Wilson[19] found similar MTU and fascicle behavior during swing, but not during stance. Finally, tendon velocity peaks were not often detected prior to muscle activation during late swing and therefore appear unrelated to increased muscle activation in this phase.

The ratio between peak MTU lengthening velocity and muscle activation in stance was also increased for CP compared to TD, supporting the hypothesis of the presence of stretch reflexes in the early stance phase. H-reflex studies support this, finding increased h-reflexes during both swing and early stance in children with CP[10] and it is also conform studies relating MTU stretch and muscle activation.[5,13] However, these findings contrast with De Niet et al.[14], who stated that stretch reflexes did not contribute to increased muscle activation in the first half of the stance phase for patients with upper motor neuron syndrome. Methodological differences, such as the exclusion of the end of swing and potential inclusion of part of the push-off in this study can cause these differences. Nevertheless, all but one child with CP in our study showed increased muscle activation during early stance, but only eight showed MTU lengthening velocity peaks prior to the increased muscle activation. Therefore, stretch of other muscle structures might also cause the increased muscle activation during early stance.

Replacing MTU stretch with fascicle stretch did not result in more peaks detected prior to the increase in early stance EMG activity, even though MTU and fascicle stretch differs during stance.[19] On the other hand, more peaks were detected when analyzing the muscle belly compared to fascicle lengthening

velocity. These findings suggest that reflexes in the stance phase emerge from stretch of the muscle belly, and potentially this is not accurately captured with the single fascicle included in our analysis. Additionally, tendon lengthening velocity often showed a peak prior to muscle activation in early stance. Tendons appear more compliant[22,54] or show unaltered stiffness[55] in children with CP compared to TD children, whereas muscle belly stiffness is generally increased.[55–57] Therefore, the relative contribution of tendon elongation is increased in children with CP during MTU stretch.[20] This is also apparent from our results, given the increased tendon lengthening velocity for CP compared to TD despite the lower walking velocity and generally decreased MTU and fascicle velocity. Thus, stretch of the tendon might also trigger stretch reflexes in early stance.

The additional analysis of the acceleration components reveals that acceleration of the fascicles, belly, and tendon might trigger reflex activity in early stance, given the high ratio and the common presence of an acceleration peak preceding the muscle activation. This is in line with the acceleration-dependency of the reflexes, as suggested previously.[29,31,32,58] At least one of the structures showed a peak preceding the increased muscle activation for both velocity and acceleration. This indicates that multiple pathways can be involved in the increased muscle activation observed during early stance and these pathways might be differently affected within the heterogeneous population.

Noteworthy, observation of the raw EMG signals often revealed sharp peaks in the spastic GM muscle activation patterns, as shown in Fig. 7.6. Most studies analyzing spastic calf muscle activation, including our quantitative assessment, assess the linear envelope of the muscle activation, which smoothens these sharp peaks. Presence of these peaks might originate from another pathway as opposed to overall increased early stance muscle activation, and might therefore influence the analysis. Presence of these peaks appears related to the presence of stretch hyper-reflexes during gait, as described in detail in Supplementary Materials 7.2.

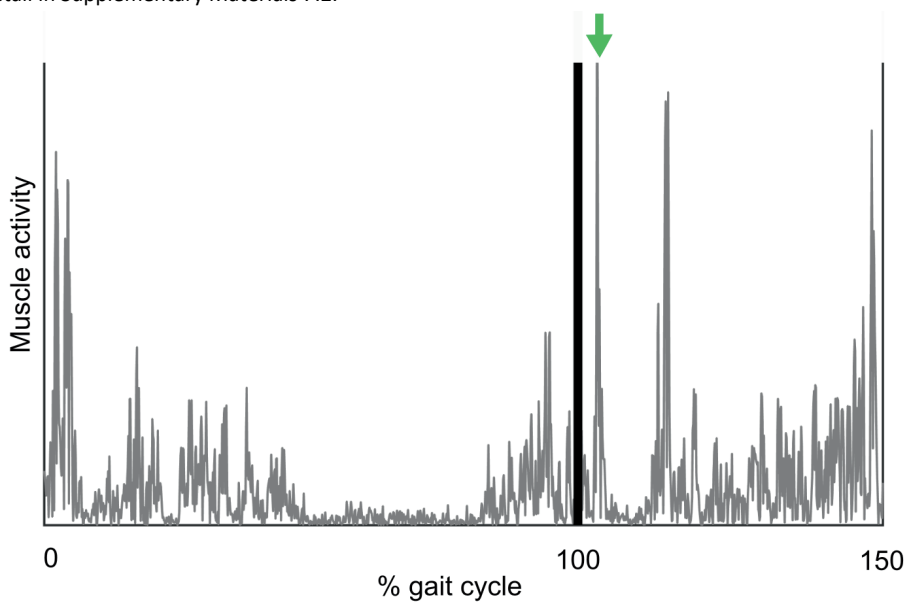

Figure 7.6. Example of loading response peak in EMG. The green arrow shows where the sudden pathological increase in EMG is seen.

There are some limitations to this study. First, different trials were used to measure muscle activation and stretch of the muscle-tendon structures. Especially in children with CP, who generally show a more variable gait pattern,[23,24,59] between-stride differences could affect relating stretch of the muscle-tendon structure to muscle activation. Nevertheless, the effect is expected to be limited as kinematics and MTU length for the different trials are generally similar.[43] To further minimize this effect, strides were matched on kinematics and stride time. Future studies could avoid the use of different trials by simultaneously recording ultrasound and EMG.[60] However, it is important to assess the interference of such devices with the gait pattern of children. A second limitation is that, because our objective was to evaluate stretch hyperreflexia during comfortable gait, the TD children in this study walked significantly faster than children with CP (Table 7.1). This could have led to an underestimation of the ratio between muscle activation and stretch of the muscle-tendon structures, since an increased walking speed for CP would increase the expression of stretch reflexes.[61] Matching walking speed would likely result in even larger differences between CP and TD. A final limitation regards the normalization method of the muscle activation to the median value over the stride-average, which could have systematically reduced the normalized RMS EMG in CP. Children with CP often display prolonged tonic activation[62] that could have resulted in a higher median value used for normalization. However, this would not have changed our conclusions as our normalization method might have led to under- rather than overestimating the muscle activation differences between CP and TD. A final limitation of this study is that we assessed stretch of the tendon as a proxy for MTU force. Tendon force can be further analyzed in future studies, for example using shear wave tensiometry.[34]

The added value of the insights from our study to clinical decision-making should be further explored. The identified relation between stretch and increased muscle activation in children with CP supports prescribing treatments to reduce stretch reflex hyper-activity that may interfere with gait. Treatments may be personalized by targeting the specific trigger for each patient. For example, if stretch reflexes are triggered by high lengthening acceleration of the tendon, treatments can be prescribed to specifically target tendon acceleration. This can, for example, be done by describing exercises that reduce tendon compliance.[63] Nevertheless, most current treatments targeting spasticity, e.g. botulin toxin type A injections or selective dorsal rhizotomy, do not consider the structures of the MTU, but rather, only the neurological system. Results from this study indicate that it is important to consider the interaction between the neurological system and the different muscle-tendon structures. Therefore, we encourage further development of clinically feasible assessments that identify the altered properties of the different MTU structures in individuals with CP. This will increase the understanding of the possible effect of these alterations on the neurological system and vice versa. Performing these assessments on an individual level will help to provide more personalized treatment in the future.

7.5 Conclusions

Results of this study are in line with the hypothesis that GM stretch reflexes are involved in unperturbed walking of children with CP. This leads to increased GM muscle activation during late swing and early stance phase of gait. During late swing, reflex activity is most likely caused by increased lengthening velocity of the GM muscle fascicles. During early stance, multiple triggers might cause increased GM reflex activity, such as belly and tendon lengthening velocity and fascicle and belly acceleration. This study provides fundamental insight into pathological muscle activation and the role of stretch reflexes during gait observed in children with CP.

Acknowledgments

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References

1. Rosenbaum P, Paneth N, Leviton A, et al. A report: The definition and classification of cerebral palsy April 2006. *Dev Med Child Neurol* 2007; 49: 8–14.
2. van den Noort JC, Bar-On L, Aertbeliën E, et al. European consensus on the concepts and measurement of the pathophysiological neuromuscular responses to passive muscle stretch. *Eur J Neurol* 2017; 24: 981-e38.
3. Scholtes VAB, Becher JG, Lankhorst GJ. Clinical assessment of spasticity in children with cerebral palsy: a critical review of available instruments. *Dev Med Child Neurol* 2006; 48: 64–73.
4. Ada L, Vattanasilp W, O'dwyer NJ, Crosbie J. Does spasticity contribute to walking dysfunction after stroke? *J Neurol Neurosurg Psychiatry* 1998; 64: 628–35.
5. Crenna P. Spasticity and 'Spastic' Gait in Children with Cerebral Palsy. *Neurosci Biobehav Rev* 1998; 22: 571–8.
6. Domagalska M, Szopa A, Syczewska M, Pietraszek S, Kidoń Z, Onik G. The relationship between clinical measurements and gait analysis data in children with cerebral palsy. *Gait Posture* 2013; 38: 1038–43.
7. Van der Krogt MM, Doorenbosch CAM, Becher JG, Harlaar J. Dynamic spasticity of plantar flexor muscles in cerebral palsy gait. *J Rehabil Med J Rehabil Med J Rehabil Med* 2010; 42: 656–63.
8. Van der Krogt MM, Doorenbosch CAM, Becher JG, Harlaar J. Walking speed modifies spasticity effects in gastrocnemius and soleus in cerebral palsy gait. *Clin Biomech* 2009; 24: 422–8.
9. Olney SJ, MacPhail HA, Hedden DM, Boyce WF. Work and Power in Hemiplegic Cerebral Palsy Gait. *Phys Ther* 1990; 70: 431–9.
10. Hodapp M, Klisch C, Mall V, Vry J, Berger W, Faist M. Modulation of Soleus H-Reflexes During Gait in Children With Cerebral Palsy. *J Neurophysiol* 2007; 98: 3263–8.
11. Bar-On L, Molenaers G, Aertbeliën E, Monari D, Feys H, Desloovere K. The relation between spasticity and muscle behavior during the swing phase of gait in children with cerebral palsy. *Res Dev Disabil* 2014; 35: 3354–64.
12. Flux E, van der Krogt MM, Harlaar J, Buizer AI, Sloot LH. Functional assessment of stretch hyperreflexia in children with cerebral palsy using treadmill perturbations. *J NeuroEngineering Rehabil 2021 181* 2021; 18: 1–17.
13. Lamontagne A, Malouin F, Richards CL. Locomotor-Specific measure of spasticity of plantarflexor muscles after stroke. *Arch Phys Med Rehabil* 2001; 82: 1696–704.
14. De Niet M, Latour H, Hendricks H, Geurts AC, Weerdesteyn V. Short-Latency Stretch Reflexes Do Not Contribute to Premature Calf Muscle Activity During the Stance Phase of Gait in Spastic Patients. *Arch Phys Med Rehabil* 2011; 92: 1833–9.
15. Sloot LH, Van Den Noort JJC, van der Krogt MMM, Bruijn SMS, Harlaar J. Can treadmill perturbations evoke stretch reflexes in the calf muscles? *PLoS One* 2015; 10. DOI:10.1371/journal.pone.0144815.
16. Crenna P, Inverno M, Frigo C, Palmieri R, Fedrizzi E. Pathological profile of gait in children with cerebral palsy. *Med Sport Sci* 1992; 36: 186–98.
17. Chow JW, Yablon SA, Stokic DS. Electromyogram-lengthening velocity relation in plantar flexors during stance phase of gait in patients with hypertonia after acquired brain injury. *Arch Phys Med Rehabil* 2012; 93: 2287–94.
18. Chow JW, Yablon SA, Stokic DS. Effect of intrathecal baclofen bolus injection on ankle muscle activation during gait in patients with acquired brain injury. *Neurorehabil Neural Repair* 2015; 29: 163–73.
19. Lichtwark GA, Wilson AM. Interactions between the human gastrocnemius muscle and the Achilles tendon during incline, level and decline locomotion. *J Exp Biol* 2006; 209: 4379–88.
20. Barber L, Carty C, Modenese L, Walsh J, Boyd R, Lichtwark G. Medial gastrocnemius and soleus muscle-tendon unit, fascicle, and tendon interaction during walking in children with cerebral palsy. *Dev Med Child Neurol* 2017; 59: 843–51.
21. Cronin NJ, Carty CP, Barrett RS, Lichtwark G. Automatic tracking of medial gastrocnemius fascicle length during human locomotion. *J Appl Physiol* 2011; 111: 1491–6.
22. Kalkman BM, Bar-On L, Cenni F, et al. Muscle and tendon lengthening behaviour of the medial gastrocnemius during ankle joint rotation in children with cerebral palsy. *Exp Physiol* 2018; 103: 1367–76.
23. Kurz MJ, Arpin DJ, Corr B. Differences in the dynamic gait stability of children with cerebral palsy and typically developing children. *Gait Posture* 2012; 36: 600–4.
24. Katz-Leurer M, Rotem H, Keren O, Meyer S. Balance abilities and gait characteristics in post-traumatic brain injury, cerebral palsy and typically developed children. *Dev Neurorehabil* 2009; 12: 100–5.

25. Goudriaan M, Papageorgiou E, Shuman BR, et al. Muscle synergy structure and gait patterns in children with spastic cerebral palsy. *Dev Med Child Neurol* 2022; 64: 462–8.
26. van Hooren B, Teratsias P, Hodson-Tole EF. Ultrasound imaging to assess skeletal muscle architecture during movements: A systematic review of methods, reliability, and challenges. *J Appl Physiol* 2020; 128: 978–99.
27. Day J, Bent LR, Birznieks I, Macefield VG, Cresswell AG. Muscle spindles in human tibialis anterior encode muscle fascicle length changes. *J Neurophysiol* 2017; 117: 1489–98.
28. Stark H, Schilling N. A novel method of studying fascicle architecture in relaxed and contracted muscles. *J Biomech* 2010; 43: 2897–903.
29. Van 't Veld RC, Van Asseldonk EHF, Kooij H Van Der, Schouten AC. Disentangling acceleration-, velocity-, and duration-dependency of the shortand medium-latency stretch reflexes in the ankle plantarflexors. *J Neurophysiol* 2021; 126: 1015–29.
30. Blum KP, Lamotte D'Incamps B, Zytnicki D, Ting LH. Force encoding in muscle spindles during stretch of passive muscle. *PLOS Comput Biol* 2017; 13: e1005767.
31. Falisse A, Bar-On L, Desloovere K, Jonkers I, De Groote F. A spasticity model based on feedback from muscle force explains muscle activity during passive stretches and gait in children with cerebral palsy. *PLoS One* 2018; 13: 1–20.
32. Sloot LH, Weide G, van der Krogt MM, et al. Applying Stretch to Evoke Hyperreflexia in Spasticity Testing: Velocity vs. Acceleration. *Front Bioeng Biotechnol* 2021; 8: 1–10.
33. Bar-On L, Molenaers G, Aertbeliën E, et al. Spasticity and its contribution to hypertonia in cerebral palsy. *Biomed Res Int* 2015; 2015. DOI:10.1155/2015/317047.
34. Ebrahimi A, Schwartz MH, Martin JA, Novacheck TF, Thelen DG. Atypical triceps surae force and work patterns underlying gait in children with cerebral palsy. *J Orthop Res* 2022; 40: 2763–70.
35. Palisano R, Rosenbaum P, Bartlett D, et al. Gross Motor Function Classification System. *Dev Med Child Neurol* 1997; 39: 214–23.
36. Scholtes VAB, Dallmeijer AJ, Becher JG. The Spasticity Test: a clinical instrument to measure spasticity in children with cerebral palsy. In: The Effectiveness of Multilevel Botulinum Toxin Type A and Comprehensive Rehabilitation in Children with Cerebral Palsy. Citeseer, 2007: 29–64.
37. Flux E, van der Krogt MM, Cappa P, Petrarca M, Desloovere K, Harlaar J. The Human Body Model versus conventional gait models for kinematic gait analysis in children with cerebral palsy. *Hum Mov Sci* 2020; 70: 102585.
38. Van Den Bogert AJ, Geijtenbeek T, Even-Zohar O, Steenbrink F, Hardin EC. A real-time system for biomechanical analysis of human movement and muscle function. *Med Biol Eng Comput* 2013; 51: 1069–77.
39. Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr Kinesiol* 2000; 10: 361–74.
40. Cenni F, Bar-On L, Monari D, et al. Semi-automatic methods for tracking the medial gastrocnemius muscle–tendon junction using ultrasound: a validation study. *Exp Physiol* 2020; 105: 120–31.
41. Matsas A, Taylor N, Mcburney H. Knee joint kinematics from familiarised treadmill walking can be generalised to overground walking in young unimpaired subjects. *Gait Posture* 2000; 11: 46–53.
42. Bénard MR, Becher JG, Harlaar J, Huijing PA, Jaspers RT. Anatomical information is needed in ultrasound imaging of muscle to avoid potentially substantial errors in measurement of muscle geometry. *Muscle Nerve* 2009; 39: 652–65.
43. Mooijekind B, Flux E, Buizer AI, van der Krogt MM, Bar-On L. The influence of wearing an ultrasound device on gait in children with cerebral palsy and typically developing children. *Gait Posture* 2023; 101: 138–44.
44. Zeni JA, Richards JG, Higginson JS. Two simple methods for determining gait events during treadmill and overground walking using kinematic data. *Gait Posture* 2008; 27: 710–4.
45. Delp SL, Anderson FC, Arnold AS, et al. OpenSim: open-source software to create and analyze dynamic simulations of movement. *IEEE Trans Biomed Eng* 2007; 54: 1940–50.
46. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 2012; 9: 671–5.
47. Cenni F, Schless SH, Adams H, Bar-On L, Desloovere K. The reliability of measuring medial gastrocnemius muscle-tendon unit lengths during gait. *Gait Posture* 2021; 90: 464–7.
48. Hof AL. Scaling gait data to body size. *Gait Posture* 1996; 4: 222–3.
49. Sousa ASP, Tavares JRMS. Surface electromyographic amplitude normalization methods: A review. In: Electromyography: new developments, procedures and applications. 2012. https://repositorio-

aberto.up.pt/bitstream/10216/64430/2/67854.pdf (accessed 19 Aug 2021).
50. Sinkjaer T, Andersen JB, Larsen B. Soleus stretch reflex modulation during gait in humans. *J Neurophysiol* 1996; 76: 1112–20.
51. Dietz V, Sinkjaer T. Spastic movement disorder: impaired reflex function and altered muscle mechanics. *Lancet Neurol* 2007; 6: 725–33.
52. Holm S. A simple sequentially rejective multiple test procedure. *Scand J Stat* 1979; : 65–70.
53. Grey MJ, Nielsen JB, Mazzaro N, Sinkjær T. Positive force feedback in human walking. *J Physiol* 2007; 581: 99–105.
54. Kruse A, Schranz C, Svehlik M, Tilp M. Mechanical muscle and tendon properties of the plantar flexors are altered even in highly functional children with spastic cerebral palsy. *Clin Biomech* 2017; 50: 139–44.
55. Theis N, Mohagheghi AA, Korff T. Mechanical and material properties of the plantarflexor muscles and Achilles tendon in children with spastic cerebral palsy and typically developing children. *J Biomech* 2016; 49: 3004–8.
56. Mathewson MA, Chambers HG, Girard PJ, Tenenhaus M, Schwartz AK, Lieber RL. Stiff muscle fibers in calf muscles of patients with cerebral palsy lead to high passive muscle stiffness. *J Orthop Res* 2014; 32: 1667–74.
57. Smith LR, Lee KS, Ward SR, Chambers HG, Lieber RL. Hamstring contractures in children with spastic cerebral palsy result from a stiffer extracellular matrix and increased *in vivo* sarcomere length. *J Physiol* 2011; 589: 2625–39.
58. Welch TDJ, Ting LH. A feedback model explains the differential scaling of human postural responses to perturbation acceleration and velocity. *J Neurophysiol* 2009; 101: 3294–309.
59. Brændvik SM, Goihl T, Braaten RS, Vereijken B. The Effect of Increased Gait Speed on Asymmetry and Variability in Children With Cerebral Palsy. *Front Neurol* 2020; 10: 1399.
60. Botter A, Beltrandi M, Cerone GL, Gazzoni M, Vieira TMM. Development and testing of acoustically-matched hydrogel-based electrodes for simultaneous EMG-ultrasound detection. *Med Eng Phys* 2019; 64: 74–9.
61. Sinkjær T, Andersen JB, Nielsen JF, Hansen HJ. Soleus long-latency stretch reflexes during walking in healthy and spastic humans. *Clin Neurophysiol* 1999; 110: 951–9.
62. Hesse S, Brandl-Hesse B, Seidel U, Doll B, Gregoric M. Lower limb muscle activity in ambulatory children with cerebral palsy before and after the treatment with Botulinum toxin A. *Restor Neurol Neurosci* 2000; 17: 1–8.
63. Kalkman BM, Holmes G, Bar-On L, et al. Resistance training combined with stretching increases tendon stiffness and is more effective than stretching alone in children with cerebral palsy: A randomized controlled trial. *Front Pediatr* 2019; 7: 333.

	Mean CP	Mean TD	P	Change
RMS_SW	73,0 ± 30,2	31,0 ± 15,4	**<0,001**	135%
RMS_ST[1]	157,2 ± 75,0	61,2 ± 18,2	**<0,001**	157%
MTUV_SW[1]	1,17 ± 0,56	2,06 ± 0,46	**<0,001**	-44%
FASV_SW	0,66 ± 0,25	1,17 ± 0,59	**0,01**	-43%
BV_SW[1]	0,74 ± 0,55	1,14 ± 0,72	0,05[2]	-36%
TV_SW[1]	0,96 ± 0,67	0,92 ± 0,37	0,56	4%
MTUV_ST	1,32 ± 0,35	1,89 ± 0,54	**<0,001**	-30%
FASV_ST	0,57 ± 0,29	0,91 ± 0,45	0,03[2]	-37%
BV_ST[1]	0,96 ± 0,47	0,97 ± 0,73	0,45	-1%
TV_ST	1,85 ± 0,76	1,28 ± 0,34	**0,01**	45%
MTUA_SW	233,6 ± 134,9	387,8 ± 133,1	**<0,001**	-40%
FASA_SW[1]	80,3 ± 44,0	152,6 ± 133,5	0,09	-47%
BA_SW[1]	134,5 ± 84,7	154,8 ± 98,5	0,56	-13%
TA_SW[1]	208,5 ± 115,4	116,9 ± 80,9	0,03[2]	78%
MTUA_ST	132,8 ± 65,1	235,3 ± 58,3	**<0,001**	-44%
FASA_ST[1]	71,8 ± 43,3	125,6 ± 117,4	0,39	-43%
BA_ST[1]	198,4 ± 66,8	166,2 ± 114,4	0,06	19%
TA_ST[1]	365,6 ± 187,7	419,5 ± 113,6	0,18	-13%
Ratio_MTUV_SW	76,0 ± 46,2	15,4 ± 7,5	**<0,001**	393%
Ratio_FASV_SW	128,6 ± 73,4	31,1 ± 16,8	**<0,001**	314%
Ratio_BV_SW	143,9 ± 98,1	33,4 ± 16,5	**<0,001**	330%
Ratio_TV_SW	104,0 ± 62,2	37,9 ± 18,6	**<0,001**	175%
Ratio_MTUV_ST[1]	124,0 ± 50,3	38,0 ± 25,5	**<0,001**	224%
Ratio_FASV_ST[1]	371,0 ± 336,8	135,8 ± 217,0	**<0,001**	173%
Ratio_BV_ST[1]	262,9 ± 301,0	91,0 ± 53,5	**0,01**	189%
Ratio_TV_ST[1]	102,3 ± 62,7	55,8 ± 39,7	**0,01**	83%
Ratio_MTUA_SW[1]	0,51 ± 0,54	0,08 ± 0,04	**<0,001**	503%
Ratio_FASA_SW[1]	1,29 ± 0,98	0,36 ± 0,30	**<0,001**	262%
Ratio_BA_SW[1]	0,89 ± 1,02	0,24 ± 0,14	**<0,001**	269%
Ratio_TA_SW[1]	0,51 ± 0,46	0,35 ± 0,18	0,62	46%
Ratio_MTUA_ST[1]	1,59 ± 1,18	0,28 ± 0,13	**<0,001**	462%
Ratio_FASA_ST[1]	3,01 ± 2,47	3,13 ± 6,02	0,03[2]	-4%
Ratio_BA_ST[1]	0,9 ± 0,56	0,49 ± 0,26	0,03[2]	83%
Ratio_TA_ST	0,54 ± 0,32	0,16 ± 0,09	**<0,001**	231%

Mean values and standard deviations for the parameters presented in Figure 4. P-values are from independent t-tests in case of normal distribution and Wilcoxon rank sum test otherwise. [1]Denotes variables without normal distribution. Bold p-values indicate significance. [2]Not significant due to Holm correction. Change indicates the percentage difference between children with cerebral palsy (CP) and typically developing children (TD), calculated as CP/TD*100-100. Abbreviations: RMS, root mean square; SW, swing; ST, stance; MTU, musculotendon unit; FAS, fascicle; B, muscle belly; T, tendon; V, peak velocity; A, peak acceleration; Ratio, peak lengthening velocity or acceleration divided by RMS EMG.

Supplementary Materials 7.2

Loading response peak in EMG activity

In the raw EMG data we often observed sharp peaks during the loading response (Fig. 7.6). These peaks were manually identified and present in 12/17 children with CP. None of the TD children showed this peak, making it unlikely that the peak originates from artefacts. Therefore we hypothesized that this peak might be related to stretch hyperreflexia. We conducted explorative analyses to gain more insight in this sharp peak, further referred to as loading response peak, to assess whether this loading response peak is related to stretch hyperreflexia.

First, we related the presence of the loading response peak to participant characteristics, i.e. SPAT scores and GMFCS levels, and foot position at initial contact. We classified this foot position as either heel, midfoot or toe contact. The relation between patient characteristics and peak occurrence was analyzed using a two-sided rank sum test for the SPAT and foot position at initial contact and a Fisher's exact test for the GMFCS levels. One child was excluded from this analysis due to technical issues with the EMG data. Second, we conducted a qualitative assessment of the relation between the loading response peak and tissue dynamics preceding this peak, similar to the qualitative assessment in the main paper (see the method of the main paper for a detailed description of the assessment). Three children were excluded from this analysis due to technical issues, conform the main paper.

Children classified with a loading response peak had on average higher SPAT values than children without this peak (Fig. S7.1, p=0.020). This suggests that children who clinically show more stretch hyperreflexia are likelier to have a loading response peak. There were no significant relations between loading response peak occurrence and GMFCS level (p=0.131) or foot position at initial contact (p=0.233, Fig. S7.1).

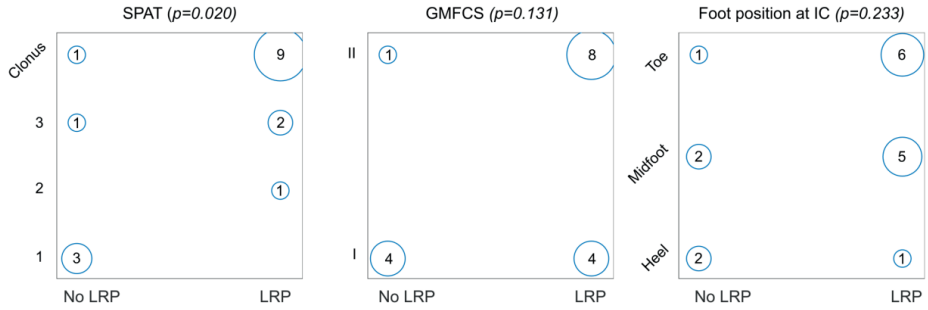


Figure S7.1. Participant characteristics related to occurrence of the loading response peak (LRP). Size of the circle and number listed within the circle indicate the number of children with CP. P-values of the corresponding analyses are enlisted above the figures. Abbreviations: SPAT, the spasticity test;[36] GMFCS, gross motor function classification system;[35] IC, initial contact.

The qualitative analysis showed that a peak in MTU velocity was most often identified in the reflex window preceding the loading response peak (Fig. S7.2). However, corresponding to our results regarding pathological stance activity as described in the main paper, velocity and acceleration peaks in several tissues preceded the loading response peak. Therefore, several pathways may trigger the loading response peak.

The presence of the loading response peak in children with CP and the absence of the peak in TD children, its relation with SPAT score, and the fact that the loading response peak was commonly preceded by stretch on the muscle-tendon tissues, further strengthens the idea that this sharp peak is related to stretch hyperreflexia. This supports the theory that stretch reflexes contribute to pathological calf muscle activation during gait in CP. As far as we know, the loading response peak has never been described elaborately in previous studies, probably because the sharp peak is filtered out in most studies. Therefore, the importance of investigating the raw signal as well as the linear envelope is highlighted.

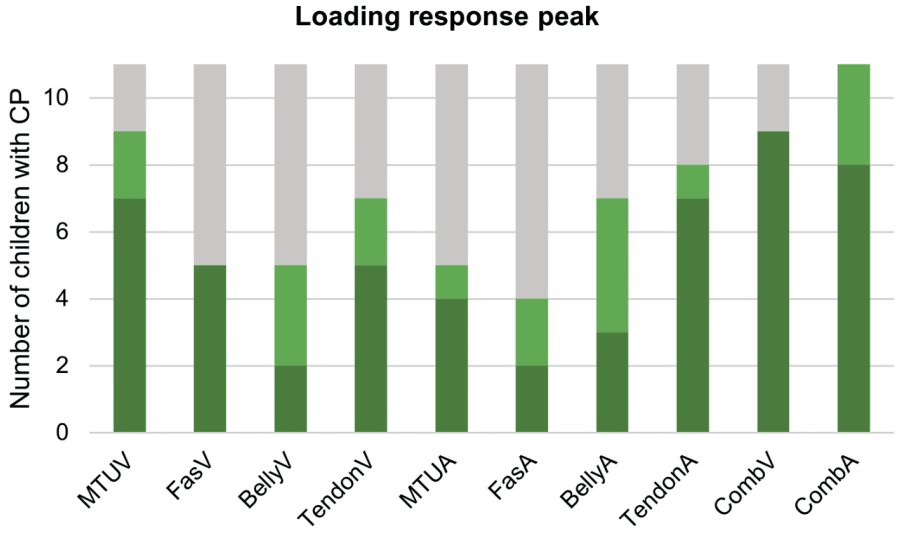
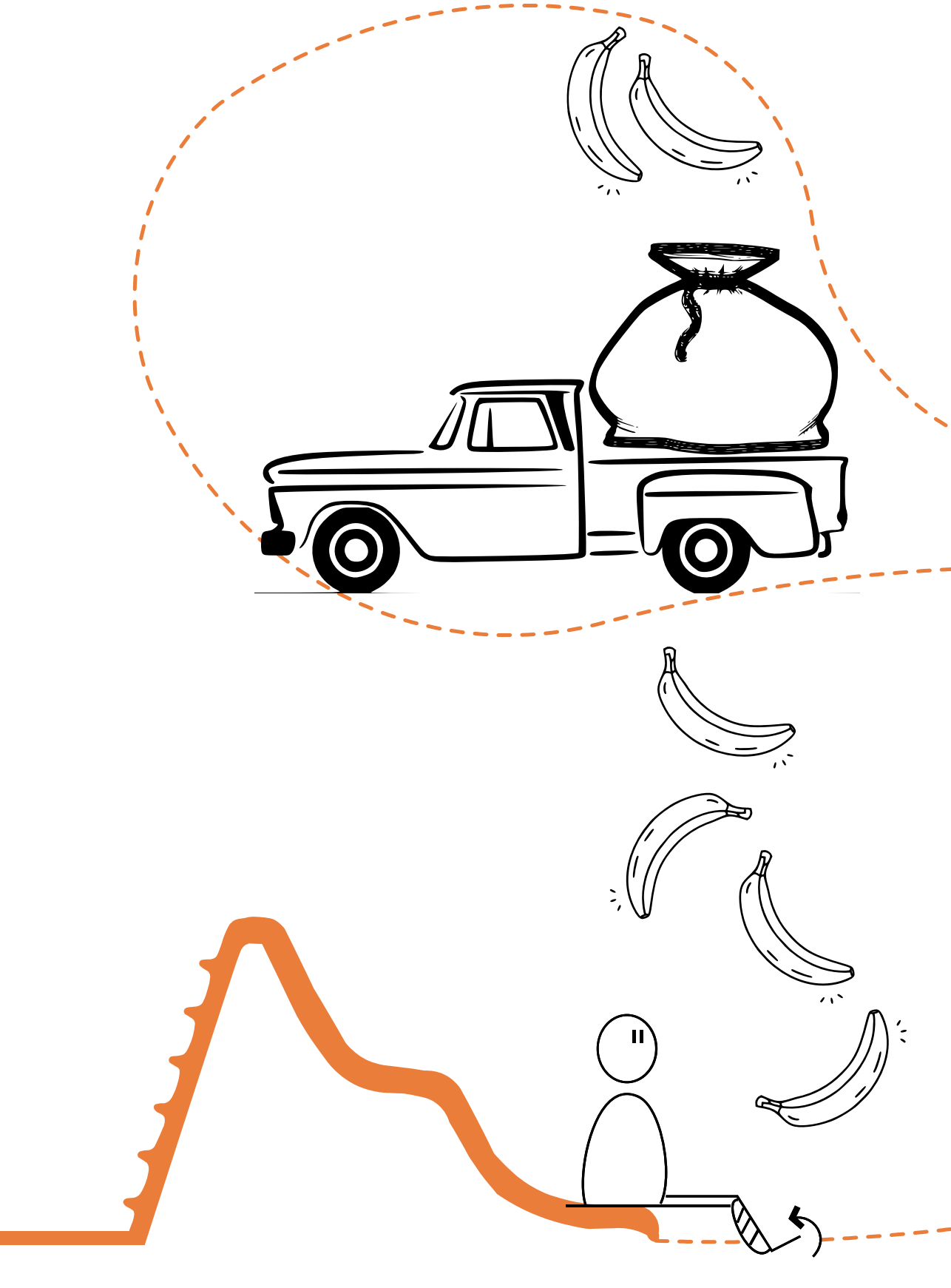
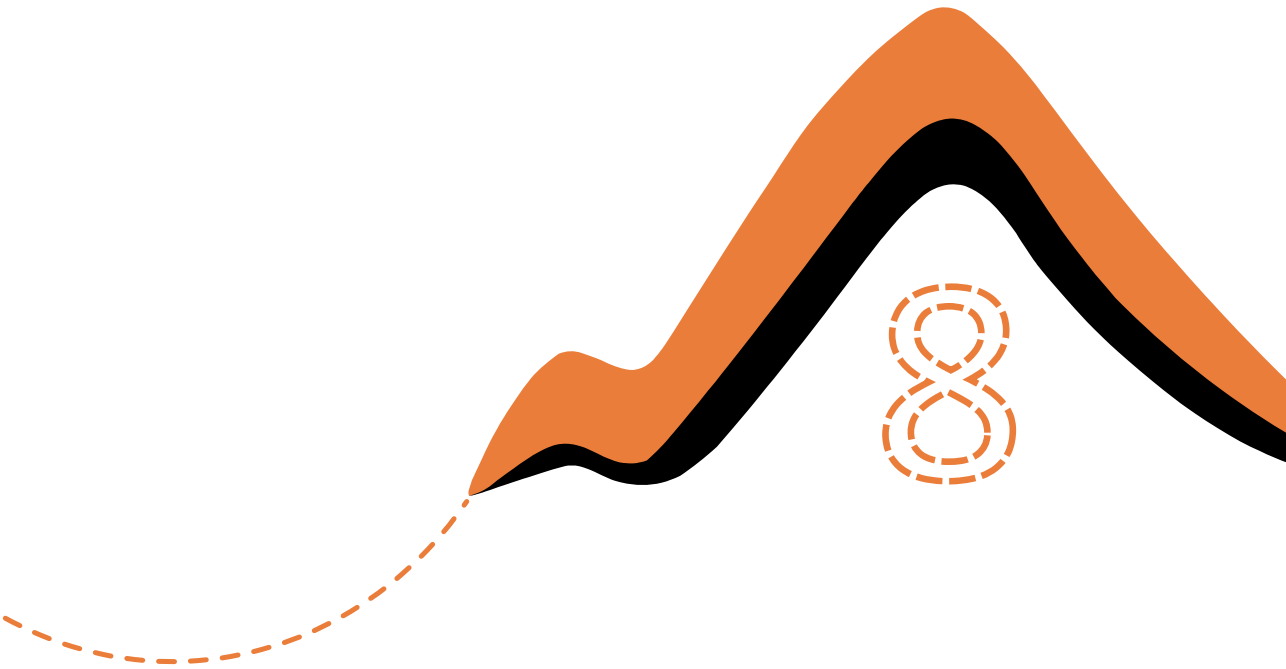


Figure S7.2. Qualitative assessment of stretch hyperreflexia during loading response. Colored bars indicate the number of children with CP who showed a peak in the tissue lengthening velocity (V) or acceleration (A) preceding an increase in muscle activation in most strides (showing a peak in both strides for musculo-tendon unit (MTU)/fascicle or at least three out of four for belly/tendon strides; dark green) or part of the strides (showing a peak in one out of two strides for MTU/fascicle or two out of four strides for belly/tendon; light green). The grey bars indicate the number of participants without a peak within the reflex window, or one out of four for the belly or tendon structures. The final two bars show the number of children who had of a peak in velocity (CombV) or acceleration (CombA) in any of the tissues.





REDUCING THE SOLEUS STRETCH REFLEX WITH CONDITIONING: EXPLORING GAME- AND IMPEDANCE-BASED BIOFEEDBACK

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Abstract

People with spasticity, i.e., stretch hyperreflexia, have a limited functional independence and mobility. While a broad range of spasticity treatments is available, many treatments are invasive, non-specific, or temporary and might have negative side effects. Operant conditioning of the stretch reflex is a promising non-invasive paradigm with potential long-term sustained effects. Within this conditioning paradigm, seated participants have to reduce the mechanically elicited reflex response using biofeedback of reflex magnitude quantified using electromyography (EMG). Before clinical application of the conditioning paradigm, improvements are needed regarding the time-intensiveness and slow learning curve. Previous studies have shown that gamification of biofeedback can improve participant motivation and long-term engagement. Moreover, quantification of reflex magnitude for biofeedback using reflexive joint impedance may obtain similar effectiveness within fewer sessions. Nine healthy volunteers participated in the study, split in three groups. First, as a reference the *"Conventional"* group received EMG- and bar-based biofeedback similar to previous research. Second, we explored feasibility of game-based biofeedback with the *"Gaming"* group receiving EMG- and game-based biofeedback. Third, we explored feasibility of game- and impedance-based biofeedback with the *"Impedance"* group receiving impedance and game-based biofeedback. Participants completed five baseline sessions (without reflex biofeedback) and six conditioning sessions (with reflex biofeedback). Participants were instructed to reduce reflex magnitude without modulating background activity. The Conventional and Gaming groups showed feasibility of the protocol in 2 and 3 out of 3 participants, respectively. These participants achieved a significant Soleus short-latency (M1) within-session reduction in at least –15% in the 4th–6th conditioning session. None of the Impedance group participants showed any within-session decrease in Soleus reflex magnitude. The feasibility in the EMG- and game-based biofeedback calls for further research on gamification of the conditioning paradigm to obtain improved participant motivation and engagement, while achieving long-term conditioning effects. Before clinical application, the time-intensiveness and slow learning curve of the conditioning paradigm remain an open challenge.

8.1 Introduction

Spasticity is a common symptom after brain and neural injuries, like spinal cord injury, stroke, and cerebral palsy.[1] Spasticity is defined as the exaggerated stretch reflex response, i.e., stretch hyperreflexia.[2] Patients with spasticity are limited in functional independence and mobility and often experience substantial pain. A broad range of spasticity treatments is available, including physical therapy, oral medication, interventional procedures, and surgical treatments.[3] Unfortunately, current treatments are invasive, non-specific, or temporary and might have negative side effects.[3] Therefore, there is a clinical need for a non-invasive spasticity treatment with long-term sustained effect.

Operant conditioning of the reflex response is a promising, non-invasive paradigm to obtain a long-term spasticity reduction.[4,5] Within the conditioning paradigm, participants are trained to either increase ("up-condition") or reduce ("down-condition") the reflex response using biofeedback of reflex magnitude. Currently, paradigm feasibility has been shown for both electrical stimulation, i.e., H-reflex conditioning, mechanical stimulation, and stretch reflex conditioning, using electromyography (EMG) biofeedback of the calf muscles.[6,7] Both forms of stimulation have shown equal effectiveness during conditioning with static posture in able-bodied participants: an average –15% short- term (within-session) and –20% long-term (across-session) down-conditioning effect was obtained after 4–6 and 12–16 conditioning sessions, respectively.[6,7] From a practical, clinical perspective, the mechanical stimulation is advantageous as it yields higher participant comfort and applicability to other joints. Besides, protocols with EMG biofeedback require accurate electrode placement, checked using electrical stimulation, to ensure that conditioning effects are not due to across-session changes in electrode placement. Removing the need for accurate electrode placement checked *via* electrical stimulation would be beneficial considering home applications. Overall, before clinical application of the conditioning paradigm, improvements are needed regarding the time-intensiveness (3 session per week) and slow learning curve (at least 16 sessions).

As potential improvements for stretch reflex conditioning, we propose the use of gamification and reflexive joint impedance biofeedback. First, gamification entails the introduction of a gaming element into non-gaming situations, like rehabilitation, to make activities more pleasurable and increase long-term engagement.[8,9] Gamification can improve participant motivation in view of the possibly demotivating conditioning paradigm,[10] given the long baseline measurements and slow learning curves.[6,7] Numerous studies have shown these improvements in motivation and engagement in patients with neurological conditions, such as cerebral palsy, stroke, and Parkinson's disease.[11,12] Alongside improved motivation, most game-based interventions ensure equal or even increased treatment effectiveness.[10-12] However, negative effects of gamification were also reported, e.g., high levels of motivation due to gamification can distract from the primary motor learning goal and encourage undesirable compensation strategies.[13] Therefore, it is important to assess whether gamification interferes with potential treatment outcomes.

Second, reflexive joint impedance biofeedback entails quantification of reflex magnitude using a mechanical-based methodology instead of the muscle-based EMG biofeedback to accelerate learning curves.[14,15] The impedance-based biofeedback disentangles the reflexive joint resistance due to the mechanical stimuli from other non-reflexive joint resistance contributions using joint torques and kinematics.[16] As such, an impedance-based conditioning treatment would not require any electrodes or

electrical stimulation. Previous study suggests a faster learning curve for impedance-based biofeedback, as participants were able to already modulate their reflex response after 2 sessions.[15] Ludvig et al.[15] used a specific online algorithm to provide biofeedback on reflex magnitude.[14] Thus, use of impedance- instead of EMG-based biofeedback can potentially improve the learning curve and practical execution.

The goal of this study is to explore the feasibility of two forms of biofeedback within the stretch reflex down-conditioning paradigm: (1) gamification of the biofeedback and (2) impedance based biofeedback. To explore feasibility, the within-session conditioning effect is investigated across six conditioning sessions. The investigation is split across three participant groups, executed in three separate phases: (1) "Conventional" receiving EMG- and bar-based biofeedback as in Mrachacz- Kersting et al.[7]; (2) "Gaming" receiving EMG- and game- based biofeedback; and (3) "Impedance" receiving impedance- and game-based biofeedback. The use of a specific biofeedback method is considered feasible when the reference –15% within- session effect reported in previous studies can be achieved across the 4th–6th conditioning session.[6,7] Each experimental phase was only started once the previous experimental phase was evaluated as being feasible. Our study aims to open the way for stretch reflex conditioning as non-invasive spasticity treatment by introducing new biofeedback methods to make improvements regarding the time-intensiveness and slow learning curve.

8.2 Materials and methods

Participants and Study Schedule

Nine volunteers with no history of neuromuscular disorders participated in the study: age 26.0 ± 5.0 yr, seven women. The EEMCS/ET ethics committee of the University of Twente approved the study, and all participants provided written informed consent. The participants were split in the three biofeedback groups in order of inclusion, see Fig. 8.1A: (1) EMG- and bar-based biofeedback ("Conventional"); (2) EMG- and game-based biofeedback ("Gaming"); and (3) impedance- and game-based biofeedback ("Impedance").

All groups completed the same study schedule, designed in similar fashion to Thompson et al.[6] and Mrachacz-Kersting et al.[7], see Fig. 8.1A. The study consisted of the following: one preparation (PRE), one acclimatization (A1), five baseline (B1- 5), and six conditioning (C1-6) sessions. The preparation session was aimed at defining all personalized hardware and software settings using a protocol distinct from all other sessions. The acclimation followed the baseline session protocol and aimed to familiarize participants with this protocol.[4,6] The baseline sessions (without reflex biofeedback) and conditioning sessions (with reflex biofeedback) formed the core data collection sessions of the paradigm, see Fig. 8.1A. Three sessions were scheduled per week (Monday, Wednesday, and Friday) with baseline and conditioning sessions typically lasting 1 h with a 1.5 h maximum. Any diurnal variation in reflexive response was minimized by scheduling all sessions at the same time of day, i.e., within the same 3 h period.

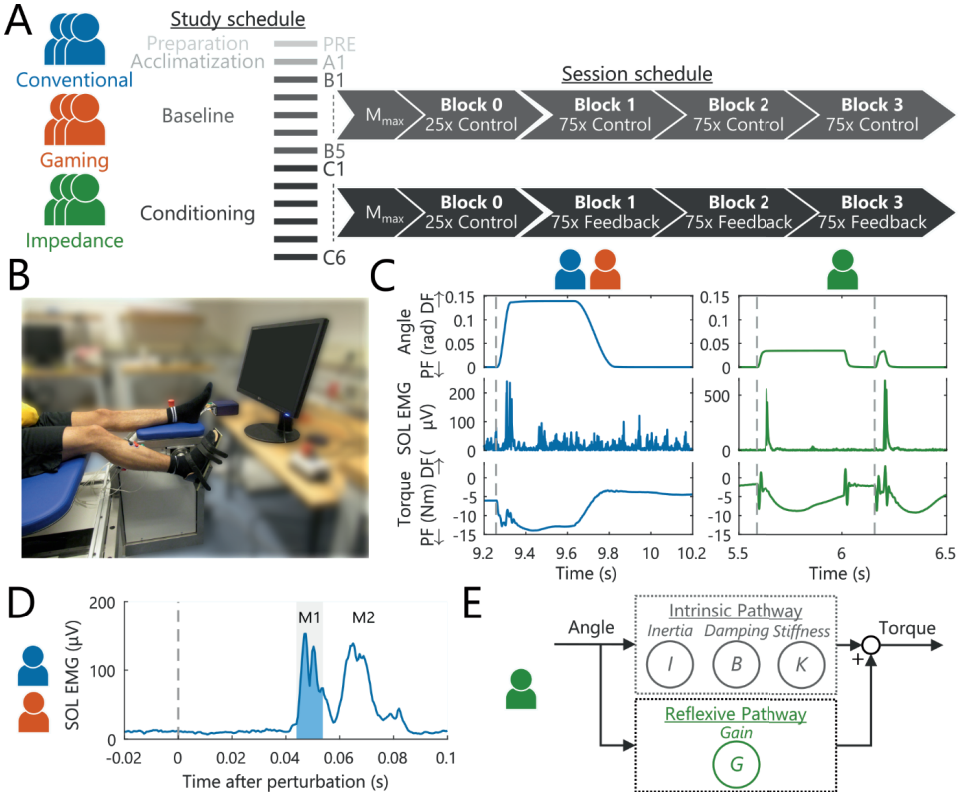

Figure 8.1. Overview experimental methodology. (A) Nine participants were split in three groups, all following the same 13 session study schedule (3 times per week). Per session, Mmax was obtained using electrical stimulation, followed by 4 blocks with stretch reflexes containing either 25 or 75 feedback instances.[7] (B) Stretch reflexes were elicited around the right ankle joint using a robotic manipulator. Participants were seated on an adjustable chair to support a static posture. (C) Dorsiflexion perturbations around the ankle joint elicited a stretch reflex response as visualized in the SOL muscle and torque. For the EMG-based groups, a discrete ramp-and-hold stretch profile was used,[7] whereas a continuous pulse-step perturbation profile was applied for the Impedance group.[14] (D) EMG-based groups received biofeedback on the SOL EMG, specifically background EMG activity and the short-latency (M1) reflex response (shaded area).[7] (E) The Impedance group received biofeedback on background torque and the estimated reflexive joint impedance gain (G). A mechanical-based methodology using recorded torques and kinematics was used to disentangle this reflexive contribution from the intrinsic contribution with parameters: inertia I, damping B and stiffness K.[14]

Experiment Setup

Ankle Manipulator and Stretch Reflex Perturbations

Stretch reflexes were elicited around the ankle joint using a one degree-of-freedom (DOF) manipulator (Moog, Nieuw-Vennep, the Netherlands) in the sagittal plane, see Fig. 8.1B. The manipulator applied dorsiflexion, ramp-and-hold perturbations to the right foot via a rigid footplate interface and Velcro straps. The encoder of the actuator of the manipulator measured foot plate angular position and velocity representing ankle angle and angular velocity. A torque sensor, located between the actuator and footplate, measured the ankle torque. Angle, velocity, and torque were recorded at 2048 Hz, all defined positive in dorsiflexion direction. To compensate for gravitational effects on the ankle and

footplate, the net torque with no voluntary participant activity was measured at the start of each block and subtracted from the torque measurements. Matlab 2017b (Mathworks, Natick, MA, USA) was used for the data collection and biofeedback during the experiment.

Participants were seated on an adjustable chair to support and control the posture during all stretch reflexes, see Fig. 8.1B. The chair supported the upper body and upper leg to control the hip and knee angles at 120° and 150°, respectively. Both knee and hip were defined at 180° for a perfectly straight posture, and angles were measured using a goniometer. All stretch perturbations started at a 90° ankle angle, defined as the angle between shank and foot. The ankle axis of rotation was visually aligned with the actuator axis, minimizing hip and knee translations due to the applied perturbations. Participants were instructed to attain background activation by pressing into the position- controlled footplate as if rotating the ankle without use of the upper leg. Session-to-session variability of the seated posture was minimized by reusing the same personalized chair settings for each participant.

For the EMG-based groups, discrete dorsiflexion perturbations were used to elicit a stretch reflex.[7] These ramp-and-hold perturbations had an 8° amplitude, 190°/s max velocity, 8000°/s^2 max acceleration and 66 ms duration. see Fig. 8.1C. Max. amplitude was held for 300 ms before the manipulator slowly returned to the 90 starting angle. For the Impedance group, continuous dorsiflexion perturbations were used to elicit a stretch reflex.[17] These ramp-and-hold perturbations had an 2° amplitude, 125°/s max velocity, velocity, 15800°/s2 max. acceleration and 40 ms duration, see Fig. 8.1C. The perturbations randomly switched between “pulses,” i.e., no hold period at max. amplitude, and “steps,” i.e., a 380 ms hold period at max. amplitude. Return toward the starting angle was with an equal and opposite profile to the dorsiflexion perturbation. The perturbation profile changes compared with the EMG-based groups were made to comply with impedance estimation procedure requirements.[14]

Reflexive joint impedance was estimated using a parallel cascade identification algorithm outlined in van ’t Veld et al.[17], see Fig. 8.1E. In short, using the recorded torques and kinematics the algorithm first estimates the intrinsic impedance parameters: inertia I, damping B, and stiffness K. These parameters capture the joint resistance in response to the mechanical perturbations from the tissue-related, non-neural origin, and tonic neural origin. The predicted intrinsic torque resulting from these parameters is subtracted from the total torque measured to estimate the reflexive torque. The gain G of the reflexive pathway is then estimated by relating this reflexive torque to the 40 ms-delayed, half-wave rectified velocity. The gain G reflects the joint resistance magnitude in response to the mechanical perturbations from a phasic neural origin. The parameters estimated within the initial 30 s of each block were discarded as the algorithm parameter estimation is unreliable within this transient period.[14]

Electromyography Measurements and Processing

Muscle activity was measured using the Porti EMG device (TMSi, Oldenzaal, the Netherlands). Bipolar electrodes (Kendall H124SG, 24 mm diameter; Covidien, Dublin, Ireland) were placed on the Soleus (SOL) and Tibialis Anterior (TA) according to the SENIAM guidelines.[18] Session-to-session variability in electrode placement was minimized by marking each electrode on the skin (four dots on each side, re-marked every session). Moreover, a drawing of the electrode placement with respect to anatomical and skin landmarks (e.g., bones, moles, scars, and vessels) was used in case the electrode markings had faded.[6,7]

Electromyography was recorded at 2048Hz, high-pass filtered (2nd-order, 5Hz, Butterworth), and rectified. SOL and TA background activity was defined as the smoothed (moving average, 100 ms window) rectified EMG.[6,7] During trials with continuous perturbations, background torque was used instead of SOL EMG, and this background activity was computed using low-pass filters (2nd-order, 0.1Hz, Butterworth {TA}; critically damped {torque}) to reduce the influence of these perturbations.[17]

Electromyography reflex magnitude was obtain using the SOL short-latency (M1) reflex response. To obtain M1 magnitude, background activity at perturbation onset was subtracted from the reflex response and the result was half-wave rectified. M1 magnitude was then defined as the root mean square (RMS) of the activity within a 10 ms window, see Fig. 8.1D.[7] This participant-specific window was manually set centered around the first peak response, typically 44–54 ms after perturbation onset, and after the last baseline sessions (B5).

Electrical Stimulation of M_{max}

To confirm correct placement of EMG electrode across-sessions, the direct motor response (M-wave) of the SOL muscle was elicited using a constant current electrical stimulator (DS7A; Digitimer, Hertfordshire, UK). The cathode (Disk electrode, 20 mm diameter; Technomed, Beek, the Netherlands) was placed in the popliteal fossa, whereas the anode (Square electrode, 41 mm height/width; MedimaxMaxpatch, UK) was placed proximal to the patella. Participants were standing with a natural, upright posture for the M-wave measurements.

The simulator delivered a 1 ms width square stimulus pulse to the tibial nerve of the right leg. The M-wave magnitude was defined after each electrical stimulus as the peak-to-peak value of the unrectified SOL EMG within a 22 ms processing window.[6,7] This participant-specific window was manually placed during the preparation session, typically 4–26 ms after stimulation. To check electrode placement, the maximum M- wave M_{max} is of interest, as a steady M_{max} indicates correct electrode placement.[6,7] To obtain M_{max}, stimulation intensity was gradually increased with 5 mA increments to find the intensity at which the M-wave magnitude plateaued. For data collection, three stimulation intensities above the plateau value were selected to obtain M_{max} and confirm that the intensities were within the range at which M-wave magnitude plateaued. These participant-specific intensities were set during the preparation session, e.g., at 20, 25, 30 mA or 60, 65, 70 mA.

Intrinsic Motivation Inventory

To assess motivation and engagement, all participants completed the intrinsic motivation inventory (IMI) questionnaire after the last conditioning session (C6).[19] The questionnaire was used to assess the participant experience with the stretch reflex perturbations only, i.e., participants were instructed to ignore the electrical stimulation element for this questionnaire.

Experimental Protocol

Preparation Session

All participants attended a preparation session to define all personalized hardware and software settings, retained through all other sessions.[6,7] A couple of trial electrical stimuli were applied to check whether participants felt comfortable with electrical stimulation. Two participants opted out of the

study due to discomfort (lightheaded and nauseous) after these trial stimuli. New volunteers were included in the study to retain the total number of participants at nine.

To normalize EMG background activity, SOL maximum voluntary contraction (MVC) was determined.[6,7] Participants were seated (hip, knee, and ankle angle all 90°) on a stool with their upper leg locked beneath a rigid structure. Participants were instructed to produce maximum SOL activity by pressing against the rigid structure, while retaining their toes on the ground, to generate a plantarflexion torque. The SOL MVC was defined as the maximum value of the smoothed (moving average, 100 ms window) rectified SOL EMG. Each participant performed three MVC trials, and the participant-specific MVC value was set as the maximum MVC across all three trials.

To match the SOL and torque background activity target levels used throughout data collection, a tonic EMG-torque mapping was obtained. Participants executed a torque tracking task using the ankle manipulator by holding isometric torque for 3 s at 0–10 Nm in increments of 2 Nm. To obtain the EMG-torque mapping, mean SOL activity at each torque level was computed. The SOL background target was defined as a 5% MVC range matching the 4 Nm level of the EMG-torque mapping, and typical ranges were 2.5-7.5% MVC and 5–10% MVC.[6,7] The torque background target was defined as a 1 Nm range set at 3.5–4.5 Nm. The TA background activity target was set at resting level, i.e., 0–7.5 μV.[6,7] Participants completed several trials with the stretch reflex perturbations and electrical stimulation, while instructed to maintain background activity within the set targets. These trials were used to check whether participants could comfortably execute these task, given all personalized settings.

Acclimatization, Baseline, and Conditioning Sessions

The acclimatization, baseline, and conditioning sessions all followed the same schedule for each participant, see Fig. 8.1A.[6,7] For all groups, 12 electrical stimuli, i.e., four repetitions at three intensities, were applied with increasing stimulation intensity to determine M_{max}. Participants were instructed to maintain steady SOL and TA background activity using bar-based biofeedback, see Fig. 8.2.[6,7] Stimuli were applied at 5–7 s intervals and only if participants complied with the background targets for the last 2 s.

In Block 0, the Control magnitude was measured, i.e., reflex magnitude before within-session conditioning.[6,7] Participants only received background biofeedback: SOL/TA biofeedback for EMG-based groups[6,7] and torque/TA biofeedback for the Impedance group.[15] For EMG-based groups, 25 discrete stretch perturbations were elicited at a 5–7 s interval and only if participants complied with the background activity targets for the last 2 s. For the Impedance group, these 25 discrete instances coupled to steady background activity were retained to create similar block duration across groups. Consequently, these instances were decoupled from the continuously applied pulse- step perturbation, resulting in roughly 250 stretch perturbations at a 0.5–0.7 s interval.

In Block 1–3, the Conditioned magnitude was measured, i.e., stretch reflex magnitude during within-session conditioning.[6,7] For baseline sessions, the protocol remained equal to Block 0 with only background biofeedback provided. For conditioning sessions, reflex biofeedback was added to the background biofeedback with the instruction to reduce reflex magnitude. Despite the use of continuous biofeedback by Ludvig et al.[15], the Impedance group received discrete reflex biofeedback to avoid any difficulty interpreting a biofeedback parameter with large variability.[20] In each block, 75

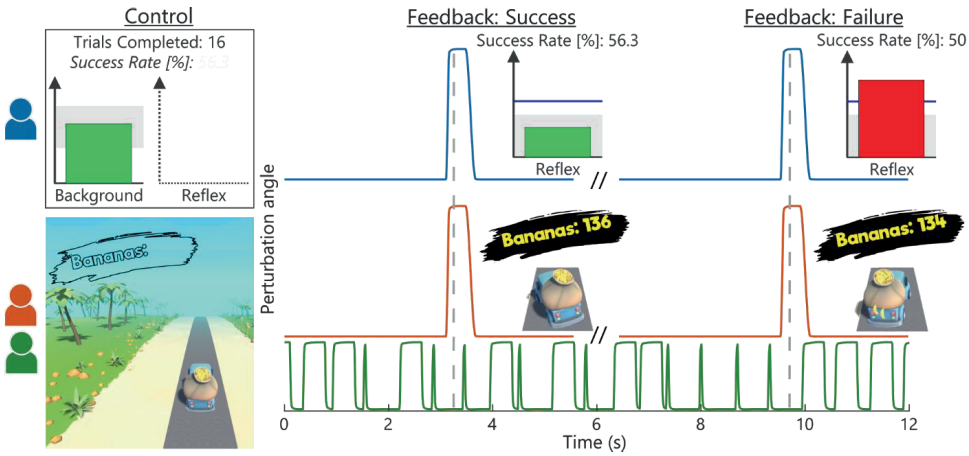

Figure 8.2. Biofeedback visualization and timing. For the (blue) Conventional group, a background (all trials) and a reflex (conditioning trials only) bar-graph directly represented current magnitudes. Moreover, a (gray) target area was displayed with the bar color visualizing whether this target was met (green) or not (red).[7] The reflex graph also showed a blue reference line based on average baseline (B1-5) reflex magnitude. The reflex biofeedback (gray-dashed vertical) was coupled to a stretch perturbation, displayed after a short data processing delay. Additionally, the completed number of trials and success rate were displayed. The game-based Gaming (red) and Impedance (green) groups had truck left-right position represent current background magnitude with the (gray) road as target area. Reflex activity controlled the number of bananas in the trunk after each feedback instance, visualized as wobble of the truck. After the wobble, all bananas were retained when the (non-visual) reflex target was met and two bananas would fall out on failure. As a result, the continuous perturbations of the Impedance group were decoupled from the feedback instances.

discrete perturbations for EMG-based groups and roughly 750 continuous perturbations for the Impedance group were applied.[6,7]

Biofeedback

Visualization and Timing

The Conventional group received bar-based biofeedback on background activity (all trials), and on reflex magnitude, average baseline (B1-5) reflex magnitude, number of trials completed, and success rate (conditioning trials only), see Fig. 8.2. Biofeedback was provided via bar size and color, based on whether the set target was met or not. The background bar color also changed whenever TA background activity was off- target, although current TA activity was not directly visualized. Background biofeedback was continuously updated at 10 Hz, whereas the reflex biofeedback update was directly coupled to a stretch perturbation.

For game-based groups, the bar-based visualization was substituted with a third-person game about a banana delivery truck, which provided biofeedback on background activity (all trials) and reflex reduction success (conditioning trials only), see Fig. 8.2. Reflex reduction success was represented by the number of bananas in the trunk: Starting at 150 bananas every block, two bananas would fall out after each failure to meet the reflex target at a feedback instance. An increased 30 Hz background update frequency was used for the game-based biofeedback to create a smooth gaming experience.

To obtain a pleasant gaming experience, the amount of biofeedback was reduced during gamification. As a result, participants did not receive information on the following: (1) background target success/failure; (2) quantified reflex magnitude; and (3) average baseline (B1-5) reflex magnitude, number of trials completed, and success rate. The experiment leaders could access this missing information during each block and communicate it to participants, e.g., success rates were regularly announced to the participants.

Reward Criterion

The reflexive target range was adaptive throughout all conditioning sessions to keep the reflex reduction target equally challenging. The upper bound of the target range was set as the 66th percentile of the previous block reflex magnitude, i.e., Block 1 based on Block 0, etc.[6,7] Participants earned a modest monetary reward if a block was completed with a success rate larger than 50%. Given the 66th percentile upper bound, a larger than 50% success rate was expected when reflex magnitude did not change between blocks.[6,7] Participants were verbally motivated to always maximize success rate, also beyond the 50% monetary threshold. Participants were not given any specific instructions or indications on reflex reduction strategies and were motivated to find their own strategy for success. Besides, participants were motivated to not purposely search for the edges of the background target ranges in order to modulate the reflex response. For additional motivation and engagement, the game-based groups also earned in-game currency per banana delivered, which could buy in-game visual upgrades for the truck and environment.

Data Analysis

Per session, the M-wave magnitudes were averaged across repetitions at each stimulation intensity with M_{max} defined as the maximum value across all intensities. Per stretch perturbation, background activity was computed over the 100 ms period before dorsiflexion perturbation onset for EMG-based groups[6,7] and a shorter 40 ms period for the Impedance group to avoid movement artifacts.[14] SOL and TA backgrounds were computed as mean rectified EMG and torque background as mean unfiltered torque.

The SOL M1 magnitudes, as defined in experiment setup, of both control (Block 0) and conditioned (Block 1-3) reflexes were normalized as % baseline, using baseline (B1-5) mean of the control and conditioned reflexes, respectively.[6,7] Per session, a within-session conditioning effect was defined as the mean normalized conditioned reflex minus mean normalized control reflex.

Besides, to support the use of reflexive gain G as biofeedback variable, the correlation between the EMG-based and impedance-based reflex magnitude was investigated. First, a set of across-block paired data points was created using the mean SOL M1 and gain G for each block per participant. Second, a set of within-block paired data points was created using the mean SOL M1 and gain G for each feedback instance per block per participant. Thus, for Block 0 (25×) and Block 1–3 (75×) all data leading up to a feedback instance were averaged for both reflexive magnitudes.
For all groups, the IMI questionnaire, taken in Session C6, consisted of four questions across four dimensions: interest-enjoyment, perceived competence, effort-importance, and tension-pressure. For each participant, all answers within a single dimension were averaged to obtain an overall score for this dimension.

Statistical Analysis

The feasibility of each biofeedback method was investigated by evaluating the within-session conditioning effect, with a –15% reference in Session C4-6 defined as success.[6,7] For each participant, a linear model (LM) was built using normalized SOL M1 (% baseline) as outcome measure (N = 2750 for Conventional & Gaming; N ≈ 27500 for Impedance). Both session (B1– C6), block (Blocks 0–3), and their interaction were used as predictor to investigate the within-session conditioning effect. Due to EMG measurement artifacts (high amplitude noise across broad frequency range), Session B1 for participant 7 and Session B5 for participant 8 were discarded. A planned contrast was used to evaluate the conditioning effect, contrasting the within- session outcome of Session C4-6 to B1-5 computed as the average of Blocks 1–3 ("Conditioned" reflex) minus Block 0 ("Control" reflex). To avoid confounding effects of the background activity, the SOL, TA, and torque background outcomes were all added to the LM as predictors to function as covariates. Per participant, the contrast was tested twice, once with and once without these covariates. Ideally, M_{max} would also be included in the LM as covariate. However, as only a single M_{max} outcome is available per session, adding M_{max} as covariate is impossible as this predictor would be collinear with the session predictor.

To support the need for an acclimatization session before starting the actual baseline, the SOL M1 was investigated further. An LM was built with data from Sessions A1 and B1-5 using only the mean control reflex (Block 0), using session as predictor. A planned reverse-Helmert like contrast was used to evaluate the difference in reflex magnitude between A1 vs. B1-5 and B1 vs. B2-5 for all participants combined.

The use of reflexive gain G as biofeedback variable was investigated using the correlation with SOL M1 magnitudes of the Impedance group (Sessions B1-C6 and Blocks 1–3). First, within- block correlation was investigated via a within-block Z-score standardization of all 75 data pairs for all 99 blocks (33 blocks per participants). The Z-score standardization allows to combine all data across-blocks and across-subjects before computing the correlation.[17] Second, the across-block correlation was investigated by using the mean of 75 data pairs per block and using a within-subject Z-score standardization to combine data across-subjects.

8.3 Results

We explored the feasibility of three different biofeedback methods to achieve a within-session reduction of SOL M1 magnitude with a Conventional, Gaming, and Impedance group. All participants completed 12 data collection sessions: 6 acclimatization/baseline sessions (A1, B1-5) and 6 conditioning sessions (C1-6). All sessions first contained a short control block (Block 0) with 25 feedback instances followed by three blocks of 75 feedback instances without (A1–B5) or with reflex biofeedback (C1–C6). Key prerequisite on SOL M1 reduction was lack of modulation in several parameters throughout data collection to avoid confounding effects: SOL M_{max}, and SOL, TA, and torque background activity.

Steadiness of M_{max} and Background Activity

Based on session averages, all M_{max} and background activity parameters were visually considered steady throughout data collection, see Fig. 8.3. Subsequently, steadiness of M_{max} was interpreted as

consistent electrode placement throughout data collection. Similarly, steady background activity was used to avoid influences on reflex magnitude via voluntary increase or decrease of tonic activation. TA background also remained below resting levels indicating that co-contraction was not present. The session averages do clearly show that the EMG- based groups (Conventional and Gaming) were provided with SOL background biofeedback to keep activity steady, whereas the Impedance group used background torque biofeedback. Although no clear trends are visible, both groups show larger across-session variability for the variables on which no biofeedback was received. Thus, it was still important to evaluate the within-session effects with an LM including background variables as covariates.

Soleus Stretch Reflex Reduction

Both EMG-based groups (Conventional and Gaming) had several successful within-session conditioning results, reaching the reference –15% target, see bottom row Fig. 8.4.[6,7] Thus, within these sessions the difference between the normalized Conditioned and Control reflex measures was at least 15%, see top rows Fig. 8.4. Contrarily, no successful within-session conditioning effect was observed for the Impedance group.

Across the full experiment, feasibility of the conditioning paradigm was confirmed in 2 (Conventional group) and 3 (Gaming group) out of 3 participants, see Table 8.1. In the Conventional group, the background-corrected results showed a –24% ($p < 0.001$) and –17% ($p < 0.001$) within-session effect for participants 1 and 3, whereas participant 2 showed a weaker SOL M1 reduction at –8.7% ($p = 0.22$). The Gaming group showed a –33% ($p < 0.001$), –22% ($p < 0.001$), and –16% ($p=0.007$) effect for the participants. Thus, gamification of the conditioning paradigm seemed feasible without interfering with conditioning outcomes.

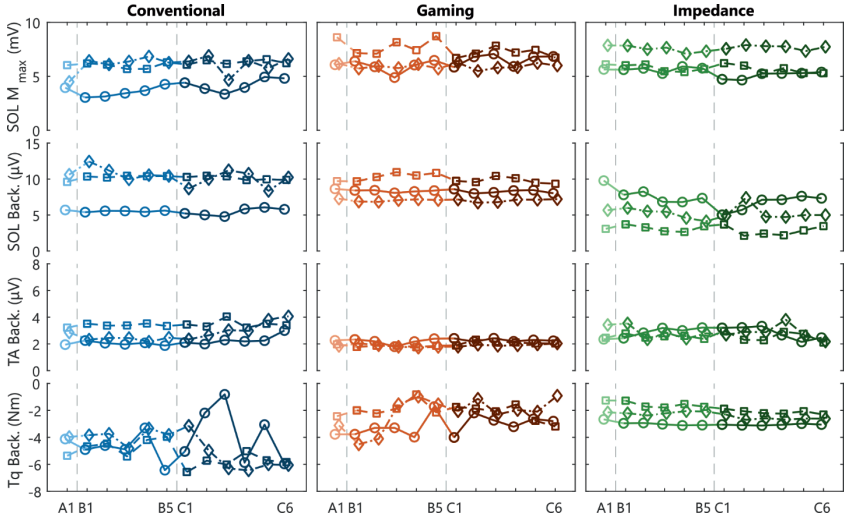

Figure 8.3. Steadiness Mmax and background activity. Individual participant traces of SOL Mmax, and SOL, TA, and torque background activity for acclimatization (A1), baseline (B1-5), and conditioning (C1-6) sessions. All variables were required to remain steady throughout data collection. Each data point reflects the average of all blocks (Block 0–3) within a single session. Conventional and Gaming groups received biofeedback on SOL activity, whereas the Impedance group received biofeedback on torque activity. For all groups, TA activity was required to remain at a resting level (<7.5 μV). Each icon (circle, square, and diamond) per group is linked to an individual participant and consistently used across figures.

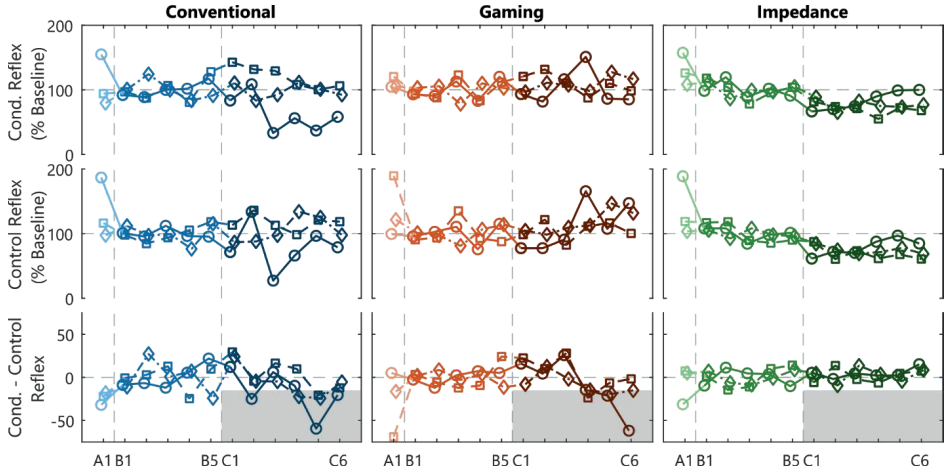

Figure 8.4. SOL M1 reflex results and within-session effect. Individual participant traces of the average conditioned reflex (mean Blocks 1-3) and control reflex (Block 0) per session for acclimatization (A1), baseline (B1-5), and conditioning (C1-6) sessions. The within-session effect is derived from the difference between the conditioned and control reflex within a session. Conventional and Gaming groups received biofeedback on SOL M1 activity, whereas the Impedance group received biofeedback on reflexive impedance gain G. A –15% within-session effect in session C4-6 was defined as success criteria to determine feasibility of the biofeedback method for each participant, see (gray) shaded target area. Each icon (circle, square, and diamond) per group is linked to an individual participant and consistently used across figures.

Table 8.1.

Contrast between B1-5 and C4-C6 for the within-session SOL M1 effect without and with covariates.

		LM:~Session×Block			LM:~Session×Block Covariates:~SOL_{back}+TA_{back}+$Torque_{back}$		
Group	**P**	**Contrasts**	**Statistics**		**Contrasts**	**Statistics**	
Conventional	#1	–30 ± 4.3	$t(2,706) = -6.93$	$p < 0.001$	–24 ± 4.5	$t(2,703) = -5.39$	$p < 0.001$
	#2	–7.7 ± 7.0	$t(2,706) = -1.10$	$p = 0.27$	–8.7 ± 7.1	$t(2,703) = -1.24$	$p = 0.22$
	#3	–17 ± 4.2	$t(2,706) = -4.08$	$p < 0.001$	–17 ± 4.3	$t(2,703) = -4.03$	$p < 0.001$
Gaming	#4	–33 ± 7.5	$t(2,706) = -4.36$	$p < 0.001$	–33 ± 7.5	$t(2,703) = -4.36$	$p < 0.001$
	#5	–11 ± 6.5	$t(2,706) = -1.64$	$p = 0.10$	–22 ± 6.6	$t(2,703) = -3.30$	$p < 0.001$
	#6	–16 ± 6.0	$t(2,706) = -2.72$	$p = 0.007$	–16 ± 6.0	$t(2,703) = -2.70$	$p = 0.007$
Impedance	#7	4.2 ± 2.5	$t(24,427) = 1.65$	$p = 0.10$	3.4 ± 2.5	$t(24,424) = 1.37$	$p = 0.172$
	#8	5.3 ± 1.2	$t(25,284) = 4.48$	$p < 0.001$	6.3 ± 1.2	$t(25,281) = 5.31$	$p < 0.001$
	#9	2.5 ± 1.9	$t(27,363) = 1.36$	$p = 0.17$	0.29 ± 1.8	$t(27,360) = 0.163$	$p = 0.87$

Within-session effect contrasts are expressed in % baseline, thus mean within-session effect for B1-5 equal zero within all participants. All contrasts were tested using a t-test for both the models without and with covariates.

Necessity Acclimatization Session

The addition of an acclimatization session before the baseline sessions was observed to potentially be beneficial for the steadiness of the reflex magnitude during baseline for all groups, see Fig. 8.4. The results of the first depicted session (A1) could be added to the baseline session (B1-5), as the protocol executed is exactly equal. However, the reflex variables generally showed an increased control and

conditioned reflexive magnitude and variability across-participants in combination with a negative within-session effect for A1 compared with B1-5. To confirm these observations, an LM of the control SOL M1 magnitude (Block 0, Session A1–B5) for all participants did indeed show a significant effect of adding the session predictor [$F(5, 48) = 5.27$, $p = 0.007$]. A contrast further showed that the reflex magnitude for Session A1 was significantly larger than sessions B1-5 35.8 ± 7.2 % baseline [$t(48) = 4.95$, $p < 0.001$]. This effect faded away when contrasting Session B1 vs. the other baseline sessions (B2-5) [$t(48) = 0.53$, $p = 0.60$]. Note, no clear discrepancies between Sessions A1 and B1-5 were observed for M_{max} and all background variables, see Fig. 8.3.

Correlation EMG and Impedance-Based Biofeedback

The observed commonality between the EMG-based and impedance-based reflex magnitudes depended on the time frame of the evaluation, see Fig. 8.5A moderate correlation (r = 0.68) was found for the across-block correlation, whereas a weak correlation (r = 0.31) was found for the within- block correlation for data of all Blocks 1–3 of the Impedance groups. The moderate across-block correlation was further corroborated given the similarity between block-averaged conditioned, control, and within-session reflex outcomes, see Fig. 8.4 and Fig. S8.1. Thus, the observed correlation was larger when data were averaged over a full block (ca. 750 stretches, 7.5 min) compared with averaged per feedback instance (ca. 10 stretches, 6 s).

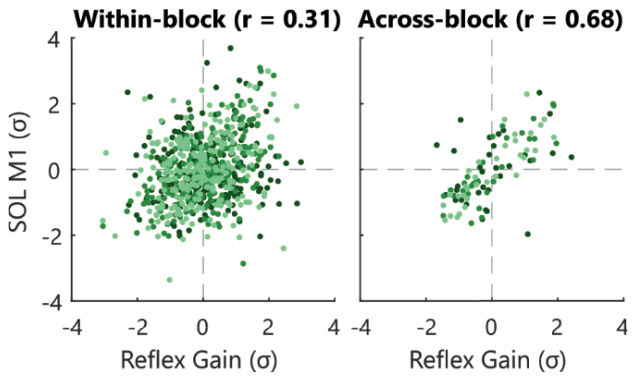

Figure 8.5. Within- and across-block correlation of reflexive biofeedback variables. Individual participants are visualized with a different color. Correlation analysis for the Impedance group for Session B1–C6 and Blocks 1–3. The within-block correlations were computed using the averaged measures per feedback instance. The across-block correlations were computed using the averaged measures per blocks. Data was Z-score standardized within-block and within-subject, respectively to allow combination of data over sessions and participants. To improve visualization only 10% of all within-block data points are shown.

Intrinsic Motivation Inventory

The IMI questionnaire showed a positive reception of the game- based conditioning paradigms, ignoring the electrical stimulation element, in terms of motivation and engagement, see Table 8.2. Participants in both game-based groups reported good scores for interest-enjoyment (8.5 and 8.0 out of 10) score and perceived competence (8.5 and 7.2). Note, these psychological results should be interpreted and compared with care, e.g., a large variation across the effort-importance scale was observed over the three groups, whereas no difference was expected.

8.4 Discussion

The goal of this study was to explore the feasibility of two forms of biofeedback to obtain a within-session reduction of the Soleus stretch reflex with conditioning. First, we explored the feasibility of gamification and second, the feasibility of combined game- and impedance-based biofeedback. For the EMG-based groups, using either bar-based or game-based biofeedback, feasibility of the conditioning paradigm was shown in 2 and 3 out of 3 participants, respectively. Contrarily, feasibility was not shown for any participant using impedance- and game-based biofeedback. Thus, whereas the combined game- and impedance- based biofeedback was not considered feasible, the gamification of EMG-based biofeedback used to improve motivation and long-term engagement was considered feasible.

Feasibility Game-Based Biofeedback

Exploring the use of EMG- and game-based biofeedback within the conditioning paradigm confirmed the feasibility of the proposed biofeedback gamification. First, the switch from bar-based to game-based biofeedback did not interfere with conditioning outcomes. Our results showed feasibility of the proposed method in all participants of the Gaming group after correcting for potentially confounding background effects.

Previous studies did not report on individual within-session effects and only reported a group-average –15% effect across the 16 (out of 17) successful participants, which achieved a long-term down-conditioning effect.[6,7] Comparing this result to the observed –24% Gaming group-average within-session effect should be done with caution due to the exploratory nature and small population size of our study. Moreover, the conditioned and control reflex were not interpreted separately, as previous studies showed no clear expected trends and large variability.[6,7] Second, feasibility of the gamification was also shown from a psychological perspective as the IMI scores of the Gaming group showed a positive evaluation for participant motivation and engagement. Given these results, improving motivation and long-term engagement of the conditioning paradigm to mitigate time-intensiveness and a slow learning curve is considered feasible.

Toward future use of gamification, the methodological differences between the game- (Gaming group) and bar- based (Conventional group) biofeedback were solely made to the biofeedback visualization. The main challenge toward a suitable gaming experience was the high information density of the bar-based biofeedback.[6,7] After gamification, participants most importantly did not receive information on the following: (1) background target success/failure and (2) quantified reflex magnitude. Whereas, the background biofeedback implementation has varied across previous studies on human stretch reflex reduction, all studies provided quantified reflex biofeedback.[7,21,22] A previous study on primate stretch reflex reduction did obtain successful conditioning results without quantified reflex magnitude using food to convey success or failure.[23] Our results show that such a binary (success/failure) biofeedback can also be considered feasible for human stretch reflex reduction paradigms.

Feasibility Combined Game- and Impedance-Based Biofeedback

Conditioning based on combined game- and impedance-based biofeedback did not yield a feasible paradigm. No participants showed a within-session reduction in reflex magnitude after impedance-based conditioning, despite positive findings in previous studies using impedance-based biofeedback outside of the conditioning paradigm.[15] Any influences of potential confounders were not observed, as

no trends in M_{max} or background activity were recorded and the psychometric scores for the Impedance group showed a positive evaluation. As such, accelerating the learning curve and improving practical execution of the conditioning paradigm remain an open challenge.

To find plausible explanations for the lack of within-session reflex reduction in the Impedance group, all methodological differences between Impedance and EMG-based groups were considered: (1) stretch reflex perturbations; (2) biofeedback gamification; (3) biofeedback processing; and (4) biofeedback visualization. First, compared with the EMG-based groups the stretch reflex required for the impedance-based biofeedback had a decreased amplitude, duration and velocity, whereas the acceleration and number of perturbations was increased. As expected from literature, the adapted perturbation parameters affected the reflex response as only M1 was observed, instead of both M1 and M2.[24] Yet, all previous stretch reflex studies focused on M1 conditioning,[7,21,22] M2 does not co-condition with the M1 reflex,[7] and H-reflex conditioning also just elicits a single reflexive response, most equivalent to M1.[4,6] Therefore, the lack of M2 is not considered a plausible explanation for the lack of reflex reduction. Contrarily, the increased acceleration of the perturbation might saturate the M1 response due to the M1 acceleration dependence,[24] which could plausibly explain the difficulty of reducing the reflex response. Besides, despite an increased number of perturbations, each stretch perturbation did elicit a stretch response as seen in similar impedance-based studies.[14,25] Consequently, while receiving an equal amount of feedback, participants in the Impedance group experienced an increased number of elicited reflexes, which might have influenced conditioning outcomes, although previous studies do not provide an indication whether increased perturbation occurrence would either improve or interfere with treatment outcome. Second, the gamified biofeedback visualization is not considered as likely explanation, as the exact same game was used for both Gaming and Impedance groups.

Third, an important difference between the biofeedback processing of the EMG- and impedance-based biofeedback was revealed through correlation analysis. A weak within- block correlation ($r = 0.31$) of the EMG- and impedance- based reflexive biofeedback was found based on 6 s data segments. Oppositely, for longer segments a moderate across- block correlation was found ($r = 0.68$; 7.5 min segments) and reported previously ($r = 0.69$; 60 s segments).[17] This difference between the correlation of short and long segments is likely related to the inherent 15 s risetime of the impedance estimation algorithm.[14] Practically, this 15 s risetime causes a slow and delayed impedance estimation compared with the direct instance-based M1 EMG processing. Consequently, the direct coupling between a feedback instance and stretch perturbation as in the EMG-based biofeedback lacks for the impedance-based biofeedback. Fourth, the biofeedback visualization used was a mix of a continuous impedance-based[15] and discrete EMG-based paradigm.[7] Ludvig et al.[15] provided continuous line-based biofeedback on magnitude, which was converted to a discrete, binary biofeedback on reflex reduction success over the last 5–7 s interval. This conversion ensured a match with the EMG-based conditioning paradigm. However, the converted impedance-based visualization did not result in a feasible paradigm, while this visualization was inspired by two previously successful studies.[7,15] This result may show the importance of quantitative or continuous impedance-based biofeedback, given the slow and delayed impedance-based biofeedback characteristics. For example, due to the variability of the reflex response, the delayed biofeedback might show reflex reduction success, while the last couple reflexes were actually too large and vice versa. Moreover, the lack of quantitative or continuous biofeedback will hide this processing effect from the participant. Overall, the lack of reflex reduction observed can potentially be

explained by the delayed and decoupled biofeedback processing and its combination with the lack of a quantitative or continuous visualization.

Study Limitations and Future Outlook

This study can solely be interpreted as exploration of the feasibility of several biofeedback methods, given the limited number of participants. Furthermore, the protocol was limited to studying short-term (within-session) effects as long-term effects have been shown to arise after 12–16 sessions.[6,7] Within these restrictions, we recommend game-based biofeedback be implemented and tested in longer study schedules, with more participants and in a neurological population. Experimental execution should include a sufficient number of preliminary trials (at least a preparation and an acclimatization session) to ensure steadiness of baseline measurements. The goal of further exploring feasibility of the gamified conditioning paradigm is to increase participant motivation and long-term engagement during this time-intensive paradigm with a slow learning curve. Furthermore, feasibility should be explored in a neurological population before clinical implementation.

Before applying the conditioning paradigm clinically, improving the time-intensiveness and slow learning curves remains an open challenge. The implementation of impedance- based biofeedback, previously used to voluntarily modulate the reflex response, within the conditioning paradigm did not result in a feasible protocol. The impedance-based biofeedback was explored combined with the game-based biofeedback, whereas an impedance- and bar-based biofeedback group was not included. Therefore, exploring impedance- and bar-based biofeedback would be useful to provide a more direct comparison between impedance- and EMG-based biofeedback. Besides, potential improvements of the impedance-based biofeedback may lie within an improved algorithm without a 15 s risetime to avoid delayed biofeedback and directly couple the biofeedback with the current actions of the participants. Moreover, an improved impedance-based algorithm may solve the reduced correlation with EMG-based reflex magnitude for short data segments. Besides impedance-based biofeedback, other paradigm changes like conditioning during locomotion have also shown promising improvements of the slow learning curves.[26]

8.5 Conclusions

We have shown the feasibility of EMG- and game-based biofeedback within the operant conditioning paradigm to obtain a within-session reduction in the SOL stretch reflex. Contrarily, we did not observe feasibility for the impedance- and game-based biofeedback. Stretch reflex conditioning should be applied clinically to potentially obtain a non- invasive spasticity treatment with long-term sustained effect. Before clinical application, the time-intensiveness and slow learning curve of the conditioning paradigm remain an open challenge. These results call for further research on gamification of conditioning paradigms to obtain improved participant motivation and engagement, while achieving long-term conditioning effects.

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References

1. Dietz V, Sinkjær T. Spastic movement disorder: impaired reflex function and altered muscle mechanics. Lancet Neurol. (2007) 6:725–33. doi: 10.1016/S1474-4422(07)70193-X
2. van den Noort JC, Bar-On L, Aertbeliën E, Bonikowski M, Braendvik SM, Broström EW, et al. European consensus on the concepts and measurement of the pathophysiological neuromuscular responses to passive muscle stretch. Eur J Neurol. (2017) 24:981–91. doi: 10.1111/ene. 13322
3. Chang E, Ghosh N, Yanni D, Lee S, Alexandru D, Mozaffar T. A review of spasticity treatments: pharmacological and interventional approaches. Crit Rev Phys Rehabil Med. (2013) 25:11–22. doi: 10.1615/CritRevPhysRehabilMed.2013007945
4. Thompson AK, Pomerantz FR, Wolpaw JR. Operant conditioning of a spinal reflex can improve locomotion after spinal cord injury in humans. J Neurosci. (2013) 33:2365–75. doi: 10.1523/JNEUROSCI.3968- 12.2013
5. Thompson AK, Wolpaw JR. Operant conditioning of spinal reflexes: from basic science to clinical therapy. Front Integr Neurosci. (2014) 8:25. doi: 10.3389/fnint.2014.00025
6. Thompson AK, Chen XY, Wolpaw JR. Acquisition of a simple motor skill: task-dependent adaptation plus long-term change in the human soleus H-reflex. J Neurosci. (2009) 29:5784–92. doi: 10.1523/JNEUROSCI.4326- 08.2009
7. Mrachacz-Kersting N, Kersting UG, de Brito Silva P, Makihara Y, Arendt- Nielsen L, Sinkjær T, et al. Acquisition of a simple motor skill: task-dependent adaptation and long-term changes in the human soleus stretch reflex. J Neurophysiol. (2019) 122:435–46. doi: 10.1152/jn.00211.2019
8. Deterding S, Sicart M, Nacke L, O'Hara K, Dixon D. Gamification: using game design elements in non-gaming contexts. In: CHI '11 Extended Abstracts on Human Factors in Computing Systems Vancouver, BC: Association for Computing Machinery (2011). p. 2425–8.
9. Turan Z, Avinc Z, Kara K, Goktas Y. Gamification and education: achievements, cognitive loads, and views of students. Int J Emerg Technol Learn. (2016) 11:64–9. doi: 10.3991/ijet.v11i07.5455
10. Proença JP, Quaresma C, Vieira P. Serious games for upper limb rehabilitation: a systematic review. Disabil Rehabil Assist Technol. (2018) 13:95–100. doi: 10.1080/17483107.2017.1290702
11. Bonnechère B, Jansen B, Omelina L, van Sint Jan S. The use of commercial video games in rehabilitation: a systematic review. Int J Rehabil Res. (2016) 39:277–90. doi: 10.1097/MRR.0000000000000190
12. Lopes S, Magalh aes P, Pereira A, Martins J, Magalh aes C, Chaleta E, et al. Games used with serious purposes: a systematic review of interventions in patients with cerebral palsy. Front Psychol. (2018) 9:1712. doi: 10.3389/fpsyg.2018.01712
13. Howcroft J, Fehlings D, Wright V, Zabjek K, Andrysek J, Biddiss, E. A comparison of solo and multiplayer active videogame play in children with unilateral cerebral palsy. Games Health J. (2012) 1:287–93. doi: 10.1089/g4h.2012.0015
14. Ludvig D, Kearney RE. Real-time estimation of intrinsic and reflex stiffness. IEEE Trans Biomed Eng. (2007) 54:1875–84. doi: 10.1109/TBME.2007.894737
15. Ludvig D, Cathers I, Kearney RE. Voluntary modulation of human stretch reflexes. Exp Brain Res. (2007) 183:201–13. doi: 10.1007/s00221-007-1030-0
16. Kearney RE, Stein RB, Parameswaran L. Identification of intrinsic and reflex contributions to human ankle stiffness dynamics. IEEE Trans Biomed Eng. (1997) 44:493–504. doi: 10.1109/10.581944
17. van 't Veld RC, Schouten AC, van der Kooij H, van Asseldonk EHF. Neurophysiological validation of simultaneous intrinsic and reflexive joint impedance estimates. J NeuroEngineering Rehabil. (2021) 18:36. doi: 10.1186/s12984-021-00809-3
18. Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for semg sensors and sensor placement procedures. J Electromyogr Kinesiol. (2000) 10:361–74. doi: 10.1016/S1050-6411(00)00027-4
19. McAuley ED, Duncan T, Tammen VV. Psychometric properties of the intrinsic motivation inventoiy in a competitive sport setting: a confirmatory factor analysis. Res Q Exerc Sport. (1989) 60:48–58. doi: 10.1080/02701367.1989.10607413
20. van 't Veld RC, Schouten AC, van der Kooij H, van Asseldonk EHF. Validation of online intrinsic and reflexive joint impedance estimates using correlation with EMG measurements. In: 7th International Conference on Biomedical Robotics and Biomechatronics. Enschede: IEEE (2018). p. 13-18. doi: 10.1109/BIOROB.2018.8488123

21. Evatt ML, Wolf SL, Segal RL. Modification of human spinal stretch reflexes: preliminary studies. Neurosci Lett. (1989) 105:350–55. doi: 10.1016/0304-3940(89)90646-0
22. Wolf SL, Segal RL. Reducing human biceps brachii spinal stretch reflex magnitude. J Neurophysiol. (1996) 75:1637–46. doi: 10.1152/jn.1996.75.4.1637
23. Wolpaw JR, O'Keefe JA. Adaptive plasticity in the primate spinal stretch reflex: evidence for a two-phase process. J Neurosci. (1984) 11:2718–24. doi: 10.1523/JNEUROSCI.04-11-02718.1984
24. Finley JM, Dhaher YY, Perreault EJ. Acceleration dependence and task- specific modulation of short- and medium-latency reflexes in the ankle extensors. Physiol Rep. (2013) 1:e00051. doi: 10.1002/phy2.51
25. Stein RB, Kearney RE. Nonlinear behavior of muscle reflexes at the human ankle joint. J Neurophysiol. (1995) 73:65–72. doi: 10.1152/jn.1995.73.1.65
26. Thompson AK, Wolpaw JR. H-Reflex conditioning during locomotion in people with spinal cord injury. J Physiol. (2019) 599: 2453–69. doi: 10.1113/JP278173

Supplementary Materials 8.1

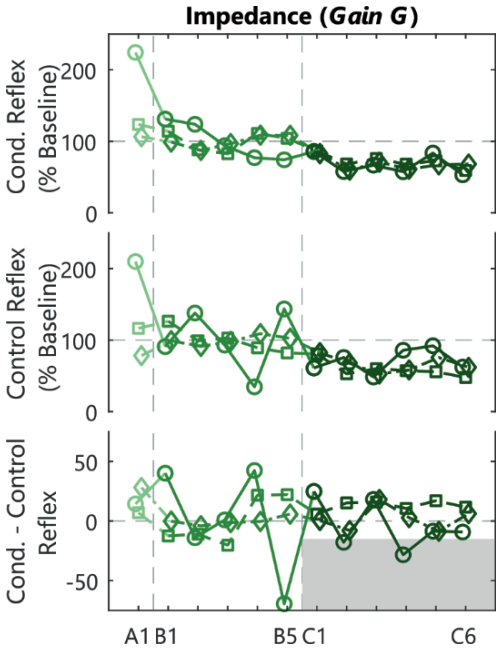
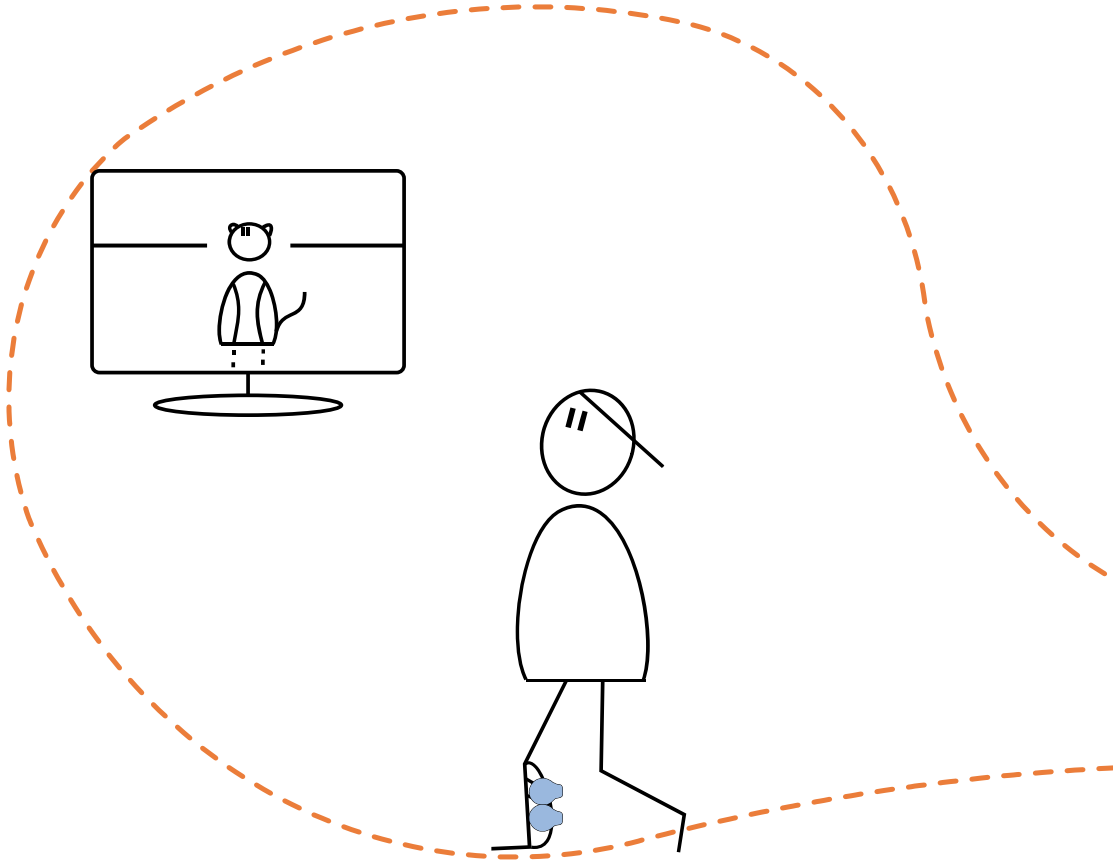
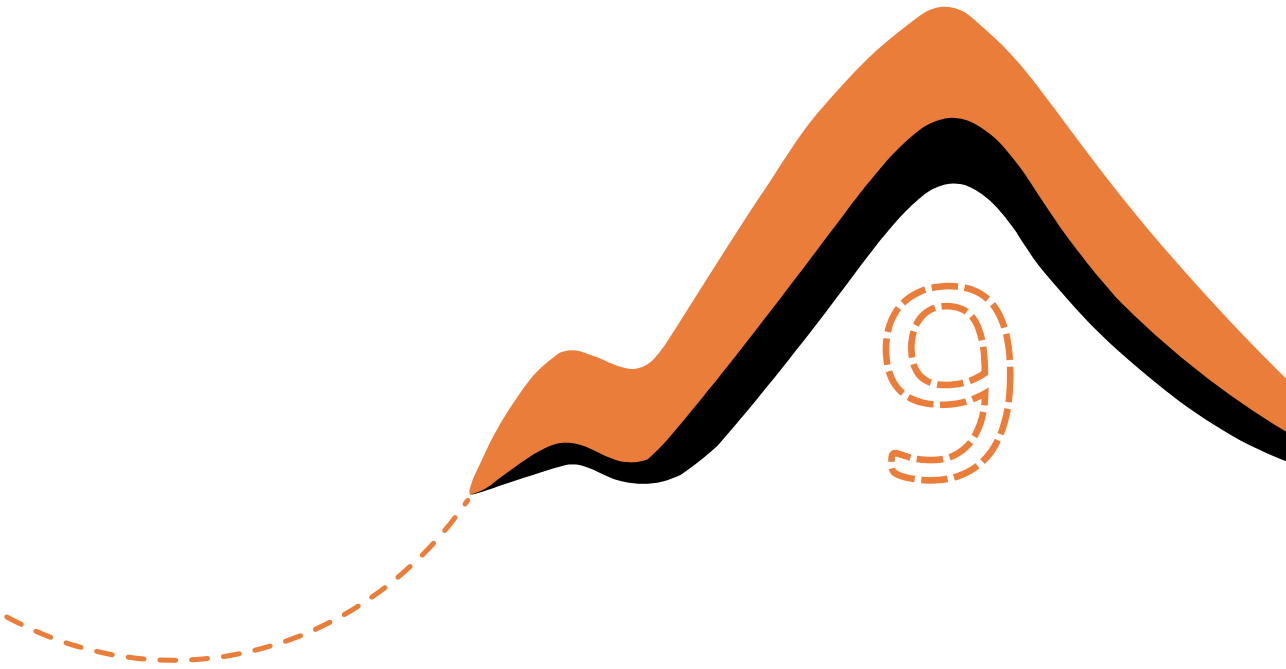


Figure S8.1. Reflexive impedance gain G results and within-session effect. Individual participant traces of the average conditioned reflex (mean Blocks 1-3) and control reflex (Block 0) per session for acclimatization (A1), baseline (B1-5) and conditioning (C1-6) sessions. The within-session effect is derived from the difference between the conditioned and control reflex within a session. The Impedance group received feedback on the depicted reflexive impedance gain G. A -15% within-session effect in session C4-6 was defined as success criteria to determine feasibility of the biofeedback method for each participant, see (grey) shaded target area. Each icon (circle, square, diamond) per group is linked to an individual participant and consistently used across figures.





ELECTROMYOGRAPHIC BIOFEEDBACK-DRIVEN GAMING TO ALTER CALF MUSCLE ACTIVATION DURING GAIT IN CHILDREN WITH SPASTIC CEREBRAL PALSY

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Abstract

Background. Children with cerebral palsy often show deviating calf muscle activation patterns during gait, with excess activation during early stance and insufficient activation during push-off.

Research question. Can children with cerebral palsy improve their calf muscle activation patterns during gait using one session of biofeedback-driven gaming?

Methods. Eighteen children (6-17y) with spastic cerebral palsy received implicit game-based biofeedback on electromyographic activity of the calf muscle (soleus or gastrocnemius medialis) while walking on a treadmill during one session. Biofeedback alternately aimed to reduce early stance activity, increase push-off activity, and both combined. Early stance and push-off activity and the double-bump-index (early stance divided by push-off activity) were determined during baseline and walking with feedback. Changes were assessed at group level using repeated measures ANOVA with simple contrast or Friedman test with post-hoc Wilcoxon signed rank test, as well as individually using independent t-tests or Wilcoxon rank sum tests. Perceived competence and interest-enjoyment were assessed through a questionnaire.

Results. Children successfully decreased their electromyographic activity during early stance feedback trials (relative decrease of 6.8±12.2 %, P=0.025), with a trend during the combined feedback trials (6.5±13.9%, P=0.055), and increased their electromyographic activity during push-off feedback trials (8.1±15.8%, P=0.038). Individual improvements were seen in twelve of eighteen participants. All children experienced high levels of interest-enjoyment (8.4/10) and perceived competence (8.1/10).

Significance. This exploratory study suggests that children with cerebral palsy can achieve small within-session improvements of their calf muscle activation pattern when provided with implicit biofeedback-driven gaming in an enjoyable manner. Follow-up gait training studies can incorporate this method to assess retention and long-term functional benefits of electromyographic biofeedback-driven gaming.

9.1 Introduction

Children with spastic cerebral palsy (CP) commonly experience difficulties in gait. Those difficulties are thought to partially arise due to exaggerated velocity-dependent stretch reflexes (stretch hyperreflexia, also referred to as spasticity),[1] causing increased calf muscle activity in early stance.[1,2] This activation limits calf muscle lengthening and ankle dorsiflexion during stance, yielding decreased push-off power.[1] Moreover, calf muscle activation at push-off is often limited by muscle weakness or impaired voluntary control.[1–4] This abnormal biphasic calf muscle activation pattern has been associated with increased energy cost[5] and decreased walking speed.[1] Current medical interventions to alter the activation pattern, such as de-innervation through botulinum toxin or neurosurgical treatment, are often invasive, non-specific, and/or temporary[6–8] and/or tend to weaken the muscle.[8,9]

Gait training is a non-invasive approach to improve mobility. Repetitive gait training can induce changes in corticomotor pathways[10,11] and is thereby expected to achieve long-term effects. Gait training can be supplemented with biofeedback to target specific factors of interest, such as stride length,[12,13] hip and knee extension,[13,14] or muscle activation patterns.[15] Impaired ankle push-off power is an attractive target for biofeedback.[1–4]

Gait training can possibly be improved when biofeedback addresses the abnormal biphasic calf muscle activation pattern of increased activity around early stance and decreased activity around push-off. Additionally, biofeedback on muscle activation, rather than kinetics, will enable translation towards physiotherapy- or home-based training, since it can be done without embedded force plates. User-friendly EMG biofeedback devices already exist,[16,17] and can be complemented with step-detection algorithms, for example through accelerometer data.[18] EMG biofeedback studies on upper extremity function have already been shown successful.[19,20] Additionally, in 1994 Colborne et al.[15] already showed that children with CP can increase their push-off power during gait by 19% when provided with electromyographic (EMG) biofeedback on the biphasic calf muscle activation pattern. However, their study presented with several limitations, such as differences in walking speed pre and post biofeedback - known to be strongly related to peak push-off power - and no quantification of actual changes in muscle activity as a result of the biofeedback.[21,22] Furthermore, they included only children with relatively mild impairments.

Over the last decades, new insights in motor learning have been developed, which may also help maximize the effects of gait training. For example, while current gait training protocols mostly use explicit forms of biofeedback,[12–15,23] a growing body of literature recognizes the importance of implicit motor learning for treatment efficacy,[24,25] especially in children with CP.[26,27] Explicit feedback entails specific movement instructions, such as visualization of the amount of knee extension. In contrast, while learning implicitly, children are challenged to develop their own strategies, resulting in longer-lasting improvements.[28] Furthermore, implicit biofeedback is thought to result in greater motivation, which is essential for treatment compliance.[29] Gamification is another tool to improve treatment efficacy,[30–32] engagement, and motivation,[33,34] and has already been successful in rehabilitation in children with CP.[30,31] Therefore, providing implicit, game-based EMG biofeedback is expected to result in a fun, engaging, effective gait training program to promote mobility.

To the best of our knowledge, training programs using game-based EMG biofeedback have not been previously studied in children with CP. Given the motor learning difficulties of children with CP,[35] it is essential to assess feasibility before assessing long-term training. Therefore, we aim to explore if children with CP can alter their calf muscle activation pattern within one session of implicit EMG biofeedback-driven gaming. More specifically, we evaluate if children can improve both deviating characteristics of calf muscle activation pattern; the early stance and the push-off activity. We furthermore assess if participant characteristics influence feasibility by assessing responder characteristics.

9.2 Methods & procedures

Participants

A convenience sample of eighteen children with spastic CP and related forms of spastic paresis participated in this observational cross-sectional feasibility study (Table 9.1). Twelve age-matched typically developing children were included for reference values. Exclusion criteria were: orthopedic leg surgery (<12 months ago), lower limb botulinum toxin-A injections (<6 months ago), selective dorsal rhizotomy, visual deficits limiting interpretation of visual feedback, frequent epilepsy, behavioral problems, or comorbidities affecting gait. The study protocol was approved by the local medical ethics committee (NL65846.029.18). All participants aged twelve years and older provided written informed consent, as well as all parents of participants under sixteen.

Study design

All measurements were performed on an instrumented treadmill in a semi-immersive virtual reality environment (Fig.8.1A). EMG electrodes were placed on the gastrocnemius medialis and soleus muscles according to SENIAM guidelines,[38] and reflective markers were placed according to the Human Body Model marker set.[39,40] EMG signals were measured at 1000 Hz via a wireless system (Wave, Cometa, Italy), motion data at 100 Hz using a motion capture system (Vicon Motion Systems, Oxford, UK), and ground reaction forces at 1000 Hz by sensors underneath both treadmill belts (R-Mill, Forcelink, The Netherlands).

Children started with at least six minutes of habituation to treadmill walking. They wore a non-weight bearing safety harness, and handrails were present for additional safety. Comfortable walking speed was determined by gradually altering belt speed until comfortable, as indicated by children and parents, and maintained throughout the experiment. Next, a comfortable walking trial of 30 seconds without biofeedback was recorded for all participants, while walking within an environment with optic flow (for typically developing children and participants 1-8 with CP) or the gaming environment (participants 9-17 with CP). Pilot analyses showed that walking in the gaming environment alone, did not alter the gait pattern. Thereafter, biofeedback trials were performed for children with CP only. Breaks were provided when necessary.

Table 9.1. Participant characteristics

	Children with cerebral palsy		Typically developing children	
Characteristic	**Inclusion criteria**	**Values**	**Inclusion criteria**	**Values**
		(mean ± std or n)		(mean ± std or n)
Age (y)	6-17	10.5±2.9	6-17	10.4±3.7
Gender (F/M)	-	(8F, 10M)	-	(3M, 9F)
Height (m)	-	1.45±0.16	-	1.45±0.23
Body mass (kg)	-	39.0±14.6	-	40.9±19.2
GMFCS	I-II	I: 9, II: 9	-	.
Distribution	-	Uni: 10, Bi: 8	-	.
Side	-	Left: 6, Right: 12	-	.
SPAT GM	≥1*	1: 4, 2: 1, 3: 3, CL:10	-	.
SPAT SO	≥1*	0:1, 1: 3, 2: 2, CL: 12	-	.
Walking speed (m/s)	-	0.72±0.14	-	1.02±0.14

*Abbreviations: GMFCS, Gross Motor Function Classification System[36]; GM, m. gastrocnemius medialis, SO: m. soleus; SPAT, scores reflect values of spasticity according to the SPAT test;[37] with the leg bent (SO) or extended (GM); CL, clonus. *One of these values should be 1 or higher.*

EMG-driven biofeedback was presented implicitly through a game (Fig. 9.1), as explained in detail in Supplementary Materials 9.1. In short, a monkey had to travel through a game, and children were instructed that the gait pattern of their most affected leg controlled the monkey. This control was based on the gastrocnemius medialis or soleus EMG of the most affected leg. Traveling of the monkey was achieved every step when children successfully decreased average early stance activity (0-50% or 15-50% of stance phase; FB_{early_stance}; see Supplementary Materials 9.1), increased push-off activity (60-90% of stance phase; $FB_{push\text{-}off}$), or both simultaneously ($FB_{combined}$). The soleus was targeted for most children, but the gastrocnemius medialis was targeted when its SPAT scores were higher than for the soleus (n=4). Feedback was provided in real-time during every stride for eighteen minutes; resulting in 700-1000 feedback occurrences per session. The session started with either early stance (n=10) or push-off (n=8) feedback as randomly assigned, and ended with combined feedback. Each condition lasted six minutes and feedback trials were recorded during the last 30 seconds of walking with feedback. Feedback was set at 67% positive feedback initially and manually adjusted if necessary to maintain motivation and maximize improvements. Motivation and perceived competence were assessed through an intrinsic motivation questionnaire[41] administered directly after the game, consisting of eight questions using a 1-10 Likert scale (Supplementary Materials 9.3).

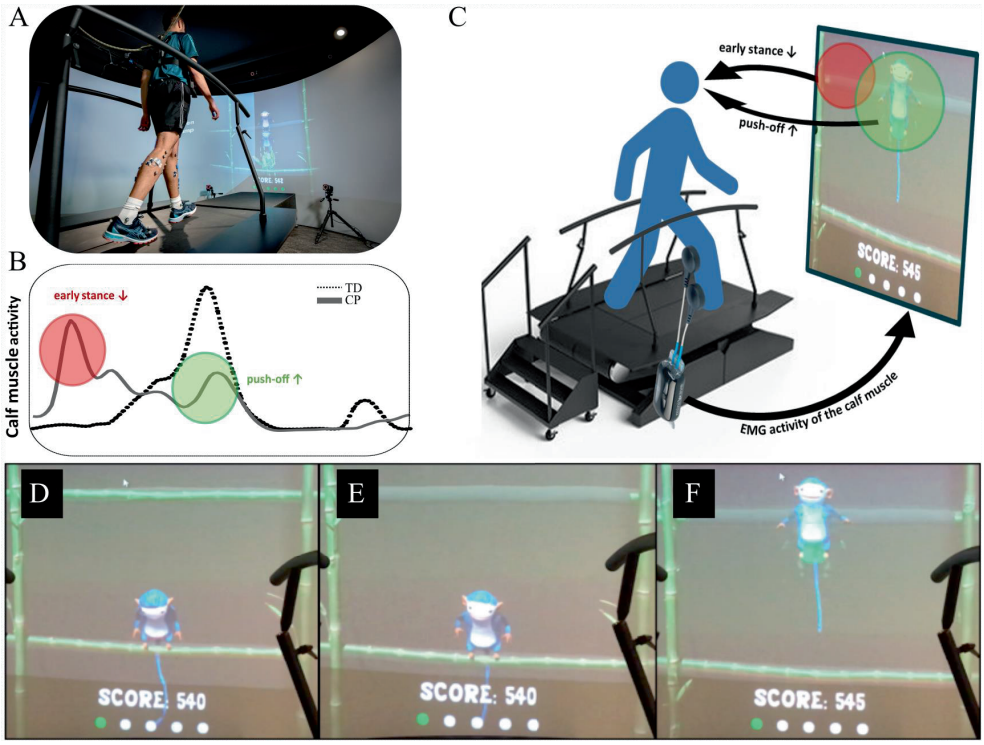

Figure 9.1. Measurement set-up and game. Panel A depicts the participant walking in the Gait Real-time Analysis Interactive Lab (Motek ForceLink, Amsterdam, Netherlands). The calf muscle activity is measured, see panel B for a typical example, and used as input for the biofeedback game, which is depicted in panels C-F: The goal is to make a monkey jump to as many branches as possible. If children decrease their early stance electromyographic (EMG) activity (panel B) the branch above the head of the monkey opens up (panels D→E), enabling a jump. The size of their EMG activity during the push-off (panel B) determines the height of the jump (panels E → F). These conditions were trained separately (EMG_{early_stance} and $EMG_{push\text{-}off}$ condition), and then combined. During early stance feedback, there was always a jump of sufficient height to reach the next branch in case it opened and during push-off feedback the branch was always open and if the jump was sufficiently high, the monkey would reach the next branch.

Data analysis

EMG signals were high-pass filtered (bidirectional 4th order Butterworth at 20Hz), rectified, and low-pass filtered (5Hz). The EMG envelopes were normalized to the mean over the entire gait cycle averaged over all baseline strides.[42] Kinematics (pelvis, hip, knee, and ankle angles) and kinetics (ankle moment and power) were calculated using the human body model,[39] and kinetics were normalized to body weight. Data were time-normalized to gait cycles using initial contact following Zeni.[43] Strides were manually excluded when movements were present that generally do not belong in gait (e.g. kicking, sliding, stepping sideways, or standing still). Furthermore, clear outliers were excluded with excessive deviations (>±3 SD from the median[13] over the trial) in maximum ankle plantar- or dorsiflexion or EMG peaks at early stance or push-off.

To quantify the improvements in EMG activity, the average peak during early stance (0-50% of stance phase), the average peak during push-off (60-90% of stance phase), and the double bump index[2] (DBI; early stance peak divided by push-off peak; see Fig. 9.2) were calculated over all steps within a recording. Furthermore, peak ankle power during push-off and ankle work during early stance and push-off were calculated. The following kinematic variables were assessed: maximum dorsiflexion during stance - expected to increase with decreased early stance EMG activity; maximum plantarflexion around push-off (60-120% of stance phase) - expected to increase along with increased push-off EMG activity; and knee extension in 20-100% of stance - expected to increase due to the plantarflexor knee extensor coupling in stance. Furthermore, the gait profile score (GPS[44]) was calculated to assess overall kinematic deviations from normal. Finally, for intrinsic motivation, answers within two dimensions (interest-enjoyment and perceived competence) were averaged to obtain one overall score for each dimension.

Statistical analysis

All outcome parameters were compared between baseline and the three feedback types using RM ANOVA with simple contrast and Friedmans test and post-hoc Wilcoxon signed rank test for

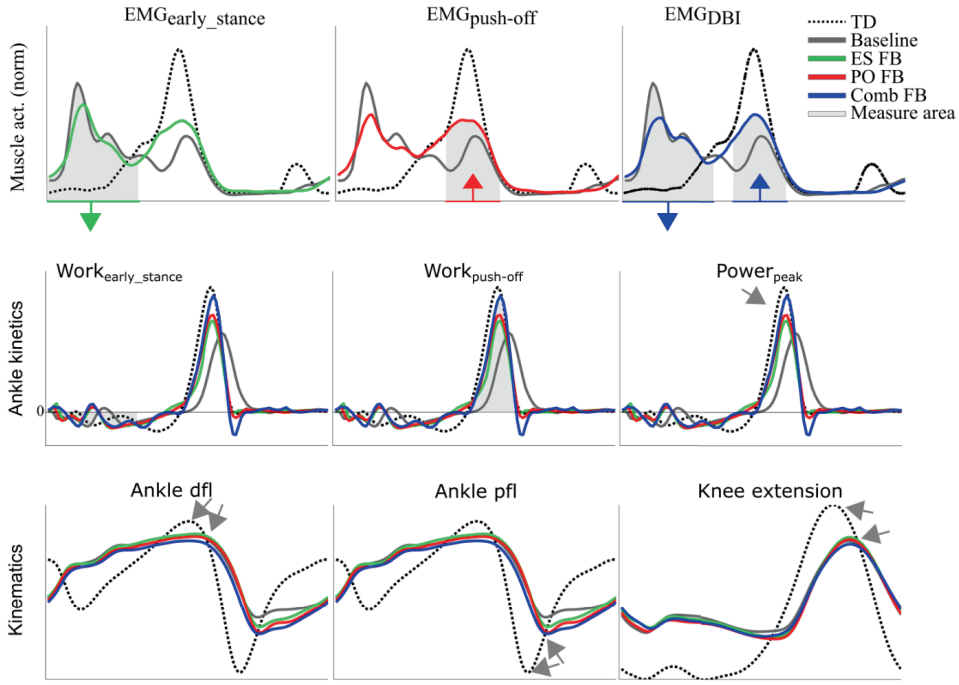

Figure 9.2. Example data from one typically developing child (dotted line) and one child with CP (solid lines) at baseline and during the three different feedback trials. The top row presents the EMG signals, with the shaded grey areas depicting the areas over which the RMS EMG were calculated which were used as input for the biofeedback and as outcome parameters. The arrows corresponding to the areas indicate the desired direction of improvement, e.g. early stance activity should be decreased. The second row represents the work around the ankle, with shaded areas and arrows representing the calculated outcome parameters. The third row represents the ankle and knee kinematics with arrows indicating the calculated outcome parameters.

parameters without normal distribution. Normality was tested by Shapirow-Wilk tests. Improvements were quantified using the percentage change, calculated as values after feedback minus baseline values divided by baseline values. For kinematics, improvements were quantified by subtracting the joint angles before and after biofeedback. Parameters at baseline and during the three feedback conditions were compared to typically developing children using an ANOVA with Dunnet's post-hoc testing. To identify responders to the three types of feedback, independent t-tests, or Wilcoxon sum rank when appropriate, were performed for each subject individually, comparing early stance EMG peaks (for FB_{early_stance}), push-off EMG peaks ($FB_{push\text{-}off}$), and DBI ($FB_{combined}$) for all feedback strides with all baseline strides. To determine if responder characteristics could be identified, we calculated correlation coefficients between improvements in DBI and subject characteristics, using Pearson correlation for age and baseline DBI, Spearman correlation for GMFCS and SPAT, and a partial eta squared for uni/bilateral involvement and most affected side. Furthermore, we correlated baseline early stance and push-off EMG peaks to changes in early stance and push-off EMG peaks. Given the explorative nature of this study, p-values below 0.10 were considered trends and p-values below 0.05 as significant.

9.3 Results

All participants were able to perform the biofeedback game and perceived the game as highly motivating (interest-enjoyment score 6.2-10, average 8.4). All but one subject felt competent (perceived-competence score 6-10, average 7.9; with one outlier of 3.3). Two participants did not complete the combined feedback trials and five participants only performed four to five minutes of biofeedback per session due to fatigue. One subject experienced an unexpectedly large increase in early stance peak once the game started and was therefore left out of group level analysis, as explained in detail in Supplementary Materials 9.2.

At group level, participants showed a significant decrease in early stance muscle activity during early stance feedback (-6.8±12.2% p=0.025) and a trend during combined feedback (-6.5±13.9%, p=0.055; Fig. 9.2, Fig. 9.3). Muscle activity around push-off improved during push-off feedback (+8.1±15.8%, p=0.039), but not during early stance or combined feedback. There was no overall effect on the DBI (p=0.102-0.186). All EMG parameters remained significantly different from TD values (*P*<0.001-0.002; see Table S9.4).

Peak ankle power increased during push-off feedback trials (10.6±19.0%, p=0.037). Knee extension in stance increased by 1-2° during all forms of feedback (*P*=0.009-0.040), but the GPS did not change (*P*=0.28-0.79). There were no significant changes in ankle kinematics (p=0.226-0.915) or work during early stance (*P*=0.295-0.831) and push-off feedback trials (p=0.185-0.580). Ankle dorsiflexion during stance, and plantarflexion and work during push-off were already not significantly different from TD at baseline (*P*=0.108-0.900) and also not during feedback. Peak push-off power remained significantly different from TD during all trials (*P*<0.018). However, ankle work during early stance was no longer significantly different from TD during combined feedback trials (p=0.064). Similarly, maximum knee extension during both combined (p=0.053) and push-off feedback trials (p=0.064) was no longer significantly different from TD, but these parameters did show a trend towards differences (p<0.10).

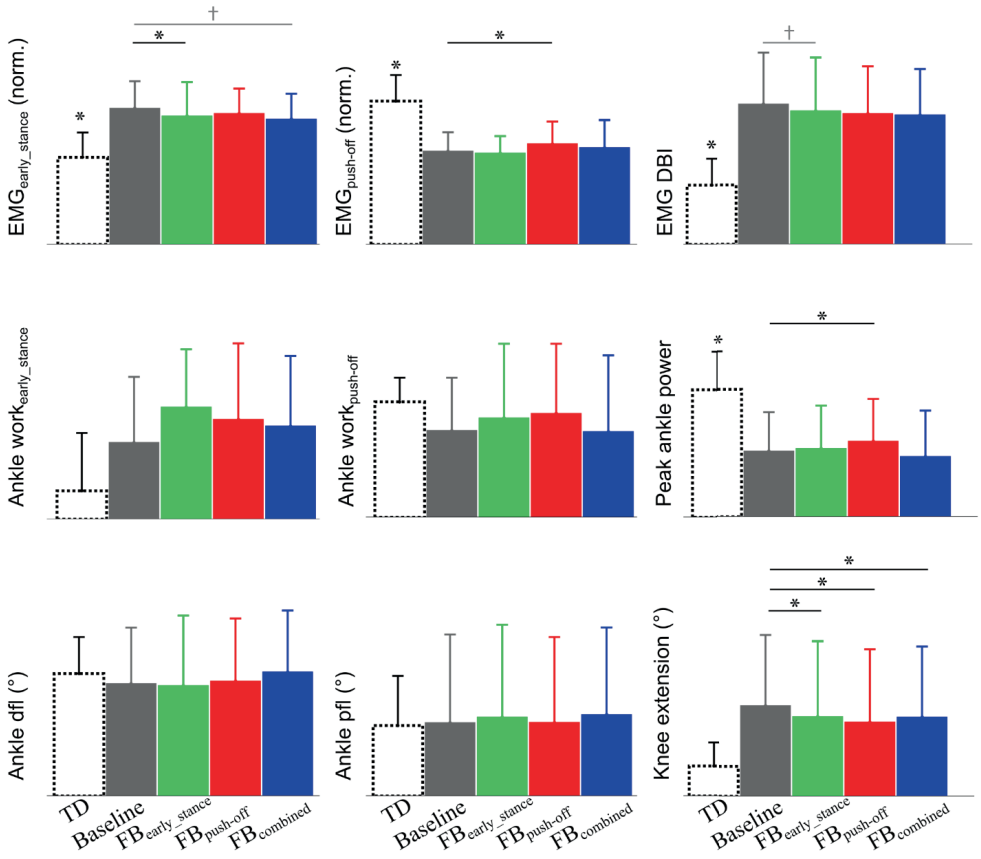

Figure 9.3. Changes in outcome parameters due to biofeedback**.** Means and standard deviations as described in the previous figure are shown for the different conditions, for EMG (top row), ankle work (middle row) and ankle and knee angles (bottom row). Bars are presented for typically developing (TD) children and children with cerebral palsy (CP) at baseline and during the three types of biofeedback (FB_{early_stance}, $FB_{push\text{-}off}$, $FB_{combined}$). Stars above typically developing (TD) data indicate parameters that were significantly different from all conditions of the children with CP. Trends ($p<0.10$) are visualized with a cross. Stars between baseline and biofeedback conditions represent improvements due to the biofeedback. Baseline values, improvements and p-values can be found in Supplementary Materials 9.4. Max ankle dorsiflexion (dfl) is measured during stance, plantarflexion (pfl) around push-off (60-120% of stance phase), and max knee extension during 20-100% of the stance phase. Abbreviations: DBI, double-bump-index, dfl, dorsiflexion; pfl, plantarflexion.

Twelve of eighteen participants were categorized as responders for at least one feedback type (Table 9.2), with 6/18 (FB_{early_stance}), 7/18 ($FB_{push\text{-}off}$), and 5/18 ($FB_{combined}$) significantly improving EMG activity (Fig. 9.2). Three participants showed significant worsening in DBI, of whom two were able to improve their push-off activity. Improvements in DBI correlated with higher DBI at baseline ($r^2=0.309$; $P=0.031$), lower peak ankle power at baseline ($r^2=0.392$, $P=0.012$), total power during push-off ($r^2=0.342$, $P=0.031$) and a trend for the right leg as the most affected side ($\eta^2=0.240$; $P=0.096$) (Fig. 9.4; Table S9.4).

Table 9.2.

Individual participants improvements and characteristics

	Early stance (*P*-values)	Push-off (*P*-values)	COMB trials (*P*-values)	Muscle	Distrib ution	Side	Motiva tion	Compet ence
P01	**0.002**$^{+}$	**0.050**	-†	SO	Bi	R	-†	-
P02	0.622	**0.005**	0.002*	SO	Uni	L	9.3	8.0
P03	**0.028**	0.351	0.695^{+}	GM	Uni	L	9.1	9.9
P04	0.266	**<0.001**	**<0.001**$^{+}$	SO	Uni	R	9.3	9.3
P05	**0.056**	0.803	0.533	GM	Uni	R	8.0	9.0
P06	**<0.001**	0.218	0.609^{+}	SO	Uni	R	6.2	7.9
P07	**<0.001**$^{+}$	**0.082**	0.108	SO	Bi	L	10.0	9.2
P08	<0.001*$^{+}$	**0.003**	<0.001*$^{+}$	SO	Bi	L	7.5	6.0
P09	0.563	0.813	0.015*$^{+}$	SO	Bi	R	7.5	9.0
P10	**<0.001**	**<0.001**	**<0.001**	SO	Bi	R	9.5	8.0
P11	0.882	0.107	**0.009**	SO	Uni	R	7.5	10
P12	0.273	0.656^{+}	0.437^{+}	GM	Uni	R	9.4	9.0
P13	0.110	**0.046**$^{+}$	**0.025**$^{+}$	SO	Bi	R	7.9	3.3
P14	**0.088**$^{+}$	**<0.001**$^{+}$	-†	GM	Bi	R	9.4	7.8
P15	0.544^{+}	0.675	**0.028**$^{+}$	SO	Bi	L	8.2	9.2
P16	0.364	0.667	0.866^{+}	SO	Bi	R	-†	-
P17	**0.013**	0.516	0.801^{+}	SO	Uni	R	9.3	8.6
P18	0.899	0.355	0.290^{+}	SO	Bi	L	7.7	7.1

*This table presents if individual participants were able to decrease their early stance EMG activity during the early stance biofeedback trial, increase their push-off activity during the push-off biofeedback trial and decrease their double-bumb-index during the combined (COMB) feedback trial. P-values are presented for all individual participants (P##). Furthermore, the assessed muscle is shown, being the soleus (SO) or gastrocnemius medialis (GM) muscle. Distribution of the subject is presented, with uni, unilateral and bi, bilateral. Side indicates the (most-) affected side upon which feedback was provided. The final columns depict the individual scores on the intrinsic motivation inventory for both subscales motivation and perceived competence. Bold values indicate significant improvements or trends, *indicate significant worsening. $^{+}$Indicates non-parametric Wilcoxon sum rank test was used. Abbreviations: ES, early stance feedback; PO, push-off feedback; COMB, combined feedback; †indicate missing values, which were therefore left out of the analyses.*

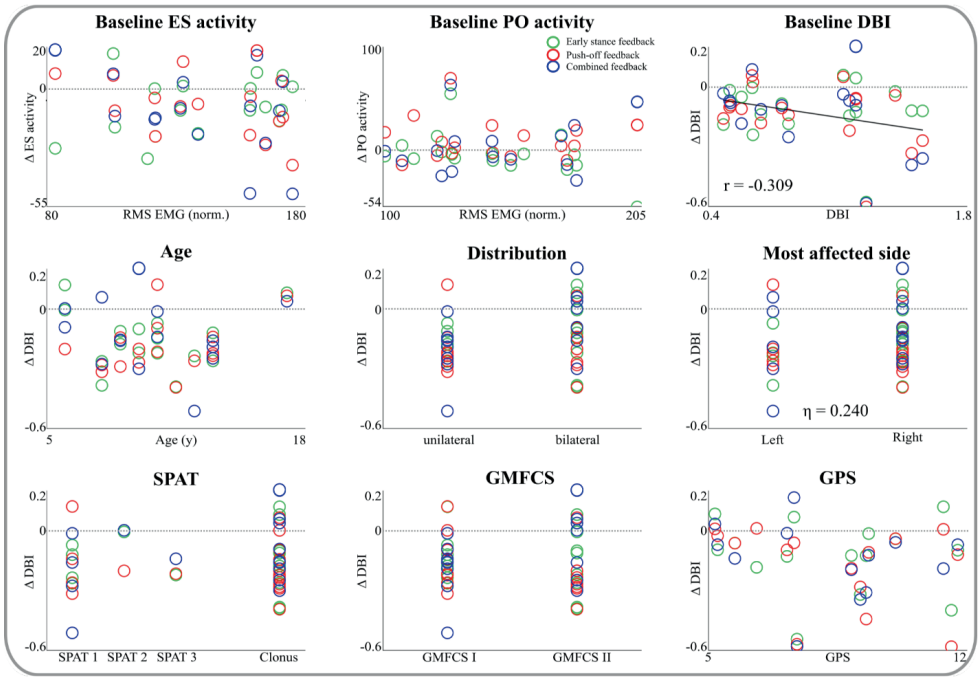

Figure 9.4. Correlation analysis to identify responder characetristics. The regression line is only presented for the variables 'baseline DBI', and the partial eta score for 'most affected side', as these were the only significant correlations. Other correlation values and the corresponding p-values can be found in Table S9.3. DBI is the double-bump-index, calculated as the ES activity/PO activity, with lower values indicating improvements. The negative correlation in Baseline DBI versus ΔDBI indicates that children with larger DBI (i.e. more deviating activity patterns) show larger improvements (DBI decreases with feedback). Abbreviations; ES, early stance; PO, push-off; SPAT, spasticity assessment; GMFCS, gross motor function classification system; GPS, gait profile score.

9.4 Discussion

This study explored the possibilities of improving calf muscle activation in children with CP through biofeedback-driven gaming. At group level, we found that children can change their activation pattern when receiving early stance and push-off biofeedback. Twelve of eighteen participants showed an immediate response to at least one type of biofeedback. Improvements were generally small (5-10%), while participants with more deviating baseline EMG patterns - according to push-off activity and double-bump-index - achieved larger improvements. Furthermore, changes in muscle activity were accompanied by some improvements in kinetics and kinematics. GPS did not change during biofeedback, suggesting that children did not show large improvements but also no major compensations in other joints. Importantly, all children enjoyed playing the game and scored high on perceived competence.

Results of our study add to the limited available evidence[15,45] that children with CP can alter their muscle activation patterns when walking with EMG biofeedback. Improvements were small, with 7-8% relative improvement in push-off and early stance activity during their corresponding feedback. Furthermore, we saw a trend toward 7% improved DBI during early stance feedback. Similar to

Colborne et al.[15], who found a 19% increased ankle power ($p<0.10$), push-off biofeedback resulted in improved peak ankle power, but other kinetic variables did not improve.

Some overlap but also some discrepancies with previous studies became apparent. Colborne et al.[15] provided similar biofeedback but over eight sessions instead of one. Despite more sessions, changes were not considerably larger than in our study, as they only found a trend towards improved ankle power. This may be because their study was performed in an overground lab, limiting the number of strides (60-70 per session, compared to 700-1000 in our study) and thereby the amount of biofeedback. Additionally, the discrepancy could be caused by the less pathological baseline values in the study of Colborne et al.[15] (e.g., average peak ankle push-off power was 91% of our norm data, compared to 50% in our study). The inclusion of less affected individuals likely limits the effect size, as we found that children with more deviating EMG patterns and lower ankle push-off power generally achieved larger improvements. This is consistent with previous findings, as Van Gelder et al.[14] found an association between baseline GPS and improvements from kinematic biofeedback in children with CP. Therefore, it appears that more severely affected patients achieve greater improvements when targeting gait with biofeedback. Supporting this, Booth et al.[13] included more severely affected children (ankle push-off 41% of norm) and provided biofeedback directly on ankle push-off power during treadmill walking. They found large increases in peak ankle push-off power (38%) already after two minutes of biofeedback.

Compared to Booth et al.[13], the improvements found in our study (7-8%) were relatively modest. Furthermore, also a recent study by Conner and Lerner[46] found large increases of 46% in soleus activation in a single session of robot-resisted gait training when adding push-off feedback. Several factors could explain this difference. First, most previous gait training studies[13,15,46] used explicit biofeedback, whereas we applied implicit biofeedback. It is noteworthy that several children already showed changes within one session, as implicit feedback is expected to take longer to yield results.[28] Although it should be confirmed in future studies for our specific application, implicit learning in general yields longer-lasting results[24,25] and increases engagement and motivation.[29] Another advantage of implicit learning is that it requires less working memory, which is often impaired due to left hemisphere lesions, as is common in children with right unilateral CP.[26] Although weak, we found a trend towards a greater response in children whose right side was more severely affected, further underlining the efficacy of implicit biofeedback for this group.

A second factor that could explain the relatively small improvements regards the requirement of consecutively decreasing and increasing the activation of the same muscle within a short time window of 400-600ms. Even though the implicit nature of the biofeedback allows for such a task, this may be complex to perform. Mastering the separate components first and only providing combined feedback later might increase improvements. Thirdly, since we kept walking speed constant, this might have impeded further increases, knowing that calf muscle activation is highly dependent on walking speed. Long-term training studies can use self-paced walking during the biofeedback conditions, allowing for greater effects. However, improvements should be assessed at matched walking speeds for a fair comparison.[21] Finally, we noticed that subjects experienced both more and less successful periods of feedback, for example due to a sudden loss of effective strategy, bursts of frustration, loss of attention, or the occurrence of fatigue. Children were on average not able to comply with the combined

feedback, which might be caused by these factors. We expect that larger within-session improvements can be achieved by fine-tuning the gaming techniques.
Although it is debatable whether the changes found in this study represent a clinically relevant improvement and effects are limited to immediate effects during biofeedback, it is promising that improvements could be seen in most children already within one session. For successful implementation in clinical care, it is likely important that children already achieve success during early stages of training. Therefore, within-session improvements are a first step, and translation toward general gait should be studied in long-term studies. Furthermore, future studies can analyze if improvements are specific to the biofeedback imposed, and what types of feedback would work best. For instance, it could be that only the focus on improving gait, regardless of the type of feedback, already leads to improvements in muscle activation. Yet, our finding that reductions in early stance activity and increases in push-off activity were specific to their respective feedback type, strengthens the idea that changes are indeed feedback-specific.

EMG biofeedback is likely easier to implement in physiotherapy- or potentially even home-based training, compared to for example kinetic biofeedback,[13] as it omits the need for expensive 3D motion tracking devices with embedded force plates. User-friendly devices already exist to provide EMG biofeedback at home.[16,17] Adding a step-detection algorithm, for example through accelerometer data,[18] can make these devices suitable to target specific phases of the gait cycle. Additionally, gamification increases long-term treatment efficacy[30–32] as well as engagement and motivation,[33,34] and indeed we measured high levels of motivation. Besides these positive effects, moving away from clinical settings towards physiotherapy or home settings will increase clinical applicability.

This explorative study assessed improvements within just one session. No post-testing was performed as retention was not expected after the short feedback session. Follow-up training studies are required to evaluate the long-term effects. Additionally, even though multiple outcome parameters normalized towards typical, we did not assess functional outcomes, such as energy expenditure or walking speed, which should be a target in a long-term study.[47] Another limitation is the single muscle currently addressed for biofeedback, whereas future applications might need to target more muscles for optimal improvements.

In conclusion, this exploratory study indicates that most participants with CP can achieve within-session improvements in their muscle activation pattern during walking with implicit EMG biofeedback-driven gaming. Furthermore, the gaming was well-tolerated and motivating for children with CP. These results indicate that it is worthwhile to assess the long-lasting functional effects of implicit EMG biofeedback-driven gaming.

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References

1. Olney SJ, MacPhail HA, Hedden DM, Boyce WF. Work and Power in Hemiplegic Cerebral Palsy Gait. *Phys Ther* 1990; 70: 431–9.
2. Van der Krogt MM, Doorenbosch CAM, Becher JG, Harlaar J. Dynamic spasticity of plantar flexor muscles in cerebral palsy gait. *J Rehabil Med J Rehabil Med J Rehabil Med* 2010; 42: 656–63.
3. Berger W, Altenmueller E, Dietz V. Normal and impaired development of children's gait. *Hum Neurobiol* 1984; 3: 163–70.
4. Barber L, Carty C, Modenese L, Walsh J, Boyd R, Lichtwark G. Medial gastrocnemius and soleus muscle-tendon unit, fascicle, and tendon interaction during walking in children with cerebral palsy. *Dev Med Child Neurol* 2017; 59: 843–51.
5. Hodapp M, Klisch C, Mall V, Vry J, Berger W, Faist M. Modulation of Soleus H-Reflexes During Gait in Children With Cerebral Palsy. *J Neurophysiol* 2007; 98: 3263–8.
6. Williams PE, Goldspink G. The effect of immobilization on the longitudinal growth of striated muscle fibres. *J Anat* 1973; 116: 45–55.
7. Williams PE, Goldspink G. Longitudinal Growth of Striated Muscle Fibres. *J Cell Sci* 1971; 9.
8. Thomas CK, Hager-Ross CK, Klein CS. Effects of baclofen on motor units paralysed by chronic cervical spinal cord injury. *Brain* 2010; 133: 117–25.
9. D'Amico JM, Condliffe EG, Martins KJB, Bennett DJ, Gorassini MA. Recovery of neuronal and network excitability after spinal cord injury and implications for spasticity. *Front Integr Neurosci* 2014; 8: 1–24.
10. Barbeau H. Locomotor training in neurorehabilitation: emerging rehabilitation concepts. *Neurorehabil Neural Repair* 2003; 17: 3–11.
11. Yen C-L, Wang R-Y, Liao K-K, Huang C-C, Yang Y-R. Gait training—induced change in corticomotor excitability in patients with chronic stroke. *Neurorehabil Neural Repair* 2008; 22: 22–30.
12. Baram Y, Lenger R. Gait improvement in patients with cerebral palsy by visual and auditory feedback. *2009 Virtual Rehabil Int Conf* 2009. DOI:10.1109/ICVR.2009.5174222.
13. Booth AT, Buizer AI, Harlaar J, Steenbrink F, van der Krogt MM. Immediate Effects of Immersive Biofeedback on Gait in Children With Cerebral Palsy. *Arch Phys Med Rehabil* 2019; 100: 598–605.
14. van Gelder L, Booth ATC, van de Port I, Buizer AI, Harlaar J, van der Krogt MM. Real-time feedback to improve gait in children with cerebral palsy. *Gait Posture* 2017; 52: 76–82.
15. Colborne GR, Wright F V, Naumann S, Anonymous. Feedback of triceps surae EMG in gait of children with cerebral palsy: a controlled study. *Arch Phys Med Rehabil* 1994; 75: 40–5.
16. Donoso Brown E V., McCoy SW, Fechko AS, Price R, Gilbertson T, Moritz CT. Preliminary Investigation of an Electromyography-Controlled Video Game as a Home Program for Persons in the Chronic Phase of Stroke Recovery. *Arch Phys Med Rehabil* 2014; 95: 1461–9.
17. Toner L V, Cook K, Elder GC. Improved ankle function in children with cerebral palsy after computer-assisted motor learning. *Dev Med Child Neurol* 1998; 40: 829–35.
18. Bach MM, Dominici N, Daffertshofer A. Predicting vertical ground reaction forces from 3D accelerometery using reservoir computers leads to accurate gait event detection. *bioRxiv* 2022; : 2022.02.14.480318.
19. Rios DC, Gilbertson T, McCoy SW, et al. NeuroGame Therapy to improve wrist control in children with cerebral palsy: a case series. *Dev Neurorehabil* 2013; 16: 398–409.
20. Bloom R, Przekop A, Sanger TD. Prolonged electromyogram biofeedback improves upper extremity function in children with cerebral palsy. *J Child Neurol* 2010; 25: 1480–4.
21. Booij MJ, Meinders E, Sierevelt IN, Nolte PA, Harlaar J, van den Noort JC. Matching walking speed of controls affects identification of gait deviations in patients with a total knee replacement. *Clin Biomech* 2021; 82: 105278.
22. Oudenhoven LM, Booth ATC, Buizer AI, Harlaar J, van der Krogt MM. How normal is normal: Consequences of stride to stride variability, treadmill walking and age when using normative paediatric gait data. *Gait Posture* 2019; 70: 289–97.
23. Booth ATC, Steenbrink F, Buizer AI, Harlaar J, van der Krogt MM. Is avatar based real-time visual feedback a feasible method to alter gait parameters of interest? *Gait Posture* 2016; 49: 98.
24. Tablerion JM, Wood TA, Hsieh KL, et al. Motor Learning in People with Multiple Sclerosis: A Systematic Review and Meta-analysis. Arch. Phys. Med. Rehabil. 2020; 101: 512–23.
25. Kal E, Prosée R, Winters M, Van Der Kamp J. Does implicit motor learning lead to greater automatization of motor skills compared to explicit motor learning? A systematic review. PLoS One. 2018; 13. DOI:10.1371/journal.pone.0203591.

26. Van Der Kamp J, Steenbergen B, Masters RSW. Explicit and implicit motor learning in children with unilateral cerebral palsy Explicit and implicit motor learning in children with unilateral cerebral palsy. *Disabil Rehabil* 2017. DOI:10.1080/09638288.2017.1360403.
27. Jongbloed-Pereboom M, Janssen AJWM, Steenbergen B, Nijhuis-van der Sanden MWG. Motor learning and working memory in children born preterm: A systematic review. Neurosci. Biobehav. Rev. 2012; 36: 1314–30.
28. McDougle SD, Bond KM, Taylor JA. Explicit and Implicit Processes Constitute the Fast and Slow Processes of Sensorimotor Learning. *J Neurosci* 2015; 35: 9568–79.
29. Beckers LWME, Geijen MME, Kleijnen J, et al. Feasibility and effectiveness of home-based therapy programmes for children with cerebral palsy: A systematic review. BMJ Open. 2020; 10. DOI:10.1136/bmjopen-2019-035454.
30. Lopes S, Magalhães P, Pereira A, et al. Games used with serious purposes: a systematic review of interventions in patients with cerebral palsy. *Front Psychol* 2018; 9: 1712.
31. Bonnechère B, Jansen B, Omelina L, Van Sint Jan S. The use of commercial video games in rehabilitation: a systematic review. *Int J Rehabil Res* 2016; 39: 277–90.
32. Proença JP, Quaresma C, Vieira P. Serious games for upper limb rehabilitation: a systematic review. *Disabil Rehabil Assist Technol* 2018; 13: 95–100.
33. Deterding S, Sicart M, Nacke L, O'Hara K, Dixon D. Gamification. using game-design elements in non-gaming contexts. In: CHI'11 extended abstracts on human factors in computing systems. 2011: 2425–8.
34. Turan Z, Avinc Z, Kara K, Goktas Y. Gamification and education: Achievements, cognitive loads, and views of students. *Int J Emerg Technol Learn* 2016; 11.
35. Jenks KM, Moor J de, Lieshout ECDM van, Maathuis KGB, Keus I, Gorter JW. The Effect of Cerebral Palsy on Arithmetic Accuracy is Mediated by Working Memory, Intelligence, Early Numeracy, and Instruction Time. *Dev Neuropsychol* 2007; 32: 861–79.
36. Palisano R, Rosenbaum P, Bartlett D, et al. Gross Motor Function Classification System. *Dev Med Child Neurol* 1997; 39: 214–23.
37. Scholtes VAB, Dallmeijer AJ, Becher JG. The Spasticity Test: a clinical instrument to measure spasticity in children with cerebral palsy. In: The Effectiveness of Multilevel Botulinum Toxin Type A and Comprehensive Rehabilitation in Children with Cerebral Palsy. Citeseer, 2007: 29–64.
38. Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr Kinesiol* 2000; 10: 361–74.
39. Flux E, van der Krogt MM, Cappa P, Petrarca M, Desloovere K, Harlaar J. The Human Body Model versus conventional gait models for kinematic gait analysis in children with cerebral palsy. *Hum Mov Sci* 2020; 70: 102585.
40. van den Bogert AJ, Geijtenbeek T, Even-Zohar O, Steenbrink F, Hardin EC. A real-time system for biomechanical analysis of human movement and muscle function. *Med Biol Eng Comput* 2013; 51: 1069–77.
41. McAuley E, Duncan T, Tammen V V. Psychometric Properties of the Intrinsic Motivation Inventory in a Competitive Sport Setting: A Confirmatory Factor Analysis. Taylor & Francis Group, 1989.
42. Sousa ASP, Tavares JRMS. Surface electromyographic amplitude normalization methods: A review. In: Electromyography: new developments, procedures and applications. 2012. https://repositorio-aberto.up.pt/bitstream/10216/64430/2/67854.pdf (accessed 19 Aug 2021).
43. Zeni JA, Richards JG, Higginson JS. Two simple methods for determining gait events during treadmill and overground walking using kinematic data. *Gait Posture* 2008; 27: 710–4.
44. Baker R, McGinley JL, Schwartz MH, et al. The Gait Profile Score and Movement Analysis Profile. *Gait Posture* 2009; 30: 265–9.
45. Bolek JE. A Preliminary Study of Modification of Gait in Real-Time Using Surface Electromyography. *Appl Psychophysiol Biofeedback* 2003; 28.
46. Conner BC, Lerner ZF. Improving Ankle Muscle Recruitment via Plantar Pressure Biofeedback during Robot Resisted Gait Training in Cerebral Palsy. *IEEE Int Conf Rehabil Robot* 2022; 2022-July: 25–9.
47. Riad J, Broström E, Langius-Eklöf A. Do movement deviations influence self-esteem and sense of coherence in mild unilateral cerebral palsy? *J Pediatr Orthop* 2013; 33: 298–302.
48. van den Noort JC, Bar-On L, Aertbeliën E, et al. European consensus on the concepts and measurement of the pathophysiological neuromuscular responses to passive muscle stretch. *Eur J Neurol* 2017; 24: 981-e38.
49. van der Velden LL, de Koff MAC, Ribbers GM, Selles RW. The diagnostic levels of evidence of instrumented devices for measuring viscoelastic joint properties and spasticity; a systematic review. *J*

Neuroeng Rehabil 2022; 19: 1–8.
50. Sackett DL, Haynes RB. The architecture of diagnostic research. *Bmj* 2002; 324: 539–41.
51. van't Veld RC, Flux E, van Oorschot W, et al. Examining the role of intrinsic and reflexive contributions to ankle joint hyper-resistance treated with botulinum toxin-A. *J Neuroeng Rehabil* 2023; 20: 1–14.
52. Flux E, van 't Veld R, van Asseldonk E, et al. A comparison of different methods to quantify stretch reflexes in children with cerebral palsy. In: Stretch hyperreflexia in children with cerebral palsy: Assessment, Contextualization and Modulation. 2023.
53. Alhusaini AAA, Dean CM, Crosbie J, Shepherd RB, Lewis J. Evaluation of spasticity in children with cerebral palsy using Ashworth and Tardieu Scales compared with laboratory measures. *J Child Neurol* 2010; 25: 1242–7.
54. Malhotra S, Cousins E, Ward A, et al. An investigation into the agreement between clinical, biomechanical and neurophysiological measures of spasticity. *Clin Rehabil* 2008; 22: 1105–15.
55. Patrick E, Ada L. The Tardieu Scale differentiates contracture from spasticity whereas the Ashworth Scale is confounded by it. *Clin Rehabil* 2006; 20: 173–82.
56. Bohannon RW, Smith MB. Interrater reliability of a modified Ashworth scale of muscle spasticity. *Phys Ther* 1987; 67: 206–7.
57. Haugh AB, Pandyan AD, Johnson GR. A systematic review of the Tardieu Scale for the measurement of spasticity. *Disabil Rehabil* 2006; 28: 899–907.
58. Scholtes VAB, Becher JG, Lankhorst GJ. Clinical assessment of spasticity in children with cerebral palsy: a critical review of available instruments. *Dev Med Child Neurol* 2006; 48: 64–73.
59. Fleuren JFM, Voerman GE, Erren-Wolters C V, et al. Stop using the Ashworth Scale for the assessment of spasticity. *J Neurol* 2009; 81: 46.
60. Charalambous CP. Interrater reliability of a modified ashworth scale of muscle spasticity. In: Classic Papers in Orthopaedics. Springer-Verlag London Ltd, 2014: 415–7.
61. Netherlands CP register. ; : https://cpregister.nl/.
62. Ludvig D, Cathers I, Kearney RE. Voluntary modulation of human stretch reflexes. *Exp Brain Res* 2007; 183: 201–13.
63. van 't Veld RC, Flux E, Schouten AC, van der Krogt MM, van der Kooij H, van Asseldonk EHF. Reducing the Soleus Stretch Reflex With Conditioning: Exploring Game- and Impedance-Based Biofeedback. *Front Rehabil Sci* 2021; 2: 1–13.
64. Mrachacz-Kersting N, Kersting UG, de Brito Silva P, et al. Acquisition of a simple motor skill: Task-dependent adaptation and long-term changes in the human soleus stretch reflex. *J Neurophysiol* 2019; 122: 435–46.
65. Mirbagheri MM, Alibiglou L, Thajchayapong M, Rymer WZ. Muscle and reflex changes with varying joint angle in hemiparetic stroke. *J Neuroeng Rehabil* 2008; 5: 1–15.
66. Alibiglou L, Rymer WZ, Harvey RL, Mirbagheri MM. The relation between Ashworth scores and neuromechanical measurements of spasticity following stroke. *J Neuroeng Rehabil* 2008; 5: 1–14.
67. van't Veld R. Integrated Spasticity Assessment and Treatment Using Disentangled Joint Resistance. Enschede: University of Twente, 2022 DOI:10.3990/1.9789036553919.
68. Mirbagheri MM, Barbeau H, Kearney RE. Intrinsic and reflex contributions to human ankle stiffness: Variation with activation level and position. *Exp Brain Res* 2000. DOI:10.1007/s002210000534.
69. Graser J V., Prospero L, Liesch M, Keller U, van Hedel HJA. Test–retest reliability of upper limb robotic exoskeleton assessments in children and youths with brain lesions. *Sci Reports 2022 121* 2022; 12: 1–15.
70. Bar-On L, Aertbeliën E, Wambacq H, et al. A clinical measurement to quantify spasticity in children with cerebral palsy by integration of multidimensional signals. *Gait Posture* 2013; 38: 141–7.
71. Bar-On L, Desloovere K, Molenaers G, Harlaar J, Kindt T, Aertbeliën E. Identification of the neural component of torque during manually-applied spasticity assessments in children with cerebral palsy. *Gait Posture* 2014; 40: 346–51.
72. Yamaguchi T, Hvass Petersen T, Kirk H, et al. Spasticity in adults with cerebral palsy and multiple sclerosis measured by objective clinically applicable technique. *Clin Neurophysiol* 2018; 129: 2010–21.
73. Willerslev-Olsen M, Choe Lund M, Lorentzen J, Barber L, Kofoed-Hansen M, Nielsen JB. Impaired muscle growth precedes development of increased stiffness of the triceps surae musculotendinous unit in children with cerebral palsy. *Dev Med Child Neurol* 2018; 60: 672–9.
74. Sloot LH, Bar-On L, van der Krogt MM, et al. Motorized versus manual instrumented spasticity assessment in children with cerebral palsy. *Dev Med Child Neurol* 2017; 59: 145–51.
75. Bar-On L, Aertbeliën E, Wambacq H, et al. A clinical measurement to quantify spasticity in children with

cerebral palsy by integration of multidimensional signals. *Gait Posture* 2013; 38: 141–7.
76. Gillett J, Greaves H, Bass A, et al. Clinically applicable assessment of passive stiffness and stretch reflex responses using a portable device in children with cerebral palsy. *Gait Posture* 2020; 81: 105–6.
77. Lamontagne A, Malouin F, Richards CL. Locomotor-Specific measure of spasticity of plantarflexor muscles after stroke. *Arch Phys Med Rehabil* 2001; 82: 1696–704.
78. Van der Krogt MM, Doorenbosch CAM, Becher JG, Harlaar J. Dynamic spasticity of plantar flexor muscles in cerebral palsy gait. *J Rehabil Med* 2010; 42: 656–63.
79. Crenna P. Spasticity and 'Spastic' Gait in Children with Cerebral Palsy. *Neurosci Biobehav Rev* 1998; 22: 571–8.
80. De Niet M, Latour H, Hendricks H, Geurts AC, Weerdesteyn V. Short-Latency Stretch Reflexes Do Not Contribute to Premature Calf Muscle Activity During the Stance Phase of Gait in Spastic Patients. *Arch Phys Med Rehabil* 2011; 92: 1833–9.
81. Nielsen JB, Christensen MS, Farmer SF, Lorentzen J. Spastic movement disorder: should we forget hyperexcitable stretch reflexes and start talking about inappropriate prediction of sensory consequences of movement? Exp. Brain Res. 2020; 238: 1627–36.
82. Sloot LH, Van Den Noort JJC, van der Krogt MMM, Bruijn SMS, Harlaar J. Can treadmill perturbations evoke stretch reflexes in the calf muscles? *PLoS One* 2015; 10. DOI:10.1371/journal.pone.0144815.
83. Flux E, van der Krogt MM, Harlaar J, Buizer AI, Sloot LH. Functional assessment of stretch hyperreflexia in children with cerebral palsy using treadmill perturbations. *J NeuroEngineering Rehabil 2021 181* 2021; 18: 1–17.
84. Flux E, Mooijekind B, Bar-On L, van Asseldonk E, Buizer AI, van der Krogt MM. Relation between gastrocnemius medialis muscle-tendon stretch and muscle activation during gait in children with cerebral palsy. In: Stretch hyperreflexia in children with cerebral palsy: assessment, contextualization and modulation. 2023.
85. Lichtwark GA, Wilson AM. Interactions between the human gastrocnemius muscle and the Achilles tendon during incline, level and decline locomotion. *J Exp Biol* 2006; 209: 4379–88.
86. Cronin NJ, Carty CP, Barrett RS, Lichtwark G. Automatic tracking of medial gastrocnemius fascicle length during human locomotion. *J Appl Physiol* 2011; 111: 1491–6.
87. Kalkman BM, Bar-On L, Cenni F, et al. Muscle and tendon lengthening behaviour of the medial gastrocnemius during ankle joint rotation in children with cerebral palsy. *Exp Physiol* 2018; 103: 1367–76.
88. Bar-On L, Flux E, van der Krogt MM, et al. Medial gastrocnemius muscle and tendon interaction during gait in typically developing children and children with cerebral palsy. In: Virtual meeting of the European Society of Movement Analysis in adults and Children 2020. 2020.
89. van Hooren B, Teratsias P, Hodson-Tole EF. Ultrasound imaging to assess skeletal muscle architecture during movements: A systematic review of methods, reliability, and challenges. *J Appl Physiol* 2020; 128: 978–99.
90. Mooijekind B, Flux E, Buizer AI, van der Krogt MM, Bar-On L. The influence of wearing an ultrasound device on gait in children with cerebral palsy and typically developing children. *Gait Posture* 2023; 101: 138–44.
91. Cenni F, Bar-On L, Monari D, et al. Semi-automatic methods for tracking the medial gastrocnemius muscle–tendon junction using ultrasound: a validation study. *Exp Physiol* 2020; 105: 120–31.
92. Cenni F, Schless SH, Adams H, Bar-On L, Desloovere K. The reliability of measuring medial gastrocnemius muscle-tendon unit lengths during gait. *Gait Posture* 2021; 90: 464–7.
93. Pitcher CA, Elliott CM, Panizzolo FA, Valentine JP, Stannage K, Reid SL. Ultrasound characterization of medial gastrocnemius tissue composition in children with spastic cerebral palsy. *Muscle Nerve* 2015; 52: 397–403.
94. Farris DJ, Lichtwark GA. UltraTrack: Software for semi-automated tracking of muscle fascicles in sequences of B-mode ultrasound images. *Comput Methods Programs Biomed* 2016; 128: 111–8.
95. Thompson AK, Chen XY, Wolpaw JR. Acquisition of a Simple Motor Skill: Task-Dependent Adaptation Plus Long-Term Change in the Human Soleus H-Reflex. *J Neurosci* 2009; 29. DOI:10.1523/JNEUROSCI.4326-08.2009.
96. Wolpaw JR, Braitman DJ, Seegal RF. Adaptive plasticity in primate spinal stretch reflex: initial development. *J Neurophysiol* 1983; 50: 1296–311.
97. Chen Y, Chen XY, Jakeman LB, Chen L, Stokes BT, Wolpaw JR. Operant Conditioning of H-Reflex Can Correct a Locomotor Abnormality after Spinal Cord Injury in Rats. *J Neurosci* 2006; 26: 12537–43.
98. Makihara Y, Segal RL, Wolpaw JR, Thompson AK. Operant conditioning of the soleus H-reflex does not

induce long-term changes in the gastrocnemius H-reflexes and does not disturb normal locomotion in humans. *J Neurophysiol* 2014; 112. http://jn.physiology.org/content/112/6/1439 (accessed 19 July 2017).
99. Thompson AK, Pomerantz FR, Wolpaw JR. Operant Conditioning of a Spinal Reflex Can Improve Locomotion after Spinal Cord Injury in Humans. *J Neurosci* 2013; 33: 2365–75.
100. Mrachacz-Kersting N, Kersting UG, de Brito Silva P, et al. Acquisition of a simple motor skill: task-dependent adaptation and long-term changes in the human soleus stretch reflex. *J Neurophysiol* 2019; 122: 435–46.
101. Flux E, Bar-On L, Buizer AI, Harlaar J, van der Krogt MM. Electromyographic biofeedback-driven gaming to alter calf muscle activation during gait in children with spastic cerebral palsy. *Gait Posture* 2023; 102: 10–7.
102. Nash J, Neilson PD, O'Dwyer NJ. Reduing spasticity to control muscle contracture of children with cerebral palsy. *Dev Med Child Neurol* 1989; 31: 471–80.
103. O'Dwyer N, Neilson P, Nash J. Reduction of spasticity in cerebral palsy using feedback of the tonic stretch reflex: a controlled study. *Dev Med Child Neurol* 1994; 35: 770–86.
104. Booth ATC, Buizer AI, Meyns P, Oude Lansink ILB, Steenbrink F, van der Krogt MM. The efficacy of functional gait training in children and young adults with cerebral palsy: a systematic review and meta-analysis. *Dev Med Child Neurol* 2018; 60: 866–83.
105. Sakzeweski L, Ziviani J, Boyd RN. Efficacy of Upper Limb Therapies for Unilateral Cerebral Palsy: A Meta-analysis. *Pediatrics* 2014; 133: 175–204.
106. Van Vulpen LF, De Groot S, Rameckers EAA, Becher JG, Dallmeijer AJ. Effectiveness of functional power training on walking ability in young children with cerebral palsy: study protocol of a double-baseline trial. *Pediatr Phys Ther* 2017; 29: 275–82.
107. Thompson AK, Wolpaw JR, Taylor J, Mrachacz-Kersting N, Thompson AK, Wolpaw JR. H-reflex conditioning during locomotion in people with spinal cord injury. *J Physiol C 2019 Authors J Physiol* 2021; 599: 2453–69.
108. Roebroeck ME, Harlaar J, Lankhorst GJ. The Application of Generalizability Theory to Reliability Assessment: An Illustration Using Isometric Force Measurements. *Phys Ther* 1993; 73: 386–95.
109. Habersack A, Zussner T, Thaller S, Tilp M, Svehlik M, Kruse A. Validity and reliability of a novel 3D ultrasound approach to assess static lengths and the lengthening behavior of the gastrocnemius medialis muscle and the Achilles tendon in vivo. *Knee Surgery, Sport Traumatol Arthrosc 2022 3012* 2022; 30: 4203–13.
110. Botter A, Beltrandi M, Cerone GL, Gazzoni M, Vieira TMM. Development and testing of acoustically-matched hydrogel-based electrodes for simultaneous EMG-ultrasound detection. *Med Eng Phys* 2019; 64: 74–9.
111. Lubbers M, Dedic A, Coenen A, et al. Calcium imaging and selective computed tomography angiography in comparison to functional testing for suspected coronary artery disease: the multicentre, randomized CRESCENT trial. *Eur Heart J* 2016; 37: 1232–43.
112. Mynark RG, Koceja DM. Down training of the elderly soleus H reflex with the use of a spinally induced balance perturbation. *J Appl Physiol* 2002; 93: 127–33.
113. Conner BC, Schwartz MH, Lerner ZF. Pilot evaluation of changes in motor control after wearable robotic resistance training in children with cerebral palsy. *J Biomech* 2021; 126: 110601.

Supplementary Materials 9.1: Instructions and protocol

Biofeedback was presented implicitly in a virtual reality gaming environment, in which children had to make a monkey jump from one branch to another. Children were instructed that they controlled the game with their most affected lower leg and that adapting their gait pattern would influence the monkey's behavior. Children were encouraged to explore different strategies but were instructed to keep walking straight and not jump. If gait deviations were too abnormal (e.g., hard kicking with the leg in the air), children were encouraged to return to their gait pattern and discover a new strategy.

Feedback was provided immediately during each step. In the early stance and combined feedback trials, if the early stance peak was sufficiently reduced, the path to the next branch opened up (figure 1D-E) allowing for a jump to the next branch (always in the early stance trial, and with sufficiently large push-off activity in the combined trial). Otherwise, the branch remained closed and, regardless of the size of the jump, the monkey would hit its head. The height of the push-off peak determined the size of the jump (figure 1D-F). So when the branch above the head was opened (always in push-off trials and in combined trials if early stance activity was sufficiently reduced), the monkey could reach a new branch with a sufficiently large push-off activity.

Early stance peak was calculated as the maximum activity during 15-50% of the stance phase for the first eight subjects and during 0-50% of the stance phase for the following subjects. This was altered during the experiment as peak activity occurred earlier than expected based on pilot data. Push-off activity was determined as the total muscle activity during 60-90% of the stance phase, calculated as the area under the curve. Stance phase of gait was defined from initial contact to push-off, according to Zeni[43].

The threshold for feedback was initially set to result in 67% positive feedback. This was done by calculating the average activity over ten strides and increasing (for early stance) and decreasing (for push-off) this by the standard deviation. If the gait pattern would remain stable, children would reach a new branch in 67% of the steps. The threshold could be adjusted during the experiment based on the success experience of the participants, as would be done in regular care. After five successful jumps, a super jump was awarded to increase motivation.

Supplementary Materials 9.2: Participant excluded

SP08, had a severe increase in early stance peak in the first biofeedback trials compared to the baseline trials, as is visualized in Fig. S9.1. This increase might be caused by the addition of dual-tasking, known to affect the quality of gait, especially in younger children with CP[1]. Fatigue could also increase the expression of stretch hyperreflexia and thereby explain the early stance peak, but is unlikely the cause in this subject, as the effect fades away after five biofeedback trials, so approximately after ten minutes of biofeedback. As the effects fade away within one session, it is not thought to obstruct long-term training. Moreover, this subject was able to show a significant increase in push-off activity in the last trial of push-off feedback. It is unknown if this large increase in early stance activity is caused by biofeedback or by walking in the gaming environment instead of the optic flow environment. Given the large increase in early stance activity and the absence of a baseline game trial, this subject was left out of statistical analyses in the article.

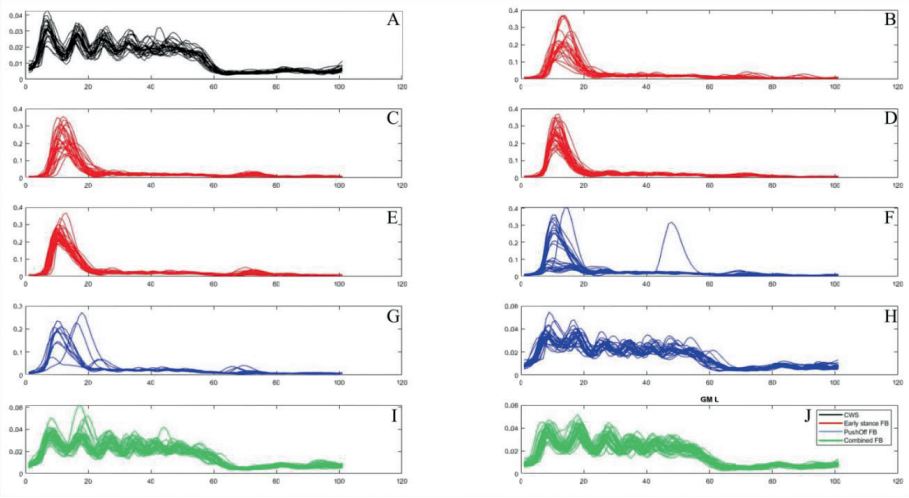
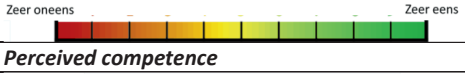


Figure S9.1. Calf muscle activity of a subject with a severe increase in early stance peak. The subject walked for approximately 15 minutes of feedback, with gait trials recorded approximately every two minutes. A) Baseline walking, B-E) walking with early stance biofeedback, F-H) walking with push-off biofeedback, I-J) walking with combined biofeedback. In B-G, an almost tenfold increase in early stance muscle activity is seen compared to A and H-J.

Supplementary Material 9.3

Intrinsic motivation questionnaire

The intrinsic motivation was assessed using a translated version of the Intrinsic Motivation Inventory (IMI). This is a flexible assessment tool in which you can include all dimensions that are applicable for a certain study. The English version of this measurement tool has been validated,[1] and is used in multiple studies analyzing intrinsic motivation.[2] A concise version from the Dutch version is used as is publically available online (https://drive.google.com/file/d/0Bw-gHVDUY3mseTFVb2Y4b0kzZmc/view), and has been used in previous studies in rehabilitation.[3] In this study, the second subscale of IMI, 'waargenomen competentie' (perceived competence) will be analyzed, since it highly influences intrinsic motivation of children over time.[4] Furthermore, we assessed the scale 'interesse en plezier' (interest-enjoyment), according to recommendations from previous research.[1] All questions were asked in random order. Values were transformed to make all positive answers high values and consequently averaged to obtain an overall score for each dimension.

Cluster 1: Interesse / Plezier	***Interest/Enjoyment***
Ik vond de opdracht erg leuk om te doen. *I enjoyed doing this activity very much.*	
Deze opdracht was erg leuk. *This activity was fun to do.*	
Ik zou deze opdracht omschrijven als erg interessant. *I would describe this activity as very interesting.*	
Deze activiteit hield mijn aandacht totaal niet vast. *This activity did not hold my attention at all.*	
Cluster 2: Waargenomen competentie	***Perceived competence***
Ik denk dat ik best wel goed ben in deze opdracht *I think I am pretty good at this activity.*	
Na het afronden van deze opdracht, voelde ik mij best wel competent *After working at this activity for a while, I felt pretty competent.*	
Ik ben best wel vaardig in het uitvoeren van deze taak *I was pretty skilled at this activity.*	
Dit was een opdracht die ik niet zo goed kon. *This was an activity that I couldn't do very well.*	

Dutch questions as asked to the participants are presented, as well as the English version of the corresponding questions.

References Supplementary Materials

1. McAuley E, Duncan T, Tammen V V. Psychometric Properties of the Intrinsic Motivation Inventory in a Competitive Sport Setting: A Confirmatory Factor Analysis. Taylor & Francis Group, 1989.
2. Ryan RM, Deci EL. Self-Determination Theory and the Facilitation of Intrinsic Motivation, Social Development, and Well-Being. Am Psychol 2000; 55: 68–78.
3. Jacobs A, Timmermans A, Michielsen M, Plaetse M Vander, Markopoulos P. CONTRAST: Gamification of Arm-Hand Training for Stroke Survivors. CHI'13 Conf preceedings 2013. https://uhdspace.uhasselt.be/dspace/bitstream/1942/16200/1/Jacobs et al 2013_CHI_Final.pdf.
4. Tsigilis N, Theodosiou A. Temporal stability of the intrinsic motivation inventory. Percept Mot Skills 2003; 97: 271–80.

Supplementary Materials 9.4: Statistical outcomes

Table S9.1.

Parameters and corresponding changes due to biofeedback

	Baseline		**FB_{early_stance}**			**$FB_{Push-off}$**			**$FB_{combined}$**		
	Values	p- ANOVA	Values	p-value	Change†	Values	p-value	Change†	Values	p-value	Change†$^{\$}$
EMG_{early_stance}	140.1±27.2	**0.038**	133.8±33.4	**0.025**	-6.8±12.2	132.2±24.5	0.170	-3.1±11.0	129.4±25.4	**0.055**	-6.5±13.9
$EMG_{push-off}$	145.8±28.7	0.106^{+}	143.0±25.3	0.227^{+}	-1.1±14.1	155.2±35.6	**0.039**$^{+}$	8.1±15.8	149.7±41.9	0.566	2.1±17.6
EMG_{DBI}	1.08±0.38	0.106^{+}	1.02±0.38	0.163^{+}	-5.2±13.2	0.98±0.34	0.102^{+}	-5.8±13.4	1.00±0.34	0.186	-5.1±16.0
$Work_{push-off}$	6.09±4.28*	0.580	6.54±4.52*	0.336	7.1±25.7	6.88±4.67*	0.185	12.3±33.5	6.31±4.44*	0.354	15.2±38.2
Ankle $Power_{peak}$	0.94±0.58*	0.454	0.97±0.55*	0.453	4.2±20.0	1.04±0.60	**0.037**	10.6±19.0	0.92±0.50	0.782	5.6±25.9
$Work_{early\_stance}$	-6.08±4.51*	0.308	-6.17±4.48*	0.831	-8.7±21.2	-6.58±4.94*	0.295	-6.1±24.1	-5.51±4.06*	0.592	-7.0±26.6
Ankle pfl	5.3±7.4°	0.226	6.5±9.1°	0.607	0.7±5.2°	6.0±8.4°	0.915	0.1±4.6°	7.6±8.4°	0.439	2.3±5.2°
Ankle dfl	12.2±5.7°	0.313^{+}	12.2±7.0°	0.193^{+}	0.2±3.8°	12.7±6.1°	0.906^{+}	0.3±3.8°	12.8±6.3°	0.978^{+}	0.6±3.2°
Knee extension	**10.5**±7.6°	**0.040**	9.4±8.6°	**0.030**	1.4±2.4°	8.7±8.5°	**0.009**	2.1±3.0°	9.8±9.0°	**0.032**	0.7±2.8°
GPS	8.38±2.14	0.410	8.64±2.08	0.319	3.3±10.1	8.49±1.89	0.686	2.5±10.8	8.74±2.35	0.185	4.6±11.3

The table depicts the different outcome parameters, with its mean values including standard deviations for the baseline trials and the three different biofeedback trials. P-values and the change as relative improvements to baseline, †expressed in percentages except for kinematics, when the difference is in degrees. *This is the amount of work during the push-off phase multiplied by 100. $^{\$}$Note that two subjects were excluded from this value due to missing values. The change is therefore relative to slightly different baseline values. $^{+}$Indicates non-parametric Friedmans test and Wilcoxon signed rank tests were used.

Table S9.2.

Comparison of parameters to typically developing values

Parameter	Baseline	FB_{early_stance}	$FB_{push\text{-}off}$	$FB_{combined}$
Early stance EMG activity	**<0.001**	**0.002**	**<0.001**	**<0.001**
Push-off EMG activity	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Double-bump-index	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Ankle work early stance	**0.047**	**0.045**	**0.034**	0.081
Ankle work push-off	0.108	0.200	0.292	0.158
Peak push-off power	**<0.001**	**<0.001**	**0.018**	**<0.001**
Dorsiflexion early stance	0.882	0.438	0.481	0.777
Plantarflexion push-off	0.900	0.118	0.518	0.647
Knee extension stance	**0.011**	**0.045**	0.064	0.053

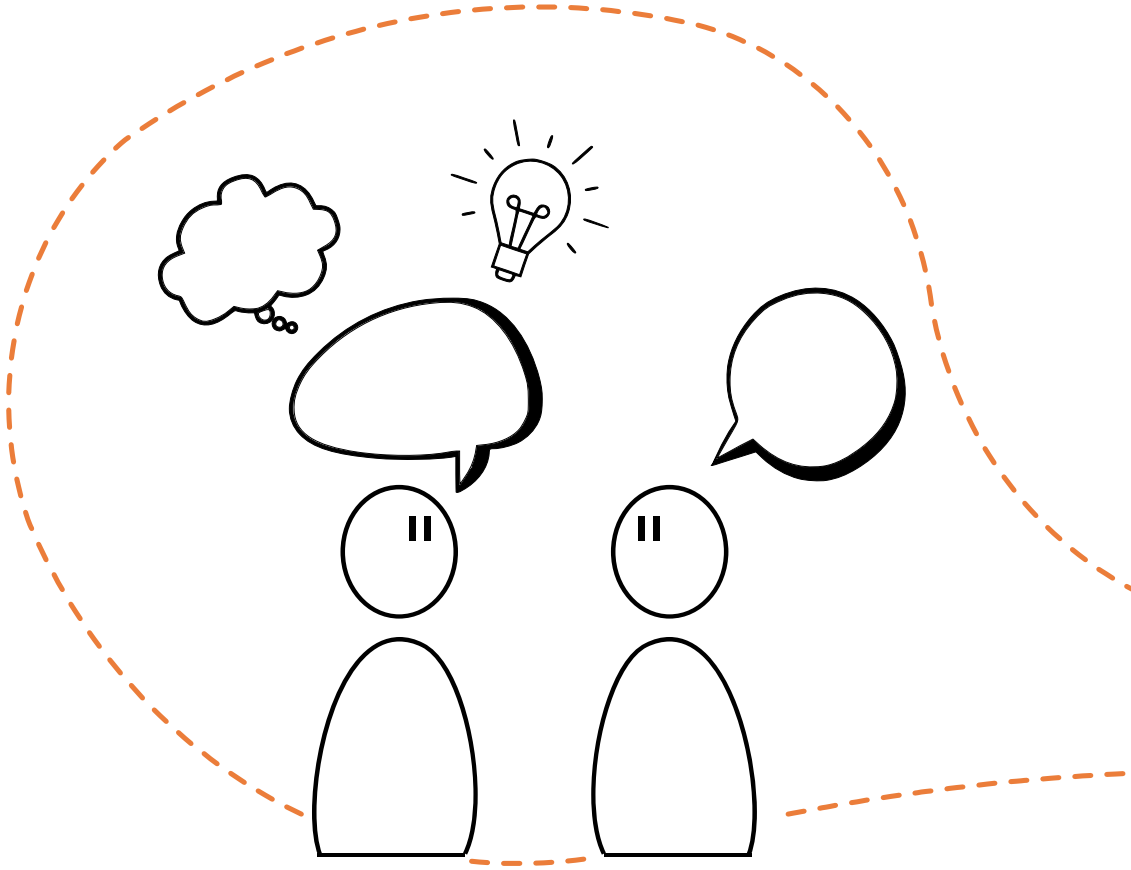
The table depicts p-values from the independent t-test between the reference values of the typically developing children and the children with cerebral palsy at baseline and after the three different feedback types.

Table S9.3.

Correlation values between baseline parameters and improvements

Parameter	Correlation	p-value
Baseline ES peak*	-0.198	0.172
Baseline PO peak*	**-0.088**	**0.549**
Baseline DBI	**-0.309**	**0.031**
Age	<0.000	0.995
Distribution	0.180	0.214
Most affected side	**-0.240**	**0.096**
SPAT	-0.216	0.137
GMFCS	-0.121	0.406
GPS	-0.019	0.899
Peak push-off power	**0.392**	**0.012**
Push-off power	**0.342**	**0.031**

*Pearson correlation for Baseline early stance, and push-off peak, DBI, age, and GPS; Spearman correlation for GMFCS level and spasticity scores (SPAT) and a partial eta squared for distribution and most affected side. *We assessed the correlation between baseline early stance and push-off peak and the relative improvements in early stance and push-off peak respectively.*



10

GENERAL DISCUSSION

10.1 Introduction

Increased joint resistance is a common phenomenon in children with spastic cerebral palsy (CP), limiting active and passive range of motion and, in ambulatory children, walking performance and independent function.[1] Joint hyper-resistance can be caused by intrinsic hyper-resistance, for example due to alterations in the muscle tissue properties, and reflexive hyper-resistance, also known as stretch hyperreflexia or spasticity. Stretch reflexes are influenced by multiple factors, such as baseline muscle activity and muscle length, making it difficult to quantify and to modulate their magnitude. Yet, unraveling the stretch hyperreflexia from intrinsic hyper-resistance remains essential for optimal intervention selection and evaluation.

Multiple diagnostic tools have been developed over the past decades to assess the magnitude of stretch hyperreflexia.[2] Diagnostic tools can be implemented in clinical decision-making when multiple levels of evidence are provided. Van der Velden et al.[2] proposed five levels of evidence required before implementing instrumented devices to measure stretch hyperreflexia. These levels were based on the four phases of diagnostic research as proposed by Sackett and Haynes.[3] Studies reporting test ranges and comparing patients with healthy controls can provide *Level I* evidence. *Level II* evidence consists of comparing heterogeneous patients, for example, by correlating test results with a reference test, analyzing the effect over time for example analyzing the effect of interventions. *Level III* analyzes if the diagnostic tool can distinguish patients with and without stretch hyperreflexia, as opposed to the group level comparisons performed in *level I* and *II*, and provides diagnostic accuracy of the assessments. In the final *level IV*, the diagnostic tool is implemented in clinical care to evaluate if the diagnostic tool improves health outcomes.[3] Furthermore, clinical feasibility is a prerequisite for all diagnostic tools and is considered *level zero* in this thesis.

This thesis aimed to investigate different methods to assess, contextualize and modulate the stretch reflex magnitude during both passive, controlled settings and active, functional settings. In this chapter, we discuss the different levels of evidence for diagnostic tools to assess stretch hyperreflexia used throughout this thesis, as summarized in Table 10.1. Furthermore, we discuss the potential for modulating reflex magnitude, clinical implications, and future directions.

In the first part of this thesis, we analyzed the clinical feasibility of methods to measure gait kinematics. Feasibility of these gait analysis methods is necessary for assessing and modulating stretch reflexes. In **chapter 2**, we found that the Human Body Model presents similar outcomes as conventional gait models for clinical gait analysis of children with CP, with maximum deviations for sagittal plane angles below the minimal clinically significant difference of five degrees. Furthermore, **in chapter 3**, we assessed the influence of wearing an ultrasound probe on the calf muscle on the gait pattern of children with CP and typically developing children. We found several minor differences, but the influence on sagittal plane kinematics remained below the five-degree difference. We concluded that the Human Body Model is suitable for assessing sagittal plane kinematics in real time and can be complemented with an ultrasound probe on the calf muscle.

In the second part, we assessed clinical feasibility and provided *level II* evidence for quantifying stretch hyperreflexia in a passive, controlled setting. **Chapter 4** compared the outcomes of two motorized assessments for adults with spinal cord injury and stroke. On group level, we found a moderate

correlation with clinical assessments (r=0.60 & 0.57) and a strong correlation (r=0.86) between the two motorized assessments, supporting the hypothesis that the motorized assessments capture similar concepts. However, as shown in **chapter 5**, the same two methods appeared uncorrelated in a population of children with spastic cerebral palsy (confidence interval of r=[-0.24, 0.80]). Nevertheless, the two methods did correlate with a third motorized assessment (r=0.84 & 0.70). Neither of the tests correlated with functional outcome measures nor with clinical assessments. Therefore, we concluded that passive and functional assessments can provide complementary information, and different methods to assess stretch hyperreflexia should not be used interchangeably.

In the third part of this thesis, we provide *level zero and I* evidence for assessing stretch hyperreflexia in the context of interest, in a dynamic, functional setting. In **chapter 6,** we demonstrated the applicability of treadmill perturbations to evoke stretch reflexes in children. Additionally, we showed that children with CP had, on average, 1.7 times larger responses to perturbations in the gastrocnemius medialis muscle compared to typically developing children. Furthermore, children with spastic CP showed increased muscle activation in late swing and early stance in response to stretch of different muscle-tendon structures during unperturbed gait, as described in **chapter 7**. The relation between muscle activation and stretch was up to 500% larger compared to typically developing children, depending on the phase of the gait cycle and the muscle-tendon structure assessed. Findings from the contextualized assessment in **chapter 6 and 7** support the hypothesis that stretch hyperreflexia is present in the gait pattern of children with CP.

In the fourth part of this thesis, we evaluated two different methods to modulate stretch reflex magnitude. First, we provided healthy adults with biofeedback on the stretch reflex magnitude in a passive, controlled setting in **chapter 8**. We found that most adults could achieve within-session decreases of 16-33% in stretch reflex magnitude with two out of three analyzed interventions. Secondly, in **chapter 9,** we assessed a method to provide biofeedback on the muscle activation pattern in a functional setting, i.e. during walking. This method appears promising, as most children with cerebral palsy could slightly adjust (5-10%) their muscle activation pattern during one session of biofeedback.

10.2 The assessment of stretch hyperreflexia in passive conditions

Within this thesis, we described multiple diagnostic tools to assess stretch hyperreflexia in a controlled setting, as well as in the context of an active, functional setting, i.e., during walking (see Fig. 10.1). We analyzed currently available clinical tests to assess stretch hyperreflexia by applying manual rotations around the passive ankle joint, and three passive, controlled assessments using a robotic device. The strengths of such motorized assessment methods are the highly reliable movement profile and their objective nature. The theoretical concept behind all these motorized, passive assessments is based on the definition of stretch hyperreflexia,[1] being a velocity-dependent increase of muscle activation in response to stretch. The theoretical concept is generally similar to currently used clinical tests such as the modified Tardieu and the spasticity assessment (SPAT), consisting of slow and fast rotations around

a joint to assess the increased velocity-dependent resistance. Therefore, the different clinical and motorized passive assessments were expected to present similar results.

The clinical tests have strong clinical feasibility, but *level II* evidence is lacking, as clinical scores did not correlate well with motorized and dynamic, i.e. contextualized assessments.[4,5] This is conform previous studies.[6–8] The modified Ashworth scale, in particular, neither fulfills *level I*, as it was unable to differentiate neural and non-neural causes of joint resistance,[6,8] and therefore does not provide test ranges for the level of stretch hyperreflexia, nor fulfills *level II*, as we found no correlation with motorized assessments.[4] Therefore, all available evidence advices against the use of the modified Ashworth scale in clinical decision-making. Manual assessments that include slow and fast velocities, such as the Tardieu scale and SPAT, were shown to differentiate better between patients with and without stretch hyperreflexia,[6,8] therefore, providing some *level II* evidence. The increased distinctive properties are probably due to the distinction between intrinsic resistance (mainly expressed during slow velocities) and reflexive resistance (mainly expressed during high velocities). Nevertheless, these tests were not, or only moderately, correlated with the severity of stretch hyperreflexia as assessed more quantitatively.[4–6,8] Moreover, the reliability of manual tests has repeatedly been shown to be poor.[9–13] Finally, *level III and IV* studies of the clinical tests are lacking, resulting in insufficient evidence for using these diagnostic tools in clinical decision-making. These results indicate that multi-level scoring of clinical scales should be discouraged in clinical decision-making. The different scores are already disregarded in the Netherlands CP register, a national registry to monitor patient progress and evaluate treatments.[14] Manual assessments are used as input for the register, but only rated as zero or one, indicating the absence or presence of stretch hyperreflexia. Therefore, we conclude that dichotomous scores of manual assessments might be as meaningful as it gets.

Table 10.1

Summary of levels of evidence for diagnostic tools analyzed in this study.

	Level zero	Level I	Level II	Level III & IV
Ashworth	*Very strong (Ch 4)*	*Cannot distinguish reflexive from intrinsic stiffness*	*Not correlated to motorized assessments (Ch 4)*	
SPAT & Tardieu	*Very strong (Ch 2, 5, 6)*	*Can differentiate between patients with & without stretch hyperreflexia*	*Not or poorly correlated with severity of stretch hyperreflexia (Ch 4, 5, §10.3)*	*Do not use in current form. Explore diagnostic values of dichotomous values*
Motorized SPAT	*Good for SCI/Stroke (Ch 4) Not for CP (Ch 5)*		*Strong correlation with severity of stretch hyperreflexia according to some (Ch 4, 5), but not all assessments (Ch 5).*	*Do not use in current form for CP, but explore instrumented SPAT. Complement with contextualized assessments*
Instrumented SPAT	According to previous studies	*According to previous studies*	*Supported by some, but not all previous studies*	
Eight-degree stretch reflex	*Ok for patients, but several device-limitations and challenges to select starting angles, especially in CP (Ch 5, 8)*		*Strong correlation with severity of stretch hyperreflexia (Ch 5)*	*Advise the instrumented SPAT for assessment in children with CP. Can be used for training purposes (Ch 8)*
PC-SI	*Similar to eight-degree stretch reflex. (Ch 4, 5, 8)*		*Strong correlation with eight-degree stretch reflex (Ch 4, 5), but no correlation with motorized SPAT (Ch 5).*	*Does not provide information over entire range of motion for children with CP (Ch 5). Do not use for training purposes (Ch 8).*
Functional: perturbations	*Can be performed without interrupting the gait pattern of children with CP (Ch 6). High technological requirements.*	*Can differentiate between CP and TD (Ch 6)*	*Not correlated to SPAT (§10.3) but children with SDR showed lowest values. Explore correlation with other tests.*	
Functional with US	*Can be performed without interupting the gait pattern of children with CP (Ch 3). High processing time should be solved.*	*Can differentiate between CP and TD on group level (Ch 7)*	*Not correlated to passive measures of stretch hyperreflexia (Ch 5). Explore correlation with other functional tests.*	*Explore added value of assessment on clinical decision making*
Loading response peak	*Very strong*	*Can differentiate between CP and TD on group level (Ch 7)*	*Presence of the peak is correlated with the SPAT (Ch 7). More research necessary.*	*Might be of value to complement clinical decision making.*

indicates positive findings, indicates negative findings, indicates conflicting or moderate findings. The magnifier glasses indicate the advice to continue or discontinue research using these methods.

Level zero indicates clinical feasibility, level I consists of comparisons between patients and healthy controls on group level, level II evidence includes correlations with gold standards and analyzing the effect of interventions, level III regards evidence if the diagnostic tool can distinguish patients with and without stretch hyperreflexia, and level IV consists of evaluating a diagnostic tool's added value for clinical decision-making. Note: the Instrumented SPAT was not assessed in this thesis but included in this table, as results from this thesis warrant further investigation of this method. Empty columns indicate that this level of evidence is not discussed within this thesis.

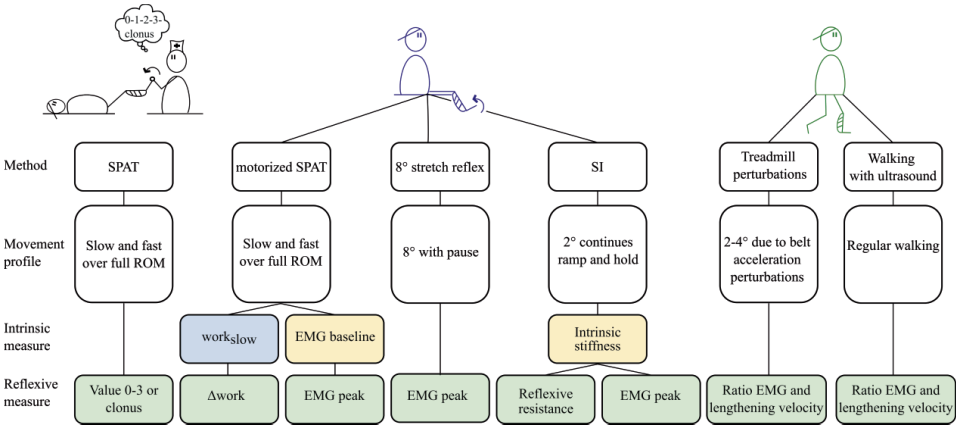


Figure 10.1. Overview of stretch hyperreflexia assessment methods used in this thesis. The clinical test most commonly used in this thesis is the manual spasticity test (SPAT).[15] The motorized SPAT, eight-degree stretch reflex, and a parallel-cascade system identification method (SI) are three passive, motorized assessment methods performed on an instrumented ankle perturbator. In addition, two functional methods included the analysis of muscle activation patterns while walking on an instrumented treadmill.

The motorized assessments described in this thesis include three methods, 1) a motorized version of the SPAT, in which slow and fast bell-shaped rotations are applied over the full range of motion of the ankle joint, 2) a short stretch of eight degrees amplitude (eight-degree stretch reflex) and 3) very short stretches of approximately two degrees ramp-and-hold perturbations, which are analyzed using a parallel-cascade system-identification (SI) technique. All proposed methods have their strengths. For example, the motorized SPAT is designed to assess reflexive resistance over the entire range of motion, whereas the eight-degree stretch reflex and SI are restricted to a small portion of the range of motion. The SI, in turn, is designed to provide real-time information on the stretch reflex size, which makes it attractive for use in biofeedback,[16] as is the eight-degree stretch reflex.[17,18] The eight-degree stretch reflex has a slightly larger movement profile, therefore providing information over a large area of the range of motion, but does not distinguish different contributes of resistance, as is done with the SI method.

Multiple studies have been performed on the SI method, providing *level I* evidence for distinguishing between patients and healthy controls.[19,20] Furthermore, the SI appears capable of distinguishing reflexive from intrinsic resistance.[21] We found a strong correlation between the SI and eight-degree stretch reflex,[4,5] supporting the *level II* evidence for this diagnostic tool. A disadvantage of the SI and potentially also the eight-degree stretch reflex is the high sensitivity to the selected initial angle due to the small range of motion of the perturbations,[5,19,22] probably resulting in the low correlation with other measures. Therefore, these methods do not appear to provide sufficient information regarding reflex magnitude over the entire functional range of motion.

Generally, the motorization of assessments allows for a controlled, precise, and highly repeatable movement profile. However, there appears to be a trade-off with clinical feasibility when using this method for children with CP. For example, the robotic device can evoke anxiety in some children. We

hypothesized that anxiety affects the measured outcomes, which hinders clinical application.[5] This hypothesis is supported in previous work on robotic devices in children with CP.[23] This especially holds for perturbations over a large range of motion, such as the motorized SPAT, but can also impact perturbations over a small range of motion, such as the SI. In addition, other population-specific limitations, such as bony deformities, hinder accurate assessment in children with CP. The eight-degree stretch reflex method can be used in biofeedback applications,[17] as further discussed in paragraph 10.4, and shows some potential for use in treatment evaluation in research settings. However, the limited information regarding the entire range of motion should be considered. We conclude that the clinical feasibility of the motorized assessments described in this thesis is low for use in diagnostics. Likewise, Graser et al.[23] question the clinical feasibility of motorized assessments in diagnostics, as they are relatively time-consuming and complex.

Several previous studies have assessed various instrumented versions of the SPAT, involving impedance-based or EMG-based assessment of the resistance to manual rotations around the joint.[24–27] The reduced anxiety levels and consideration of bony deformities will probably counteract the reduced movement repeatability due to the manual opposed to motorized rotations, specifically when movement profiles are standardized.[28] Moreover, the instrumented assessment will be easier to implement in clinical care, given the portability of an instrumented manual assessment device. Considering the clinical feasibility, further study better focus on validating instrumented assessments, such as the instrumented SPAT[29] or portable spasticity assessment device (PSAD),[26,30] as a diagnostic tool.

10.3 Contextualization of stretch hyperreflexia

This thesis centers on the idea that children with CP have deviating gait patterns due to stretch reflexes. For example, a gait pattern with toe-landing might be caused by excessive plantarflexion due to calf muscle stretch reflexes during the late swing phase of gait. This is supported by previous studies,[31–33] but also debated by several researchers.[34,35] From h-reflex studies, we know that passive reflexes are increased, but modulation between and within activities is also impaired in children with CP.[36] Therefore, contextualizing the assessment methods to the functional task of interest will probably improve the diagnostic tool and our insights into the role of stretch hyperreflexia on the gait pattern of children with CP.

Treadmill perturbations are a potential tool to assess stretch reflexes in the calf muscles during the stance phase of gait.[37] We were the first to demonstrate the clinical feasibility of this method to evoke stretch reflexes during gait in typically developing children and children with CP.[38] We found that children with CP generally have increased responses to perturbations compared to typically developing children, and therefore concluded that the sensitivity of the stretch reflex loop is generally increased during gait. Additionally, our findings suggest that children have decreased responses to treadmill perturbations after SDR surgery, which should be a topic of future studies to assess *level III* evidence.

Furthermore, we described a functional assessment method to analyze the presence of stretch reflexes during unperturbed gait.[39] We compared the muscle activation with the lengthening velocity and acceleration of the musculotendon unit during unperturbed walking. We found increased muscle-activation-stretch ratios in children with spastic cerebral palsy compared to typically developing

children. This complies with the hypothesis that children with CP experience stretch hyperreflexia during comfortable walking. The hypothesis is further supported as we showed that increased muscle activation is commonly preceded by stretch on the musculotendon unit in children with CP. We were the first to complement a functional assessment of stretch hyperreflexia during walking with dynamic ultrasound imaging, which can be used to assess stretch of the muscle belly, fascicles, and tendon separately.[40–44] The dynamic imaging revealed that not only stretch of the fascicles, but also stretch of the tendon might be responsible for the stretch reflexes in children with CP. Therefore, ultrasound imaging can add value to diagnostic tools to assess stretch hyperreflexia.

Additional evidence must be provided before these two functional assessments are clinically implemented as diagnostic tools. We provided level I evidence as both tests could distinguish patients from controls on group level. However, both functional measures showed high variability between children with CP, and several children diagnosed with spastic CP had lower stretch reflexes than typically developing children, according to the functional measures. Additionally, we did not find level II evidence, as the stretch hyperreflexia measures during unperturbed walking were uncorrelated to the clinically used SPAT and passive, motorized assessments of stretch hyperreflexia.[5] Unpublished analysis showed similar findings for the correlation between the response to treadmill perturbations and the SPAT in children with CP. The lack of correlation can be due to either the limitations of the SPAT and the motorized assessments, as described in paragraph 10.2, or the high number of parameters that can influence the magnitude of stretch reflexes, as presented in the box "stretch reflex magnitudes", including body posture and muscle activation. However, it can also be caused by the fact that not the height of the passive values is most important, but more so the decreased ability to modulate stretch reflexes between activities. In other words: children with high stretch reflexes during functional tasks might have low stretch reflexes in passive, controlled conditions, but lack the ability to modulate reflex activity between different conditions. These results therefore further emphasize the importance of contextualizing assessments.

Of the two proposed contextualized assessments, the treadmill perturbations will likely be more robust to capture the sensitivity of the stretch reflex loop, compared to the analysis of unperturbed comfortable walking. Multiple parameters can differ between strides, resulting in amongst others changes in joint angles, muscle-tendon lengths, and muscle activation levels. Reduced levels of muscle-tendon stretch can result in the absence of stretch reflexes in some steps, as is also shown by the uncertainty in the qualitative assessments.[39] The response to treadmill perturbations appeared more robust, as muscle-tendon stretch is caused by external perturbations. Furthermore, we found no differences between the perturbations during two separate walking trials, supporting the repeatability of this method. We encourage development of this assessment towards a clinically feasible assessment method, to be able to assess level II and III evidence to aid clinical decision-making.

The treadmill perturbation protocol can be complemented with dynamic ultrasound imaging. However, the analysis of dynamic ultrasound data presents with several challenges. First, the probe must be adequately aligned to the muscle for accurate measurements.[45] We used a probe holder (Probefix Dynamic T, USONO, The Netherlands) with multiple degrees of movement to facilitate correct alignment and fixation of the probe. We showed that adding a probe with such a probe holder does not largely interfere with the gait pattern of typically developing children and children with cerebral palsy.[46] Additionally, the probe holder allows for accurate placement of the probe, supporting

the feasibility of dynamic ultrasound imaging. A second challenge is the narrow field of view. Even with a long probe of 59 mm as used in our studies,[39,46] the fascicle length exceeded the width of the image in some taller children. This obstructed tracking of the entire fascicle length. We solved this by estimating the fascicle length based on the pennation angle and muscle width. In an unpublished pilot experiment, we assessed the reliability of this method by comparing estimated fascicle length with measured fascicle length. For this experiment, we included five typically developing children with sufficiently short fascicles, allowing us to capture fascicle length throughout. There was a good agreement between the methods (Spearman's rho=0.981). Furthermore, we did not find significant differences between estimated and measured average fascicle length, range of fascicle length, as assessed using a paired-samples t-test, and between fascicle lengthening profiles as assessed with statistical parametric mapping. Given the high correlations, we do not expect this to influence the results found in our study. However, it should be assessed in future studies whether this method can be used for adults, who generally have longer fascicles, and for other muscles with less pennate muscles and/or longer fascicles. A remaining challenge of dynamic ultrasound imaging is the high processing time. Algorithms exist for semi-automatic assessment of the MTJ displacement with generally good reliability.[47] However, fascicles tracking is more difficult[48] and automatic tracking of fascicle length is further complicated due to out-of-image movements and high echo intensity, which is generally increased in children with CP.[49] We were therefore unable to use existing semi-automatic fascicle tracking algorithms,[50] but instead performed manual tracking of fascicle length for all ultrasound data used in this thesis. Despite these difficulties, we found a high interrater agreement (intraclass correlation coefficient of 0.992) of the manual tracking of the fascicle length in a pilot study, similar to values reported in literature.[45] It can be concluded that dynamic ultrasound can be used to image fascicle, belly, and tendon stretch in children with CP, but reducing processing time will benefit clinical implementation.

Additionally to passive and contextualized assessments, we introduce the loading response peak as a potential diagnostic tool for the presence of stretch hyperreflexia. The loading response peak describes a sharp, sudden increase in EMG activity during the loading response, visible in the raw EMG data.[39] No previous studies have reported the use of this phenomenon in diagnostics. In our hospital, presence of the loading response peak is clinically considered a characteristic of stretch hyperreflexia during gait. This is supported by *level I* evidence from this thesis, as none of the typically developing children showed the loading response peak, as opposed to twelve out of seventeen children with CP.[39] Additionally, we showed that the loading response peak is commonly preceded by muscle and/or tendon stretch. This supports the hypothesis that stretch hyperreflexia can cause deviating muscle activation patterns in children with CP. Furthermore, children with CP classified with a loading response peak had, on average, higher SPAT values than children with CP without this peak, supporting *level II* evidence. However, this should be confirmed using different assessments of stretch hyperreflexia, given the limitations of the SPAT, as discussed in paragraph 10.2. A downside of the loading response peak detection is the low sensitivity, as it can only differentiate between presence or absence of the peak, resulting in limited applicability for evaluation of treatment efficacy. Treadmill perturbations can provide this higher sensitivity, but have a lower clinical feasibility due to the high technological requirements of the treadmill. The clinical feasibility of the loading response peak method is generally high, as it only requires visualization of the raw EMG signal for the specific muscle of interest, complemented with spatiotemporal information of initial contacts. The functional assessment used for treatment evaluation can depend on the application. However, given our findings

and our hospital's clinical experience, we advise exploring the added value of the loading response peak detection for clinical decision-making.

10.4 Modulation of stretch hyperreflexia

All work presented in this thesis indicated that stretch reflexes are generally increased in children with CP. Specifically, the contextualized assessment of stretch hyperreflexia during unperturbed gait showed that this likely influences the muscle activation pattern during gait in the late swing and early stance phase of gait.[39] This encourages us to develop methods to decrease stretch hyperreflexia and improve walking.

A promising method is through operant conditioning using biofeedback on reflex magnitudes.[18,51–54] Thompson et al.[51] showed that healthy adults could decrease their reflexes to 84% of baseline on average. Similarly, adults with spinal cord injury could modulate their stretch hyper-reflexes to around 76% of baseline, which led to improvements in the gait pattern, e.g. increased walking speed of 123%, improved gait symmetry, and improved modulation of h-reflex sensitivity throughout the gait cycle.[55] Although promising, a major downside of operant conditioning methods is their time-intensive nature. Therefore, we studied all prerequisites for applying conditioning methods before evaluating efficacy for children with spastic CP.

First, we assessed if reflex biofeedback can be performed in a child-friendly way.[17] Thompson et al.[51] use the h-reflex, the electrical analog of the stretch reflex, as biofeedback. Although not commonly reported in publications, h-reflexes are known to cause vasovagal syncope (e.g. feeling unwell or fainting) in some people, which decreases the clinical applicability of this method for children. This is also shown by the two drop-outs in our study using h-reflexes in a population of healthy adults.[17] Furthermore, translation toward physiotherapy-, school- or home-based settings is complicated when requiring h-reflexes, which is especially important for compliance in children. Therefore, we explored the possibilities of substituting h-reflexes with stretch reflexes and showed that healthy adults could achieve short-term decreases in their stretch reflexes.[17] Similarly, Mrachacz-Kersting[56] showed long-term decreases in stretch reflexes of healthy adults. A positive side-effect is that the result of stretch-reflex biofeedback appears faster compared to h-reflex biofeedback.[17,51,56] This might be caused by the involvement of gamma motor neurons in the stretch-reflex loop (see box "stretch reflex neural pathways"), expanding the neurophysiological modulation possibilities. We also tried decreasing response time by substituting EMG biofeedback with impedance-based biofeedback. We used the parallel-cascade system identification method described in paragraph 10.2 for this. Faster results were expected based on literature,[16] but unfortunately, we did not find any decreases in stretch reflexes, let alone a faster effect. Therefore, we advised against the impedance-based biofeedback. Regardless of the biofeedback method, long-term changes in stretch reflexes will probably require several sessions due to the slow plasticity of the spinal cord. Therefore, we explored the gamification of biofeedback. We showed that adults could still comply with the EMG biofeedback when presented in a gaming environment and experienced high motivation levels when playing the game for twelve sessions.[17] Thus, game-based EMG-driven biofeedback shows potential for children.

As a third step, we assessed the clinical feasibility of two biofeedback methods to improve the pathological gait pattern of children with CP. The first method consists of biofeedback on stretch

reflexes in a passive, controlled setting, and the second on the deviating muscle activation pattern during gait. The biofeedback of the second method is contextualized as it directly targets muscle activation during a functional task. Twelve children with spastic CP participated in an unpublished pilot study to assess the feasibility of the passive, controlled conditioning method, which is generally similar to our work in healthy controls.[17] Stretch reflexes were evoked in the calf muscle using the eight-degree stretch reflex, as presented in our previous work.[5] The resulting muscle activation was used as input for the biofeedback and was provided through a gaming environment similar to our pilot study in healthy adults.[17] We did not assess whether children could modulate their passive stretch reflexes, as improvements were not expected within one session.[17] However, results from an intrinsic motivation inventory (IMI[57]) showed that the children with CP felt very competent (8.3, range 6-10) and liked it a lot (8.7, range 6.3-10) while playing the game. The high motivation levels at the onset of training are important for treatment compliance.[58] Future studies can explore whether long-term improvements can be achieved using the passive, controlled conditioning method. We additionally explored the feasibility of the contextualized feedback paradigm.[59] Faster results were expected compared to controlled, passive conditioning due to the functional task. Results suggested that children with cerebral palsy can achieve small within-session improvements in their calf muscle activation patterns when provided with EMG-driven biofeedback during walking. Furthermore, children experienced high levels of competence (7.9, range 6-10 with one outlier of 3.3) and enjoyment (8.4, range 6.2-10). These results add to the evidence that children with CP can comply with biofeedback.[60–65] The actual effect of long-term training still needs to be studied, like in many biofeedback studies.[66] However, we studied several prerequisites, and from all results presented in this thesis, we conclude that, from the patho- and neurophysiology, there is no reason to believe that it is not possible to decrease stretch hyperreflexia in children with CP. In other words: We have never tried it before, but we think we should definitely be able to do it. Therefore we advise incorporating biofeedback aimed at reducing stretch hyperreflexia in follow-up long-term training studies.

Clinical feasibility likely differs between the passive and functional biofeedback paradigms. Within this thesis, we noted that children experienced similar levels of competence ($p=0.79$, CI=[-1.42, 1.10]) and enjoyment ($p=0.54$, CI=[-1.41, 0.76]) during the two different feedback paradigms. However, functional training is generally accepted to outperform passive or static training in rehabilitation.[66,67] Training efficacy increases with easily identifiable goals.[68] This will probably be difficult for the implicit biofeedback on the passive stretch reflexes. Furthermore, the many feedback sessions required before functional improvements further limit clinical feasibility. A solution can be to perform the EMG biofeedback alongside traditional therapies such as functional power training.[69] This way, children will likely already experience short-term success, albeit due to the traditional therapies, and therefore show better compliance. On the other hand, biofeedback during walking already showed within-session improvements in the first session in 12 out of 18 children.[59] Although the EMG biofeedback during walking less specifically targets stretch hyperreflexia, it is probably more effective. This statement is supported by findings from Thompson et al.[70], who showed that h-reflexes decreased faster when biofeedback was presented during walking instead of a controlled, seated position in patients with SCI.[70] Overall, biofeedback studies during walking appear most promising for ambulatory children with CP. Nevertheless, biofeedback during static training could be helpful in non-ambulatory children and adults.

A final advantage of EMG biofeedback during gait is that the specific biofeedback design can be adjusted to the patient's need. For example, we showed that some children with CP experience stretch reflexes during late swing, whereas others experience it during the early stance phase of gait.[39] The advantage of the EMG-biofeedback method is that it can target multiple phases of gait. Likewise, the EMG-biofeedback during gait can be easily translated towards other muscles, therefore showing high clinical feasibility.

10.5 Clinical implication

Most studies performed within this thesis lay a foundation for further research. However, several conclusions can be drawn regarding clinical implications. First, sagittal plane kinematics can be analyzed using the human body model for most clinical and research purposes. However, given the minor differences between kinematic models, it remains important to standardize the method for clinical gait analysis with pre-post evaluation.

Results from this thesis add to the available evidence that manual clinical stretch hyperreflexia tests have poor validity. Therefore, a recommendation can be to disregard the current clinical scales and instead constrain manual clinical tests of stretch hyperreflexia to indicate the presence of stretch hyperreflexia, i.e. classifying patients as with or without stretch hyperreflexia. Furthermore, results from this thesis do not support the use of motorized assessments for stretch hyperreflexia in children with CP, but instead support the recommendation to explore the added value of instrumentation of stretch hyperreflexia methods, for example the instrumented SPAT or portable spasticity assessment device.

It remains questionable whether passive assessments of stretch hyperreflexia indeed indicate the presence of stretch hyperreflexia during gait. However, treadmill perturbations show the potential to quantify stretch hyperreflexia directly during gait for children with CP. It should be kept in mind that multiple pathways might trigger stretch reflexes during gait. Additionally, a sharp peak during the loading response appears to coincide with stretch hyperreflexia during gait of children with CP, and may be a good addition to diagnostic evaluations. Finally, the identified relation between stretch and increased muscle activation in children with CP supports prescribing treatments to reduce stretch reflex hyperactivity that may interfere with gait.

Children with CP appear to comply with biofeedback on muscle activation, which may be of value in pediatric rehabilitation. Moreover, children showed high levels of intrinsic motivation and perceived competence while playing biofeedback-driven games. Therefore, gamification of biofeedback is recommended in pediatric rehabilitation.

10.6 Further directions

We discourage using motorized assessments as a diagnostic tool in children with CP, but instead encourage research with instrumented manual assessments. Specifically, repeatability and sensitivity should be assessed, for example through a generalizability study.[71] This is specifically important as the magnitude of reflexes is highly dependent on multiple factors (see box "stretch reflex magnitude"), and even motorized assessments, which provide a very controlled measurement, show highly varying repeatability.[23] Thus, assessing whether between-subject differences exceed within-subject

differences over different sessions is essential. Thereafter, studies should focus on providing level III and IV evidence for the use of such diagnostic tools in clinical decision-making.

Treadmill perturbations appear promising for functional assessment of stretch hyperreflexia in children with CP. The analysis might be complemented with dynamic ultrasound imaging. However, measurement errors of dynamic ultrasound are relatively large[72], and the feasibility of the imaging should therefore be assessed. Moreover, ultrasound imaging adds another layer of complexity to measurements, which might interfere with clinical implementation. Hence, the potential added value should be evaluated clearly. When using dynamic ultrasound imaging, (semi-) automatic methods for fascicle tracking should be improved to decrease processing time. We furthermore advise using ultrasound-compatible transparent EMG electrodes[73] in future studies. Future studies can also explore the potential of individualized models based on static ultrasound imaging,[74] to model the fascicle and tendon lengthening during gait.

We encourage the exploration of the diagnostic value of the presence of the loading response peak in the gait pattern of children with spastic CP. As a first step, the presence of the loading response peak can be identified when clinical gait analysis, including EMG, is performed for children with CP. This can be added to clinical databases such as the Netherlands CP register.[14] Comparison with other assessment methods to identify the presence of stretch hyperreflexia, such as dichotomous values of the SPAT, can be compared with the presence of the loading response peak. Subsequently, a *level IV* study, like a prospective diagnostic randomized clinical trial, can be performed.[75] In such studies, patients are randomized to the addition of the presence of the loading response peak in diagnostics, and clinical outcomes are evaluated. These study designs have the advantage of omitting the need for a gold standard.

Results from this thesis are encouraging to support further studies on biofeedback-driven gaming in children with CP, for example providing feedback on the muscle activation pattern[59]. Future studies should assess whether long-term improvements can be achieved. It is best to develop hardware and improve the software to perform training at home or school to optimize compliance. To further maximize compliance, we advise including a user group with patients, caregivers, and physiotherapists in developing such training. It is furthermore essential to assess functional improvements of such training studies. Moreover, assessment of the effect of biofeedback on stretch reflex magnitudes, for example through treadmill perturbations, can provide insight into the working mechanism of biofeedback.

This thesis focuses on the ankle joint. Translation towards other joints is not warranted, but several possibilities exist for most applications. For example, the treadmill perturbations can be translated from the ankle to the knee joint by applying treadmill accelerations during the late stance phase. Furthermore, EMG-driven biofeedback might be given on multiple muscles, for example on activation of the hamstring muscles in the late swing phase of gait.

Future studies should assess if findings from this thesis can be extrapolated to the general population of children with CP. Only children with GMFCS levels I and II were included in this study. Furthermore, included children had to be able to follow instructions, and included children might not represent the

entire population. It should be assessed if results can be extrapolated, especially for the feasibility of biofeedback.

We furthermore propose a different methodology that might be worthwhile to investigate, which first requires some introduction. The effect of functional training likely increases with the urgency of a patient to adjust specific muscle activation patterns. Mynark et al. (2002) conducted an experiment on elderly in which significant h-reflexes resulted in activation of the plantar flexors and caused balance perturbations. All participants could decrease the balance perturbations by decreasing their h-reflexes by around 20% within one session. This is opposed to results found by Thompson et al. (2013), who only found results in down-conditioning h-reflexes after 6 sessions. In the latter study, the down-conditioning served no direct function in the training protocol. Therefore, the inclusion of urgency, through easily identifiable functional advantages from training, seems meaningful. Additionally, one of the recommendations from this thesis is to contextualize stretch reflex assessments, as children with CP have difficulties in the modulation of stretch reflexes between and within different tasks. Likewise, adults with SCI have shown to modulate their reflexes faster when the biofeedback was performed during walking, as opposed to a controlled, standing position.[55,70] Therefore, future studies should focus on developing training methods using the easily identifiable functional advantages, as in the method from Mynark et al,[76] in a context of interest for the patients. Recently, Conner et al.[62] developed a training method complying with these requirements. They developed an actuated ankle orthosis that applies adaptive increased resistance to plantarflexion movement. Young adults with CP were encouraged to increase their push-off activity to overcome this increased resistance. Large increases in calf muscle activation during push-off were observed already within one session.[62,77] This method might indirectly decrease stretch reflexes during the late swing and early stance phase, to allow for the increased push-off activity. Targeting the stretch reflex activation directly may further improve this method. Given the promising results from this study, we propose a novel methodology, combining all recommendations from this thesis.

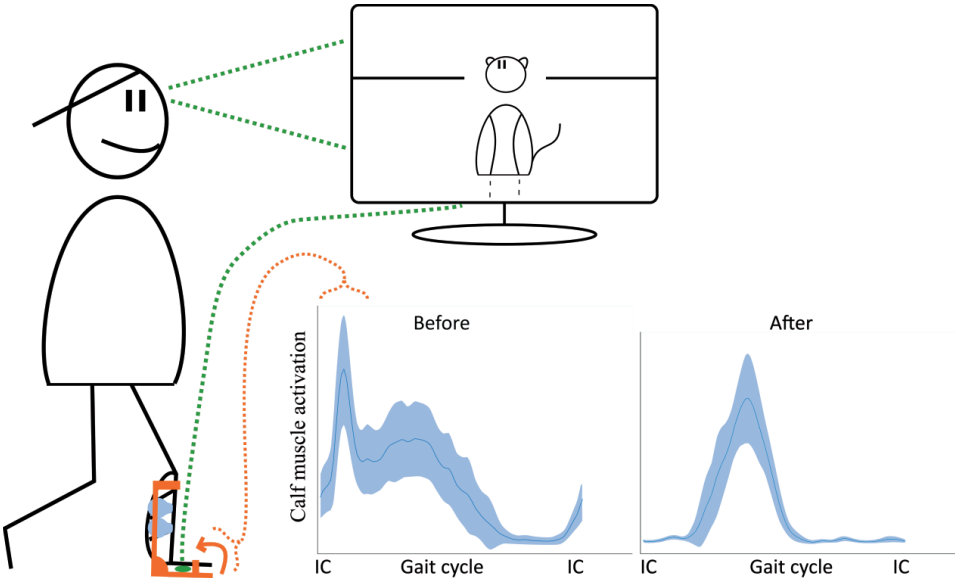


Figure 10.2. Refluxioning: A reflex reduction training, using an actuated ankle orthosis to provide biofeedback on the magnitude of stretch reflex activity. The amount of calf muscle activation – as measured using EMG - during late swing or early stance guides the amount of resistance towards plantarflexion by the actuated ankle orthotic during push-off: i.e. larger reflex activity during early stance limits the plantarflexion movement during push-off. The increased resistance limits propulsion, therefore providing an urgency to improve the gait pattern. Furthermore, the amount of plantar pressure is used for biofeedback-driven gaming, to improve the push-off and therefore the propulsion. A hypothetical EMG pattern of the m. gastrocnemius medialis is depicted before and after biofeedback training.

The reflex reduction training, in short "Refluxioning", is a contextualized training method aimed at improving the gait pattern of children with CP by reducing stretch reflex activity in late swing and early stance and increasing muscle activation around push-off (Fig. 10.2). The goal is therefore similar to the EMG biofeedback-driven gaming presented in this thesis.[59] However, Refluxioning includes biofeedback presented with urgency: reflexive activity of the calf muscles triggers the resistance applied by an actuated ankle orthosis, i.e. the higher the reflex activity in late swing/early stance, the more resistance is applied by the orthosis during the push-off. This way, the increased resistance exaggerates the "error", complicating the generation of propulsion. To keep walking, children will be forced to decrease their stretch reflexes. This training can be performed alongside the methodology proposed by Conner et al.[62,77] to increase the calf muscle activation during push-off, by providing biofeedback on the plantar pressure. In addition, gamification can be added to this protocol to increase intrinsic motivation. We challenge researchers to assess these possibilities and take the next step in treating stretch hyperreflexia for children with CP.

10.7 General conclusions

- The human body model can be used to measure sagittal plane kinematics for most clinical and research purposes.
- Gait analysis can be complemented with dynamic ultrasound imaging of the calf musculotendon junction and fascicles without significant interference with the gait pattern.
- Different diagnostic tools to assess stretch hyperreflexia yield substantially different outcomes.
- Motorized assessments for stretch hyperreflexia in children with CP have limited clinical feasibility, but instead we advise exploring the added value of instrumented manual assessments in clinical decision-making.
- Manual tests to assess stretch hyperreflexia appear unable to assess the severity of stretch hyperreflexia.
- Treadmill perturbations show the potential to quantify stretch hyperreflexia during gait.
- Findings in this thesis support the hypothesis that children with spastic CP have deviating walking patterns due to the presence of stretch hyperreflexia.
- Multiple reflex pathways might be involved in stretch hyperreflexia during gait of children with CP.
- In the calf muscle activation pattern, a sharp peak during the loading response appears to coincide with stretch hyperreflexia during gait of children with CP and may have potential as a diagnostic tool.
- EMG-biofeedback-driven gaming shows the potential to improve the gait pattern of children with CP.
- Biofeedback should focus on the task of interest for the child, i.e. walking for ambulatory children.
- Gamification in pediatric rehabilitation is recommended, as it increases motivation.

References

1. van den Noort JC, Bar-On L, Aertbeliën E, et al. European consensus on the concepts and measurement of the pathophysiological neuromuscular responses to passive muscle stretch. *Eur J Neurol*. 2017;24(7):981-e38. doi:10.1111/ene.13322
2. van der Velden LL, de Koff MAC, Ribbers GM, Selles RW. The diagnostic levels of evidence of instrumented devices for measuring viscoelastic joint properties and spasticity; a systematic review. *J Neuroeng Rehabil*. 2022;19(1):1-8. doi:10.1186/S12984-022-00996-7/FIGURES/2
3. Sackett DL, Haynes RB. The architecture of diagnostic research. *Bmj*. 2002;324(7336):539-541.
4. van't Veld RC, Flux E, van Oorschot W, et al. Examining the role of intrinsic and reflexive contributions to ankle joint hyper-resistance treated with botulinum toxin-A. *J Neuroeng Rehabil*. 2023;20(1):1-14. doi:10.1186/s12984-023-01141-8
5. Flux E, van 't Veld R, van Asseldonk E, et al. A comparison of different methods to quantify stretch reflexes in children with cerebral palsy. In: *Stretch Hyperreflexia in Children with Cerebral Palsy: Assessment, Contextualization and Modulation*. ; 2023.
6. Alhusaini AAA, Dean CM, Crosbie J, Shepherd RB, Lewis J. Evaluation of spasticity in children with cerebral palsy using Ashworth and Tardieu Scales compared with laboratory measures. *J Child Neurol*. 2010;25(10):1242-1247.
7. Malhotra S, Cousins E, Ward A, et al. An investigation into the agreement between clinical, biomechanical and neurophysiological measures of spasticity. *Clin Rehabil*. 2008;22(12):1105-1115.
8. Patrick E, Ada L. The Tardieu Scale differentiates contracture from spasticity whereas the Ashworth Scale is confounded by it. *Clin Rehabil*. 2006;20(2):173-182.
9. Bohannon RW, Smith MB. Interrater reliability of a modified Ashworth scale of muscle spasticity. *Phys Ther*. 1987;67(2):206-207. doi:10.1093/PTJ/67.2.206
10. Haugh AB, Pandyan AD, Johnson GR. A systematic review of the Tardieu Scale for the measurement of spasticity. *Disabil Rehabil*. 2006;28(15):899-907. doi:10.1080/09638280500404305
11. Scholtes VAB, Becher JG, Lankhorst GJ. Clinical assessment of spasticity in children with cerebral palsy: a critical review of available instruments. *Dev Med Child Neurol*. 2006;48(1):64-73. doi:10.1017/S0012162206000132
12. Fleuren JFM, Voerman GE, Erren-Wolters C V, et al. Stop using the Ashworth Scale for the assessment of spasticity. *J Neurol*. 2009;81(1):46. doi:10.1136/jnnp.2009.177071ï
13. Charalambous CP. Interrater reliability of a modified ashworth scale of muscle spasticity. In: *Classic Papers in Orthopaedics*. Springer-Verlag London Ltd; 2014:415-417. doi:10.1007/978-1-4471-5451-8_105
14. Netherlands CP register. https://cpregister.nl/.
15. Scholtes VAB, Dallmeijer AJ, Becher JG. The Spasticity Test: a clinical instrument to measure spasticity in children with cerebral palsy. In: *The Effectiveness of Multilevel Botulinum Toxin Type A and Comprehensive Rehabilitation in Children with Cerebral Palsy*. Citeseer; 2007:29-64.
16. Ludvig D, Cathers I, Kearney RE. Voluntary modulation of human stretch reflexes. *Exp Brain Res*. 2007;183:201-213. doi:10.1007/s00221-007-1030-0
17. van 't Veld RC, Flux E, Schouten AC, van der Krogt MM, van der Kooij H, van Asseldonk EHF. Reducing the Soleus Stretch Reflex With Conditioning: Exploring Game- and Impedance-Based Biofeedback. *Front Rehabil Sci*. 2021;2(October):1-13. doi:10.3389/fresc.2021.742030
18. Mrachacz-Kersting N, Kersting UG, de Brito Silva P, et al. Acquisition of a simple motor skill: Task-dependent adaptation and long-term changes in the human soleus stretch reflex. *J Neurophysiol*. 2019;122(1):435-446. doi:10.1152/jn.00211.2019
19. Mirbagheri MM, Alibiglou L, Thajchayapong M, Rymer WZ. Muscle and reflex changes with varying joint angle in hemiparetic stroke. *J Neuroeng Rehabil*. 2008;5:1-15. doi:10.1186/1743-0003-5-6
20. Alibiglou L, Rymer WZ, Harvey RL, Mirbagheri MM. The relation between Ashworth scores and neuromechanical measurements of spasticity following stroke. *J Neuroeng Rehabil*. 2008;5(1):1-14. doi:10.1186/1743-0003-5-18/FIGURES/8
21. van't Veld R. *Integrated Spasticity Assessment and Treatment Using Disentangled Joint Resistance*. University of Twente; 2022. doi:10.3990/1.9789036553919
22. Mirbagheri MM, Barbeau H, Kearney RE. Intrinsic and reflex contributions to human ankle stiffness: Variation with activation level and position. *Exp Brain Res*. Published online 2000. doi:10.1007/s002210000534

23. Graser J V., Prospero L, Liesch M, Keller U, van Hedel HJA. Test–retest reliability of upper limb robotic exoskeleton assessments in children and youths with brain lesions. *Sci Reports 2022 121*. 2022;12(1):1-15. doi:10.1038/s41598-022-20588-8
24. Bar-On L, Aertbeliën E, Wambacq H, et al. A clinical measurement to quantify spasticity in children with cerebral palsy by integration of multidimensional signals. *Gait Posture*. 2013;38(1):141-147. doi:10.1016/j.gaitpost.2012.11.003
25. Bar-On L, Desloovere K, Molenaers G, Harlaar J, Kindt T, Aertbeliën E. Identification of the neural component of torque during manually-applied spasticity assessments in children with cerebral palsy. *Gait Posture*. 2014;40(3):346-351. doi:10.1016/j.gaitpost.2014.04.207
26. Yamaguchi T, Hvass Petersen T, Kirk H, et al. Spasticity in adults with cerebral palsy and multiple sclerosis measured by objective clinically applicable technique. *Clin Neurophysiol*. 2018;129(9):2010-2021. doi:10.1016/j.clinph.2018.07.004
27. Willerslev-Olsen M, Choe Lund M, Lorentzen J, Barber L, Kofoed-Hansen M, Nielsen JB. Impaired muscle growth precedes development of increased stiffness of the triceps surae musculotendinous unit in children with cerebral palsy. *Dev Med Child Neurol*. 2018;60(7):672-679.
28. Sloot LH, Bar-On L, van der Krogt MM, et al. Motorized versus manual instrumented spasticity assessment in children with cerebral palsy. *Dev Med Child Neurol*. 2017;59(2):145-151. doi:10.1111/dmcn.13194
29. Bar-On L, Aertbeliën E, Wambacq H, et al. A clinical measurement to quantify spasticity in children with cerebral palsy by integration of multidimensional signals. *Gait Posture*. 2013;38(1):141-147.
30. Gillett J, Greaves H, Bass A, et al. Clinically applicable assessment of passive stiffness and stretch reflex responses using a portable device in children with cerebral palsy. *Gait Posture*. 2020;81:105-106.
31. Lamontagne A, Malouin F, Richards CL. Locomotor-Specific measure of spasticity of plantarflexor muscles after stroke. *Arch Phys Med Rehabil*. 2001;82(12):1696-1704. doi:10.1053/apmr.2001.26810
32. Van der Krogt MM, Doorenbosch CAM, Becher JG, Harlaar J. Dynamic spasticity of plantar flexor muscles in cerebral palsy gait. *J Rehabil Med*. 2010;42(7):656-663. doi:10.2340/16501977-0579
33. Crenna P. Spasticity and "Spastic" Gait in Children with Cerebral Palsy. *Neurosci Biobehav Rev*. 1998;22(4):571-578.
34. De Niet M, Latour H, Hendricks H, Geurts AC, Weerdesteyn V. Short-Latency Stretch Reflexes Do Not Contribute to Premature Calf Muscle Activity During the Stance Phase of Gait in Spastic Patients. *Arch Phys Med Rehabil*. 2011;92(11):1833-1839. doi:10.1016/J.APMR.2011.05.025
35. Nielsen JB, Christensen MS, Farmer SF, Lorentzen J. Spastic movement disorder: should we forget hyperexcitable stretch reflexes and start talking about inappropriate prediction of sensory consequences of movement? *Exp Brain Res*. 2020;238(7-8):1627-1636. doi:10.1007/s00221-020-05792-0
36. Hodapp M, Klisch C, Mall V, Vry J, Berger W, Faist M. Modulation of Soleus H-Reflexes During Gait in Children With Cerebral Palsy. *J Neurophysiol*. 2007;98:3263-3268. doi:10.1152/jn.00471.2007
37. Sloot LH, Van Den Noort JJC, van der Krogt MMM, Bruijn SMS, Harlaar J. Can treadmill perturbations evoke stretch reflexes in the calf muscles? *PLoS One*. 2015;10(12). doi:10.1371/journal.pone.0144815
38. Flux E, van der Krogt MM, Harlaar J, Buizer AI, Sloot LH. Functional assessment of stretch hyperreflexia in children with cerebral palsy using treadmill perturbations. *J NeuroEngineering Rehabil 2021 181*. 2021;18(1):1-17. doi:10.1186/S12984-021-00940-1
39. Flux E, Mooijekind B, Bar-On L, van Asseldonk E, Buizer AI, van der Krogt MM. Relation between gastrocnemius medialis muscle-tendon stretch and muscle activation during gait in children with cerebral palsy. In: *Stretch Hyperreflexia in Children with Cerebral Palsy: Assessment, Contextualization and Modulation*. ; 2023.
40. Lichtwark GA, Wilson AM. Interactions between the human gastrocnemius muscle and the Achilles tendon during incline, level and decline locomotion. *J Exp Biol*. 2006;209(21):4379-4388. doi:10.1242/JEB.02434
41. Barber L, Carty C, Modenese L, Walsh J, Boyd R, Lichtwark G. Medial gastrocnemius and soleus muscle-tendon unit, fascicle, and tendon interaction during walking in children with cerebral palsy. *Dev Med Child Neurol*. 2017;59(8):843-851. doi:10.1111/dmcn.13427
42. Cronin NJ, Carty CP, Barrett RS, Lichtwark G. Automatic tracking of medial gastrocnemius fascicle length during human locomotion. *J Appl Physiol*. 2011;111(5):1491-1496. doi:10.1152/japplphysiol.00530.2011
43. Kalkman BM, Bar-On L, Cenni F, et al. Muscle and tendon lengthening behaviour of the medial gastrocnemius during ankle joint rotation in children with cerebral palsy. *Exp Physiol*.

2018;103(10):1367-1376. doi:10.1113/EP087053
44. Bar-On L, Flux E, van der Krogt MM, et al. Medial gastrocnemius muscle and tendon interaction during gait in typically developing children and children with cerebral palsy. In: *Virtual Meeting of the European Society of Movement Analysis in Adults and Children 2020*. ; 2020.
45. van Hooren B, Teratsias P, Hodson-Tole EF. Ultrasound imaging to assess skeletal muscle architecture during movements: A systematic review of methods, reliability, and challenges. *J Appl Physiol*. 2020;128(4):978-999. doi:10.1152/JAPPLPHYSIOL.00835.2019
46. Mooijekind B, Flux E, Buizer AI, van der Krogt MM, Bar-On L. The influence of wearing an ultrasound device on gait in children with cerebral palsy and typically developing children. *Gait Posture*. 2023;101:138-144. doi:10.1016/J.GAITPOST.2023.02.007
47. Cenni F, Bar-On L, Monari D, et al. Semi-automatic methods for tracking the medial gastrocnemius muscle–tendon junction using ultrasound: a validation study. *Exp Physiol*. 2020;105(1):120-131. doi:10.1113/EP088133
48. Cenni F, Schless SH, Adams H, Bar-On L, Desloovere K. The reliability of measuring medial gastrocnemius muscle-tendon unit lengths during gait. *Gait Posture*. 2021;90:464-467. doi:10.1016/J.GAITPOST.2021.09.198
49. Pitcher CA, Elliott CM, Panizzolo FA, Valentine JP, Stannage K, Reid SL. Ultrasound characterization of medial gastrocnemius tissue composition in children with spastic cerebral palsy. *Muscle Nerve*. 2015;52(3):397-403. doi:10.1002/MUS.24549
50. Farris DJ, Lichtwark GA. UltraTrack: Software for semi-automated tracking of muscle fascicles in sequences of B-mode ultrasound images. *Comput Methods Programs Biomed*. 2016;128:111-118. doi:10.1016/J.CMPB.2016.02.016
51. Thompson AK, Chen XY, Wolpaw JR. Acquisition of a Simple Motor Skill: Task-Dependent Adaptation Plus Long-Term Change in the Human Soleus H-Reflex. *J Neurosci*. 2009;29(18). doi:10.1523/JNEUROSCI.4326-08.2009
52. Wolpaw JR, Braitman DJ, Seegal RF. Adaptive plasticity in primate spinal stretch reflex: initial development. *J Neurophysiol*. 1983;50(6):1296-1311.
53. Chen Y, Chen XY, Jakeman LB, Chen L, Stokes BT, Wolpaw JR. Operant Conditioning of H-Reflex Can Correct a Locomotor Abnormality after Spinal Cord Injury in Rats. *J Neurosci*. 2006;26(48):12537-12543. doi:10.1523/JNEUROSCI.2198-06.2006
54. Makihara Y, Segal RL, Wolpaw JR, Thompson AK. Operant conditioning of the soleus H-reflex does not induce long-term changes in the gastrocnemius H-reflexes and does not disturb normal locomotion in humans. *J Neurophysiol*. 2014;112(6). Accessed July 19, 2017. http://jn.physiology.org/content/112/6/1439
55. Thompson AK, Pomerantz FR, Wolpaw JR. Operant Conditioning of a Spinal Reflex Can Improve Locomotion after Spinal Cord Injury in Humans. *J Neurosci*. 2013;33(6):2365-2375. doi:10.1523/JNEUROSCI.3968-12.2013
56. Mrachacz-Kersting N, Kersting UG, de Brito Silva P, et al. Acquisition of a simple motor skill: task-dependent adaptation and long-term changes in the human soleus stretch reflex. *J Neurophysiol*. 2019;122(1):435-446. doi:10.1152/jn.00211.2019
57. McAuley E, Duncan T, Tammen V V. *Psychometric Properties of the Intrinsic Motivation Inventory in a Competitive Sport Setting: A Confirmatory Factor Analysis*. Vol 60. Taylor & Francis Group; 1989:48-58. doi:10.1080/02701367.1989.10607413
58. Beckers LWME, Geijen MME, Kleijnen J, et al. Feasibility and effectiveness of home-based therapy programmes for children with cerebral palsy: A systematic review. *BMJ Open*. 2020;10(10). doi:10.1136/bmjopen-2019-035454
59. Flux E, Bar-On L, Buizer AI, Harlaar J, van der Krogt MM. Electromyographic biofeedback-driven gaming to alter calf muscle activation during gait in children with spastic cerebral palsy. *Gait Posture*. 2023;102:10-17. doi:10.1016/J.GAITPOST.2023.02.012
60. Booth AT, Buizer AI, Harlaar J, Steenbrink F, van der Krogt MM. Immediate Effects of Immersive Biofeedback on Gait in Children With Cerebral Palsy. *Arch Phys Med Rehabil*. 2019;100(4):598-605.
61. van Gelder L, Booth ATC, van de Port I, Buizer AI, Harlaar J, van der Krogt MM. Real-time feedback to improve gait in children with cerebral palsy. *Gait Posture*. 2017;52:76-82. doi:10.1016/j.gaitpost.2016.11.021
62. Conner BC, Lerner ZF. Improving Ankle Muscle Recruitment via Plantar Pressure Biofeedback during Robot Resisted Gait Training in Cerebral Palsy. *IEEE Int Conf Rehabil Robot*. 2022;2022-July:25-29. doi:10.1109/ICORR55369.2022.9896581

63. Colborne GR, Wright F V, Naumann S, Anonymous. Feedback of triceps surae EMG in gait of children with cerebral palsy: a controlled study. *Arch Phys Med Rehabil*. 1994;75(1):40-45.
64. Nash J, Neilson PD, O'Dwyer NJ. Reduing spasticity to control muscle contracture of children with cerebral palsy. *Dev Med Child Neurol*. 1989;31:471-480. doi:10.1111/j.1469-8749.1989.tb04025.x
65. O'Dwyer N, Neilson P, Nash J. Reduction of spasticity in cerebral palsy using feedback of the tonic stretch reflex: a controlled study. *Dev Med Child Neurol*. 1994;35:770-786. Accessed August 31, 2017. https://www.researchgate.net/profile/Nicholas_Odwyer/publication/15273563_Reduction_of_spasticity_in_cerebral_palsy_using_feedback_of_the_tonic_stretch_reflex_A_controlled_study/links/544f8b200cf2bca5ce92a3b0.pdf
66. Booth ATC, Buizer AI, Meyns P, Oude Lansink ILB, Steenbrink F, van der Krogt MM. The efficacy of functional gait training in children and young adults with cerebral palsy: a systematic review and meta-analysis. *Dev Med Child Neurol*. 2018;60(9):866-883. doi:10.1111/dmcn.13708
67. D'Amico JM, Condliffe EG, Martins KJB, Bennett DJ, Gorassini MA. Recovery of neuronal and network excitability after spinal cord injury and implications for spasticity. *Front Integr Neurosci*. 2014;8(May):1-24. doi:10.3389/fnint.2014.00036
68. Sakzeweski L, Ziviani J, Boyd RN. Efficacy of Upper Limb Therapies for Unilateral Cerebral Palsy: A Meta-analysis. *Pediatrics*. 2014;133:175-204. Accessed July 14, 2017. http://pediatrics.aappublications.org/content/pediatrics/early/2013/12/18/peds.2013-0675.full.pdf
69. Van Vulpen LF, De Groot S, Rameckers EAA, Becher JG, Dallmeijer AJ. Effectiveness of functional power training on walking ability in young children with cerebral palsy: study protocol of a double-baseline trial. *Pediatr Phys Ther*. 2017;29(3):275-282.
70. Thompson AK, Wolpaw JR, Taylor J, Mrachacz-Kersting N, Thompson AK, Wolpaw JR. H-reflex conditioning during locomotion in people with spinal cord injury. *J Physiol C 2019 Authors J Physiol*. 2021;599:2453-2469. doi:10.1113/JP278173
71. Roebroeck ME, Harlaar J, Lankhorst GJ. The Application of Generalizability Theory to Reliability Assessment: An Illustration Using Isometric Force Measurements. *Phys Ther*. 1993;73(6):386-395.
72. Habersack A, Zussner T, Thaller S, Tilp M, Svehlik M, Kruse A. Validity and reliability of a novel 3D ultrasound approach to assess static lengths and the lengthening behavior of the gastrocnemius medialis muscle and the Achilles tendon in vivo. *Knee Surgery, Sport Traumatol Arthrosc 2022 3012*. 2022;30(12):4203-4213. doi:10.1007/S00167-022-07076-2
73. Botter A, Beltrandi M, Cerone GL, Gazzoni M, Vieira TMM. Development and testing of acoustically-matched hydrogel-based electrodes for simultaneous EMG-ultrasound detection. *Med Eng Phys*. 2019;64:74-79. doi:https://doi.org/10.1016/j.medengphy.2018.12.002
74. Veerkamp K, van der Krogt MM, Harlaar J, et al. Personalisation of Plantarflexor Musculotendon Model Parameters in Children with Cerebral Palsy. *Ann Biomed Eng*. Published online November 15, 2022:1-13. doi:10.1007/S10439-022-03107-8/FIGURES/6
75. Lubbers M, Dedic A, Coenen A, et al. Calcium imaging and selective computed tomography angiography in comparison to functional testing for suspected coronary artery disease: the multicentre, randomized CRESCENT trial. *Eur Heart J*. 2016;37(15):1232-1243.
76. Mynark RG, Koceja DM. Down training of the elderly soleus H reflex with the use of a spinally induced balance perturbation. *J Appl Physiol*. 2002;93(1):127-133. doi:10.1152/japplphysiol.00007.2001
77. Conner BC, Schwartz MH, Lerner ZF. Pilot evaluation of changes in motor control after wearable robotic resistance training in children with cerebral palsy. *J Biomech*. 2021;126:110601.

Summary

Cerebral palsy (CP) is a neurological disorder and the most frequent cause of motor impairment in children in Europe. Around 85% of children with CP experience stretch hyperreflexia, also known as “spasticity”. Stretch hyperreflexia is an excessive response to muscle stretch, leading to increased joint resistance. The joint hyper-resistance causes limitations in activities such as walking. Multiple methods have been developed to measure stretch hyperreflexia, but evidence supporting the use of these methods for diagnostics and treatment evaluation in children with CP is insufficient. Furthermore, most methods are designed to assess stretch reflexes in passive conditions, which might not translate to the limitations encountered due to stretch reflexes during activities. Furthermore, while a broad range of stretch hyperreflexia treatments is available, many are invasive, non-specific, or temporary and might have adverse side effects. Training methods to reduce stretch reflexes using biofeedback are promising non-invasive methods with potential long-term sustained effects. Still, clinical feasibility needs to be improved before implementation in clinical rehabilitation of children with CP. This thesis aimed to develop methods to assess stretch hyperreflexia of the calf muscles during passive conditions, as well as in the context of walking. Additionally, this thesis aimed to develop clinically feasible methods to modulate stretch hyperreflexia in the calf muscle of children with CP.

In Chapter 2 we analyze the Human Body Model (HBM), a kinematic model optimized for real-time computing of kinematics, which can be used in biofeedback training methods. The study comprises of a comparison of HBM outcomes with two commonly used models for clinical gait analysis: the Newington Model, also known as Plug-in-Gait (PiG), and the calibrated anatomical system technique (CAST). 3D instrumented gait analyses were performed for twenty-five children with CP. Statistical parametric mapping and RMSE values revealed that sagittal plane differences between the three models were mostly less than the clinically meaningful 5°. For frontal and transverse planes, differences between all three models for almost all segment and joint angles exceeded the value of minimal clinical significance. It remains undecided which model holds the most accurate information, since none of the three models represents a ground truth. Meanwhile, it can be concluded that all three models are equivalent in representing sagittal plane gait kinematics in clinical gait analysis. The HBM was subsequently used in Chapter 3, 7 and 9.

In **Chapter 3** we assessed the potential to complement gait analysis with ultrasonography, without interference with the gait pattern. The ultrasonography will enable dynamic imaging of medial gastrocnemius (MG) muscles and tendons during gait, and can be used to gain insight in stretch reflexes during gait. Eighteen children with spastic CP and 16 age-matched typically developing (TD) children walked at comfortable walking speed on an instrumented treadmill. One baseline gait condition (BASE) and two conditions with an ultrasound probe and custom-made probe holder were measured: on the mid-muscle fascicles (FAS) and on the muscle-tendon junction (MTJ). A two-way repeated measures ANOVA revealed that children took wider steps during FAS (CP,TD) and MTJ (TD) compared to BASE, and during FAS compared to MTJ (CP). Hip extension was lower (RMSE of 2.7°) during terminal stance for MTJ compared to FAS for TD only. There was less swing knee flexion (FAS 4.9°; MTJ 4.0°) and ankle plantarflexion around toe-off (FAS 3.0°; MTJ 2.4°) for both ultrasound placements, which did not differ between CP and TD. Power absorption during loading response was slightly increased for both ultrasound placements (0.12W/kg), with again no difference between CP and TD. The entire muscle-tendon unit shortened less in swing for both ultrasound placements (FAS 3.6mm; MTJ 3.7mm), with no group effect. It was concluded that wearing an ultrasound probe causes minimal lower-limb gait

alterations and muscle-tendon unit length changes, which are mostly similar in CP and TD. The ultrasound probe and probe holder were added to gait analysis in Chapter 7.

In **chapter 4** we compared different methods to assess stretch hyperreflexia in adults with spinal cord injury or stroke. Additionally, we used these assessment methods to investigate the effect of botulinum neurotoxin-A (BoNT-A) injections. We hypothesized that the overall joint resistance and reflexive contribution decreased 6 weeks after injection, while returning close to baseline after 12 weeks. Nine participants with stretch hyperreflexia after spinal cord injury or stroke were evaluated across three sessions: 0, 6, and 12 weeks after BoNT-A injection in the calf muscles. Evaluation included clinical measures (Modified Ashworth Scale (MAS), Tardieu Scale) and motorized assessment using the motorized spasticity test (motSPAT) and parallel-cascade (PC) system identification. The two motorized assessments were not correlated with the MAS (r= -0.01 for the motSPAT and -0.08 for the PC), but showed a moderate to good correlation with the Tardieu scale (r=0.60 for the motSPAT and 0.57 for the PC). Furthermore, the two methods were strongly correlated with each other (r=0.86), supporting the validity of these methods in adults after SCI/stroke. On group-level, the hypothesized BoNT-A effect, the combination of a reduced resistance (week 6) and return towards baseline (week 12), was not confirmed by the motSPAT and the PC, nor by the Tardieu scale. Surprisingly, the MAS did show a significantly reduced resistance at week 6. Individually, the reduction in stretch hyperreflexia was observed in the MAS (5 participants), Tardieu Scale (2 participants), motSPAT (1 participant) and PC (4 participants). Therefore, we concluded that it is essential to perform further quantification of the individual contributions to joint resistance changes using instrumented measures across a large sample size, to understand the heterogeneous response to BoNT-A injections.

In **Chapter 5** we compared different methods to assess stretch hyperreflexia in children with spastic CP. Although most methods are designed based on the same concept of stretch hyperreflexia, it is unknown whether they give similar outcomes. Five assessment methods were compared in 18 children with spastic CP. Four tests apply passive stretches to evoke reflexes, and one functional test analyzes stretch reflexes during walking. The spasticity test (SPAT), a currently used clinical test, is based on manual perturbations of the ankle joint to evoke calf muscle stretch. Three different motorized tests, a motorized SPAT, an eight-degree stretch reflex, and a system-identification method, assessed the responses to perturbations around the ankle joint, with perturbations applied using a robotic device. The functional assessment method consists of analyzing stretch reflexes during the swing and stance phase of gait, by calculating the ratio between muscle activation and fascicle lengthening velocity. The outcomes of the different methods were compared using Spearman and Pearson correlations and a bootstrapping procedure to define 95% confidence intervals (CI). The eight-degree stretch reflex was strongly correlated with the motorized SPAT (r=0.70, bootstrap confidence interval 0.00 - 0.96) and with the system-identification method (r=0.84, CI 0.48 - 0.98), but the motorized SPAT was not correlated with the system-identification method (r=0.10, CI -0.24 - 0.80). In addition, neither the SPAT, nor the functional assessments were correlated to any other assessment method. We concluded that different assessments methods cannot be used interchangeably, despite the similar assumptions used to develop the methods. Population-specific limitations, such as anxiety for motorized assessments, can partly explain these results. In addition, the concept of stretch hyperreflexia might be too complex to capture with one assessment method.

Chapter 6 describes a method to assess stretch hyperreflexia during walking, by applying treadmill belt acceleration perturbations. This study systematically explored the feasibility, reliability, and validity of

sudden treadmill perturbations to evoke and quantify calf muscle stretch reflexes during walking in children with neurological disorders. We performed an observational cross-sectional study including 24 children with CP (6-16 years) and 14 TD children (6-15 years). Short belt accelerations were applied at three different intensities while children walked at a comfortable speed. Lower leg kinematics, musculo-tendon lengthening and velocity, muscle activity, and spatiotemporal parameters were measured to analyze perturbation responses. We first demonstrated protocol feasibility: the protocol was completed by all but three children, who ceased participation due to fatigue. All remaining children were able to maintain their gait pattern during perturbation trials without anticipatory adaptations in ankle kinematics, spatiotemporal parameters, and muscle activity. Second, we showed the protocol's reliability: there was no systematic change in muscle response over time ($p=0.21$-0.54), and a bootstrapping procedure indicated sufficient number of perturbations, as the last perturbation repetition only reduced variability by ~2%. Third, we evaluated construct validity by showing that responses comply with neurophysiological criteria for stretch reflexes: perturbations superimposed calf muscle lengthening ($p<0.001$ for both CP and TD) in all but one participant. This elicited increased calf muscle activity (359±190% for CP and 231±68% for TD, both $p<0.001$) in the gastrocnemius medialis muscle, which increased with perturbation intensity ($p<0.001$), according to the velocity-dependent nature of stretch reflexes. Finally, construct validity was shown from a clinical perspective: stretch reflexes were 1.7 times higher for CP than TD for the gastrocnemius medialis muscle ($p=0.017$). The feasibility and reliability of the protocol, as well as the construct validity - shown by the exaggerated velocity-dependent nature of the measured responses - strongly support the use of treadmill perturbations to quantify stretch hyperreflexia during gait. We therefore provided a framework which can be used to inform clinical decision making and treatment evaluation.

In **Chapter 7** we describe a method to assess stretch hyperreflexia during unperturbed, comfortable walking. This is done by investigating the relation between gastrocnemius medialis muscle-tendon stretch and muscle activation during gait in children with CP and TD children. 3D gait analysis including electromyography (EMG) was performed on a treadmill. Stretch of gastrocnemius medialis fascicle, belly, and tendon during treadmill walking were measured using dynamic ultrasound imaging, and musculotendon-unit stretch was estimated using OpenSim. EMG/lengthening velocity and EMG/lengthening acceleration ratios were up to 500% higher for CP (n=14) than TD (n=15) for most structures. Increased late swing muscle activation in CP was often preceded by fascicle and musculotendon-unit peak lengthening velocity, and early stance muscle activation by peaks in multiple structures. The increased muscle activation in CP is likely associated with muscle-tendon stretch during gait. Late swing muscle activation in CP appears velocity-dependent, whereas early stance activation can be velocity- and acceleration-dependent. These insights into stretch reflex mechanisms during gait can assist in the development of targeted interventions.

Chapter 8 describes a training method to modulate stretch reflexes. Within this training method, seated participants have to reduce the mechanically elicited stretch reflex response using biofeedback of stretch reflex magnitude, as quantified using electromyography (EMG). Before clinical application of the training method, improvements are needed regarding the time-intensiveness and slow learning curve. Previous studies have shown that gamification of biofeedback can improve participant motivation and long-term engagement. Moreover, quantification of stretch reflex magnitude using reflexive joint impedance may obtain similar effectiveness of biofeedback training within fewer sessions. Nine healthy volunteers participated in the study, split in three groups. First, the "Conventional" group received EMG- and bar-based biofeedback similar to previous research. Second, we explored feasibility of game-based

biofeedback with the “Gaming” group receiving EMG- and game-based biofeedback. Third, we explored feasibility of game- and impedance-based biofeedback with the “Impedance” group receiving impedance and game-based biofeedback. Participants completed five baseline sessions (without reflex biofeedback) and six conditioning sessions (with reflex biofeedback). Participants were instructed to reduce the stretch reflex magnitude without modulating background activity. The Conventional and Gaming groups showed feasibility of the protocol in 2 and 3 out of 3 participants, respectively. These participants were able to reduce the Soleus short-latency stretch reflex with at least 15% during the 4th–6th conditioning session. None of the Impedance group participants were able to reduce their Soleus stretch reflex within the sessions. The feasibility in the EMG- and game-based biofeedback calls for further research on gamification of the conditioning paradigm to obtain improved participant motivation and engagement, while achieving long-term conditioning effects. Before clinical application, the conditioning paradigm's time-intensiveness and slow learning curve remain an open challenge.

In **Chapter 9** we explore if children with CP can improve their calf muscle activation patterns during gait when provided with biofeedback-driven gaming. Eighteen children (6-17y) with spastic CP received implicit game-based biofeedback on electromyographic activity of the calf muscle (soleus or gastrocnemius medialis) while walking on a treadmill during one session. Biofeedback alternately aimed to reduce early stance activity, increase push-off activity, and both combined. Early stance and push-off activity and the double-bump-index (early stance divided by push-off activity) were determined during baseline and walking with feedback. Perceived competence and interest-enjoyment were assessed through a questionnaire. Repeated measures ANOVA and Friedman's tests revealed that children successfully decreased their electromyographic activity during early stance feedback trials (relative decrease of 6.8±12.2 %, P=0.025), with a trend during the combined feedback trials (6.5±13.9%, P=0.055), and increased their electromyographic activity during push-off feedback trials (8.1±15.8%, P=0.038). Individual significant improvements were seen in twelve of eighteen participants. All children experienced high levels of interest-enjoyment (8.4/10) and perceived competence (8.1/10). The results of this study suggest that children with CP can achieve small within-session improvements of their calf muscle activation pattern when provided with implicit biofeedback-driven gaming in an enjoyable manner. Follow-up gait training studies can incorporate this method to assess retention and long-term functional benefits of electromyographic biofeedback-driven gaming.

All in all, the work presented in this thesis shows that sagittal plane clinical gait analysis can be performed using the human body model and can be complemented with ultrasound imaging of the calf muscle. Motorized methods to assess stretch hyperreflexia in passive conditions might be useful for evaluation in adults after SCI/Stroke. Still, limitations regarding feasibility and validity limit clinical application for children with CP. Furthermore, this thesis provides additional evidence that the deviating muscle activation patterns during walking, particularly the increased activation around initial contact, are caused by stretch hyper-reflexes in children with CP. The deviating muscle activation patterns, with increased activation during early stance and reduced activation around push-off, can be modulated within one session by several children with CP. Therefore, the next step is to develop a training program to modulate the activation pattern and potentially decrease stretch hyper-reflexes in children with CP to improve the gait pattern

Samenvatting

Cerebrale parese (CP) is een neurologische aandoening en de meest voorkomende oorzaak van motorische beperkingen bij kinderen in Europa. Ongeveer 85% van de kinderen met CP ervaart overmatige rekreflexen, ook wel bekend als “spasticiteit”. Overmatige rekreflexen zijn te gevoelige reacties op het oprekken van de spieren, wat leidt tot verhoogde gewrichtsweerstand.

De verhoogde gewrichtsweerstand leidt tot beperkingen in activiteiten zoals lopen. Er zijn verschillende methoden ontwikkeld om overmatige rekreflexen te meten, maar het bewijs ter ondersteuning van het gebruik van deze methoden voor diagnose en behandelevaluatie bij kinderen met CP is ontoereikend. Bovendien zijn de meeste methoden ontworpen om rekreflexen te beoordelen in passieve omstandigheden, wat mogelijk niet overeenkomt met de beperkingen die worden ondervonden als gevolg van rekreflexen tijdens activiteiten. Hoewel er verschillende soorten behandelingen beschikbaar zijn voor patiënten met overmatige rekreflexen, zijn vele daarvan invasief, niet-specifiek of tijdelijk en kunnen ze bovendien nadelige bijwerkingen hebben. Training gericht op het verminderen van overmatige rekreflexen met behulp van biofeedback (modulatie) is een veelbelovende niet-invasieve methode met potentieel langdurige effecten. De klinische haalbaarheid van deze methode moet echter eerst worden verbeterd voor implementatie kan plaatsvinden in de klinische revalidatie van kinderen met CP. Dit proefschrift had tot doel methoden te ontwikkelen om overmatige rekreflexen van de kuitspieren te meten tijdens passieve omstandigheden, evenals in de context van lopen. Daarnaast had dit proefschrift tot doel klinisch haalbare methoden te ontwikkelen om overmatige rekreflexen in de kuitspier van kinderen met CP te moduleren.

In **hoofdstuk 2** analyseerden we het Human Body Model (HBM), een kinematisch model geoptimaliseerd voor het real-time berekenen van kinematica, dat kan worden gebruikt in biofeedback-trainingsmethoden. De studie omvat een vergelijking van de uitkomsten van HBM met twee veelgebruikte modellen voor klinische gangbeeldanalyse: het Newington Model, ook bekend als Plug-in-Gait (PiG), en de calibrated anatomical system technique (CAST). Bij vijfentwintig kinderen met CP werd een 3D-geïnstrumenteerde gangbeeldanalyse uitgevoerd. Statistical parametric mapping en RMSE-waarden toonden dat de verschillen tussen de drie modellen in het sagittale vlak grotendeels onder de klinische relevante 5° bleven. In het frontale en transversale vlak waren de verschillen groter dan de 5° minimale klinische significantie voor bijna alle segment- en gewrichtshoeken. Wij konden met deze studie niet aantonen welk model de meest nauwkeurige informatie geeft, aangezien geen van de drie modellen een absolute waarheid vertegenwoordigt. Ondertussen kon wel worden geconcludeerd dat alle drie de modellen vergelijkbare uitkomsten geven voor de klinische gangbeeldanalyse in het sagittale vlak. Het HBM is vervolgens gebruikt in hoofdstuk 3, 7 en 9.

In **hoofdstuk 3** hebben we bekeken of het mogelijk is om klinische gangbeeldanalyse aan te vullen met echografie, zonder daarmee het looppatroon te verstoren. Met de echografie kunnen we dynamische beeldvorming verkrijgen van de m. gastrocnemius medialis (GM) spier en pees tijdens het lopen. Dit kan gebruikt worden om inzicht te krijgen in rekreflexen tijdens het lopen. Achttien kinderen met spastische CP en 16 leeftijdsgenoten met een gezonde ontwikkeling (GO) liepen op een geïnstrumenteerde loopband op een comfortabele loopsnelheid. Er werd één normaal looppatroon gemeten, evenals twee condities met een echografie probe bevestigd aan het onderbeen door middel van een op maat gemaakte probehouder: op de middelste spiervezels en op de spier-peesovergang (SPO) van de GM. Een two-way repeated measures ANOVA liet zien dat kinderen bredere passen

namen tijdens lopen met een echografie probe geplaatst op de spiervezels (CP+GO) en spier-peesovergang (alleen GO) in vergelijking met het normale looppatroon, en tijdens plaatsing op de spiervezels ten opzichte van de spier-peesovergang (CP). Er was minder heupextensie (RMSE van 2.7°) tijdens de late standfase voor spier-peesovergang plaatsing in vergelijking met plaatsing op de spiervezels voor GO. Er was minder knieflexie tijdens de zwaaifase (spiervezels 4.9°; spier-peesovergang 4.0°) en minder enkel plantairflexie rond het loskomen van de tenen (spiervezels 3.0°; spier-peesovergang 2.4°) voor beide probe plaatsingen en dit verschilde niet tussen CP en GO. Er was een licht verhoogde vermogensabsorptie in de enkel tijdens de loading response voor beide probe plaatsingen (0.12 W/kg), opnieuw zonder verschil tussen CP en GO. Het gehele spier-pees complex verkortte minder tijdens de zwaaifase bij beide plaatsingen (spiervezels 3.6 mm; spier-peesovergang 3.7 mm), zonder groepseffect. Geconcludeerd werd dat het dragen van een echografie probe resulteert in minimale veranderingen in het looppatroon en in de verlenging van het gehele spier-pees complex, die grotendeels vergelijkbaar zijn bij CP en GO. De echografie probe en probehouder werden gebruikt tijdens de gangbeeld analyse in hoofdstuk 7.

In **hoofdstuk 4** hebben we verschillende methoden vergeleken om overmatige rekreflexen te meten bij volwassenen met een dwarslaesie of beroerte. Bovendien hebben we deze meetmethoden gebruikt om het effect van botuline neurotoxine-A (BoNT-A) injecties te onderzoeken. De hypothese was dat de algehele gewrichtsweerstand en de reflexieve bijdrage aan deze weerstand zouden zijn afgenomen 6 weken na de injectie, en weer nagenoeg terug op het oude niveau zouden zijn na 12 weken. Negen deelnemers met overmatige rekreflexen na een dwarslaesie of beroerte werden onderzocht in drie sessies: 0, 6 en 12 weken na BoNT-A injectie in de kuitspieren. De evaluatie omvatte klinische metingen (modified Ashworth-schaal (MAS), Tardieu-schaal) en gemotoriseerde metingen met behulp van de gemotoriseerde spasticiteitstest (SPAT) en parallel-cascade systeemidentificatie. De twee gemotoriseerde metingen vertoonden geen correlatie met de MAS (r=-0.01 voor de gemotoriseerde SPAT en -0.08 voor de systeemidentificatie), maar vertoonden een matige tot goede correlatie met de Tardieu-schaal (r=0.60 voor de gemotoriseerde SPAT en 0.57 voor de systeemidentificatie). Bovendien vertoonden de twee gemotoriseerde methoden een sterke correlatie met elkaar (r=0.86), wat de validiteit van deze methoden ondersteunt voor het meten van reflexen bij volwassenen na een dwarslaesie of beroerte. Op groepsniveau werd het verwachte effect van BoNT-A, de combinatie van verminderde weerstand (week 6) en terugkeer naar het oorspronkelijke niveau (week 12), niet bevestigd door de gemotoriseerde SPAT en de systeemidentificatie, en ook niet door de Tardieu-schaal. Verrassend genoeg toonde de MAS wel een significant verminderde weerstand rond week 6. Op individueel niveau werd een reductie waargenomen in de overmatige rekreflexen door de MAS (5 deelnemers), Tardieu-schaal (2 deelnemers), gemotoriseerde SPAT (1 deelnemer) en systeemidentificatie (4 deelnemers). We concludeerden dat het essentieel is om de verder onderzoek te doen naar de verschillende componenten die bijdragen aan de verhoogde gewrichtsweerstand. Daarbij is het belangrijk de componenten te meten door middel van geïnstrumenteerde metingen over een grote steekproefgrootte, om de heterogene respons op BoNT-A injecties te begrijpen.

In **hoofdstuk 5** hebben we verschillende methoden vergeleken om overmatige rekreflexen te meten bij kinderen met spastische CP. Hoewel de meeste methodes zijn gebaseerd op hetzelfde concept van overmatige rekreflexen, is onbekend of ze vergelijkbare resultaten opleveren. Vijf meetmethoden werden vergeleken bij 18 kinderen met spastische CP. Vier meetmethoden zijn gebaseerd op passieve vormen van rek op de spieren voor het opwekken van reflexen, en één meetmethode analyseert rekreflexen op een functionele manier tijdens het lopen. De spasticiteitstest (SPAT), een meetmethode

die momenteel in de kliniek gebruikt wordt, is gebaseerd op handmatige verstoringen van het enkelgewricht om reflexen in de kuitspieren op te wekken. Drie verschillende gemotoriseerde tests, een gemotoriseerde SPAT, een acht-graden rekreflex en een parallel-cascade systeemidentificatiemethode, werden gebruikt om de reacties op verstoringen rond het enkelgewricht te beoordelen, waarbij verstoringen werden toegepast met behulp van een gemotoriseerd apparaat. De functionele beoordelingsmethode bestaat uit het analyseren van rekreflexen tijdens de zwaai- en stand fase van het lopen, door de verhouding tussen spieractivatie en de reksnelheid van de spiervezel te berekenen, zoals beschreven in hoofdstuk 7. De resultaten van de verschillende meetmethoden werden vergeleken met behulp van Spearman- en Pearson-correlaties en een bootstrapping-procedure om 95% betrouwbaarheidsintervallen (BI) te definiëren. De acht-graden rekreflex vertoonde een sterke correlatie met de gemotoriseerde SPAT (r=0.70, BI 0.00 - 0.96) en met de systeemidentificatiemethode (r=0.84, BI 0.48 - 0.98), maar de gemotoriseerde SPAT vertoonde geen correlatie met de systeemidentificatiemethode (r=0.10, BI -0.24 - 0.80), in tegenstelling tot de resultaten in hoofdstuk 4. Bovendien vertoonden noch de manuele SPAT, noch de functionele beoordelingen een correlatie met enige andere meetmethode. We concludeerden dat verschillende meetmethoden niet onderling uitwisselbaar zijn, ondanks de vergelijkbare aannames die zijn gebruikt om de methoden te ontwikkelen. Deze resultaten kunnen deels verklaard worden door populatie-specifieke beperkingen, zoals angst voor de gemotoriseerde meetmethoden. Bovendien is het concept van overmatige rekreflexen mogelijk te complex om met één meetmethode vast te leggen.

Hoofdstuk 6 beschrijft een methode om overmatige rekreflexen te meten tijdens het lopen, door verstoringen toe te passen aan de snelheid van de loopband. In deze studie werd systematisch de haalbaarheid, betrouwbaarheid en validiteit bekeken van plotselinge verstoringen van de loopband om rekreflexen op te wekken en te kwantificeren tijdens het lopen bij kinderen met neurologische aandoeningen. We voerden een observationele cross-sectionele studie uit met 24 kinderen met CP (6-16 jaar) en 14 GO-kinderen (6-15 jaar). Korte versnellingen van de loopband werden toegepast op drie verschillende intensiteiten terwijl de kinderen op een comfortabele snelheid liepen. De enkel- en kniehoek, de verlenging en reksnelheid van het spier-pees complex, de spieractiviteit en spatiotemporele parameters werden gemeten om de reacties op de verstoring te analyseren. We toonden eerst de haalbaarheid van het protocol aan: het protocol werd voltooid door alle kinderen op drie na, die stopten vanwege vermoeidheid. Alle overgebleven kinderen konden hun looppatroon behouden tijdens de verstoringen zonder anticiperende aanpassingen in enkelkinematica, spatiotemporele parameters en spieractiviteit. Daarna toonden we de betrouwbaarheid aan van het protocol: er was geen systematische verandering in spierrespons in de loop van de tijd (p=0.21-0.54), en een bootstrapping-procedure gaf aan dat er voldoende verstoringen werden geleverd, aangezien bij de laatste herhaling van de verstoring de variabiliteit met slechts ~2% verminderde. Vervolgens evalueerden we de constructvaliditeit door te laten zien dat de reacties voldeden aan neurofysiologische criteria voor rekreflexen: verstoringen resulteerden in verlenging van de kuitspier (p<0.001 voor zowel CP als GO) bij op één na alle deelnemers. Dit leidde tot een verhoogde activiteit in de m. gastrocnemius medialis (359±190% voor CP en 231±68% voor GO, beide p<0.001), die toenam met de intensiteit van de verstoringen (p<0.001), in lijn met de snelheidsafhankelijke aard van rekreflexen. Ten slotte werd de constructvaliditeit ook vanuit een klinisch perspectief aangetoond: rekreflexen waren 1,7 keer hoger voor CP dan voor GO in de m. gastrocnemius medialis (p=0.017). De haalbaarheid en betrouwbaarheid van het protocol, evenals de constructvaliditeit - aangetoond door de verhoogde snelheidsafhankelijke aard van de gemeten reacties - ondersteunen sterk het gebruik

van loopbandverstoringen om rekreflexen te kwantificeren tijdens het lopen. We hebben daarom een kader geboden dat kan worden gebruikt bij klinische besluitvorming en behandelevaluatie.

In **hoofdstuk 7** beschrijven we een methode om overmatige rekreflexen te beoordelen tijdens onverstoord, comfortabel lopen. Dit werd gedaan door de relatie te onderzoeken tussen de rek van de m. gastrocnemius medialis en de activatie van de spier tijdens het lopen bij kinderen met CP en GO-kinderen. 3D gangbeeldanalyse met elektromyografie (EMG) werd uitgevoerd op een loopband. Rek van de m. gastrocnemius medialis spiervezels, spierbuik en pees werden gemeten met behulp van dynamische echografie tijdens het lopen op de loopband, en de rek van het volledige spier-pees complex werd geschat met behulp van modellering in OpenSim. De verhouding EMG/verlengingssnelheid en EMG/verlengingsversnelling was tot 500% hoger voor CP (n=14) dan voor GO (n=15) voor de meeste structuren. Verhoogde spieractivatie in de late zwaaifase bij CP werd vaak voorafgegaan door pieken in spiervezel- en spier-pees complex verlengingssnelheid, en spieractivatie in de vroege standfase door pieken in meerdere structuren. De verhoogde spieractivatie bij CP hangt waarschijnlijk samen met spier-pees-verlenging tijdens het lopen. Spieractivatie in late zwaai bij CP lijkt afhankelijk van de reksnelheid, terwijl spieractivatie in de vroege standfase afhankelijk kan zijn van reksnelheid en versnelling. Deze inzichten in de rekreflexmechanismen tijdens het lopen kunnen helpen bij de ontwikkeling van gerichte interventies.

In **hoofdstuk 8** wordt een trainingsmethode beschreven om overmatige rekreflexen te beïnvloeden. Binnen deze trainingsmethode moeten deelnemers een mechanisch opgewekte rekreflex verminderen met behulp van biofeedback over de grootte van de rekreflex, zoals gekwantificeerd met EMG. Voordat de trainingsmethode klinisch kan worden toegepast, zijn verbeteringen nodig met betrekking tot de tijdsintensiteit en de trage leercurve. Eerdere studies hebben aangetoond dat motivatie en langdurige betrokkenheid van deelnemers verbeterd kan worden door gamificatie van biofeedback. Bovendien kan mogelijk een vergelijkbaar trainingseffect bereikt worden in minder sessies wanneer de rekreflexen gemeten worden door middel van reflexieve gewrichtsimpedantie. Negen gezonde vrijwilligers namen deel aan het onderzoek, verdeeld in drie groepen. Ten eerste ontving de "Conventionele" groep EMG-biofeedback, vergelijkbaar met eerdere onderzoeken. Ten tweede onderzochten we de haalbaarheid van gamificatie van de biofeedback, waarbij de "Gaming" groep EMG-biofeedback ontving gevisualiseerd in een game. Ten derde onderzochten we de haalbaarheid van gamificatie en impedantie-gebaseerde biofeedback, waarbij de "Impedance" groep impedantie-biofeedback ontving gevisualiseerd in een game. Deelnemers voltooiden vijf basissessies (zonder reflex biofeedback) en zes conditioneringssessies (met reflex biofeedback). Deelnemers kregen de instructie om de grootte van de rekreflex te verminderen zonder achtergrond spieractiviteit te moduleren. De Conventionele en Gaming groepen toonden haalbaarheid van het protocol bij respectievelijk 2 en 3 van de 3 deelnemers. Deze deelnemers konden de rekreflex van de m. soleus met minstens 15% verminderen tijdens de 4e-6e conditioneringssessie. Geen van de deelnemers in de Impedance-groep slaagde erin hun soleus rekreflex te verminderen tijdens de sessies. De haalbaarheid van EMG-biofeedback zoals gevisualiseerd in een game vraagt om verder onderzoek naar gamificatie van conditioneringsprotocollen om de motivatie en betrokkenheid van deelnemers te verbeteren, terwijl langdurige conditioneringseffecten kunnen worden bereikt. Voor klinische toepassing blijven de tijdsintensiteit en trage leercurve van het conditioneringsparadigma een uitdaging.

In **hoofdstuk 9** onderzoeken we of kinderen met CP hun patroon van kuitspieractivatie kunnen verbeteren tijdens het lopen wanneer ze biofeedback-gestuurde spellen spelen op de loopband. Achttien kinderen (6-17 jaar) met spastische CP ontvingen impliciete gegamificeerde biofeedback over de EMG-activiteit van de kuitspier (m. soleus of gastrocnemius medialis) terwijl ze op een loopband liepen gedurende één sessie. De biofeedback was afwisselend gericht op het verminderen van spieractiviteit tijdens de vroege standfase, het verhogen van spieractiviteit tijdens de afzetfase, en beide gecombineerd. Spieractiviteit tijdens de vroege standfase en de afzetfase, en de "double-bump-index" (vroege standfase gedeeld door spieractiviteit tijdens de afzetfase) werden bepaald tijdens het standaard lopen en tijdens het lopen met feedback. Waargenomen competentie en interesse enplezier werden gemeten aan de hand van een vragenlijst. Repeated measures ANOVA en Friedman-tests toonden aan dat kinderen met succes hun EMG activiteit konden verminderen wanneer zij feedback kregen op de vroege standfase (relatieve afname van 6.8±12.2%, p=0.025), met een trend tijdens de gecombineerde feedback (6.5±13.9%, p=0.055), en dat zij hun EMG-activiteit konden verhogen tijdens de feedback op de afzetfase (8.1±15.8%, p=0.038). Op individueel niveau werden significante verbeteringen gevonden bij twaalf van de achttien deelnemers. Alle kinderen ervaarden hoge niveaus van interesse/plezier (8.4/10) en waargenomen competentie (8.1/10). De resultaten van deze studie suggereren dat kinderen met CP op een plezierige manier kleine verbeteringen kunnen bereiken in hun kuitspieractivatiepatroon wanneer ze impliciete biofeedback krijgen door middel van een game. Vervolgstudies kunnen deze methode opnemen om retentie en functionele voordelen op de lange termijn te analyseren van EMG biofeedback gevisualiseerd in een game.

Op basis van dit proefschrift kan geconcludeerd worden dat klinische gangbeeldanalyse in het sagittale vlak kan worden uitgevoerd met behulp van het human body model en kan worden aangevuld met echografie van de kuitspier. Gemotoriseerde methoden om overmatige rekreflexen in passieve omstandigheden te beoordelen kunnen nuttig zijn voor de evaluatie bij volwassenen na een dwarslaesie of beroerte. Anderzijds lijken er beperkingen te zijn met betrekking tot de haalbaarheid en validiteit voor de klinische toepassing bij kinderen met CP. Dit proefschrift levert aanvullend bewijs dat de afwijkende spieractivatiepatronen tijdens het lopen bij kinderen met CP worden veroorzaakt door overmatige rekreflexen, met name de toegenomen activatie rond het initiële voet contact. De afwijkende spieractivatiepatronen, met verhoogde activatie tijdens de vroege standfase en verminderde activatie rond de afzet, kunnen binnen één sessie worden gemoduleerd door de meerderheid van de kinderen met CP. Daarom is de volgende stap het ontwikkelen van een trainingsprogramma om het activatiepatroon te moduleren en mogelijk overmatige rekreflexen bij kinderen met CP te verminderen om het looppatroon te verbeteren.

PhD portfolio

Courses and workshops (11 ECTS)	
Neurocontrol Summer School in Delft, NL (1 ECTS)	2017
Imaging Course, Master Human Movement Science (3 ECTS)	2018
Summer School in NeuroRehabilitation, Baiona, ES (2 ECTS)	2018
BROK course (2 ECTS)	2018
Neurocontrol Summer School in Chicago, USA (1 ECTS)	2019
Writing a Data Management Plan (1 ECTS)	2019
Drawing for work purposes (0.5 ECTS)	2020
Presenting online with impact (0.5 ECTS)	2020
Teaching (3 ECTS)	
Clinical Gait Analysis, lecture on kinematics and assisting in practicums *Bachelor Human Movement Sciences, VU*	2017-2020
Supervising (10 ECTS)	
Lisa van de Wiel. Influencing muscle activation during gait using biofeedback. *Movement Technology, The Hague University of Applied Sciences*	2017-2018
Sanne Ettema & Jari de Rover. Validation and Repeatability of Online Reflex Activity Measures *Bachelor Human Movement Sciences, VU University Amsterdam*	2018
Esther Kret. A simplified algorithm for tracking medial gastrocnemius fascicles during gait in typically developing children *Master Human Movement Sciences, VU University Amsterdam*	2018-2019
Catherine Hooper. What contributes to lengthening of the medial gastrocnemius during gait in typically developing children? *Master Human Movement Sciences, VU University Amsterdam*	2018-2019
Jeanine van Brugge. Validation of online intrinsic and reflexive joint impedance estimates. *Master Biomedical Engineering, University of Twente*	2018-2019
Nienke Heida. Verband tussen twee geïnstrumenteerde methodes om de reflexactiviteit te meten. *Movement Technology, The Hague University of Applied Sciences*	2019
Olaf Atteveld. Can children with spastic paresis improve their triceps surae activation during gait within one session using EMG-driven biofeedback? *Master Human Movement Sciences, VU University Amsterdam*	2019-2020
Wiek Dirks. Motor variability and Improvements in Muscle Activity During Gait of Children with Spastic Paresis within One Session Using EMG Biofeedback. *Internship & research: Master Human Movement Sciences, VU University Amsterdam*	2019-2021
Babette Mooijekind. Dynamic hyperreflexia during gait in children with spastic paresis based on ultrasound-derived fascicle lengthening *Research Master Human Movement Sciences, VU University Amsterdam*	2020-2021

Conference and symposium presentations (26 ECTS)	
A comparison of three models for gait analysis in children with cerebral palsy. *VvBN Student Day, Groningen, NL*	2017
Reducing knee joint crosstalk using PCA correction. *ESMAC conference, Trondheim, Norway (Oral).*	2017
Reflexioning: down-conditioning reflexes in patients with spinal cord injury and children with cerebral palsy. *Amsterdam Movement Sciences, PhD day (Oral)*	2018
Validation and Repeatability of Online Reflex Activity Measures. *Summer School in NeuroRehabilitation, Baiona, Spain (Poster)*	2018
A comparison of three models for gait analysis in children with cerebral palsy. *Dutch BME, Egmond aan Zee, NL (Poster)*	2019
Validation of an online reflex activity measure. *Dutch BME, Egmond aan Zee, NL (Oral)*	2019
Assessment of stretch hyperreflexia during gait through treadmill perturbations and ultrasound. *GRAIL Symposium (Invited speaker, Oral)*	2019
Validity and repeatability of a real-time reflex measure. *Amsterdam Movement Science conference (Poster)*	2019
Validation of an online reflex activity measure for use in feedback training and clinical decision making. *ESMAC conference, Amsterdam, NL* (Poster)	2019
Treadmill perturbations to evoke stretch reflexes during gait in children with cerebral palsy. *Conference for Society for Neuroscience, Chicaco, USA (Poster)*	2019
Treadmill perturbations to evoke stretch reflexes during gait in children with cerebral palsy. *VvBN*	2019
Biofeedback-driven gaming to improve EMG patterns during gait in children with cerebral palsy. *ESMAC conference (virtual poster presentation)*	2020
Biofeedback-driven gaming to reduce muscle stretch reflexes. *ESMAC conference (virtual poster presentation)*	2020
Comfortable walking with an ultrasound device: can children maintain a typical gait pattern? *ESMAC conference (virtual poster presentation)*	2020
Webinar: The use of HBM for (real-time) clinical gait analysis; comparison with other models, and normative gait data	2020
Gait analysis of tomorrow: Precision diagnostics using instrumented treadmill technology. Functional assessment of hyperreflexia using treadmill perturbations and the use of dynamic ultrasound in children with cerebral palsy. *Mini symposium on DCRM (Virtual)*	2021
Grants and awards	
Gerrit-Jan van Ingen Schenau promising young scientist-award	2015
Jury best oral presentation, VvBN Student day, Groningen, NL	2017
Jury best oral presentation, Amsterdam Movement Sciences PhD day, Amsterdam, NL	2018
Audience best presentation award, VvBN PhD day, Groningen, NL	2019
COVR Grant: Safety Validation of Haptic Rehabilitation Robots (co-applicant)	2020
Amsterdam UMC Young Talent Fund	2021

Experiences (11 ECTS)	
Jury for best presentation at the VvBN student day	2017
Neurocontrol consortium meetings	2017-2021
Science transmission meetings	2017-2023
Author of an approved proposal for the ethical committee	2018
Author of an approved proposal for the medical ethical committee	2018
Organisation committee ESMAC conference in Amsterdam	2019
Operant Conditioning Special Interest Group meetings	2019-2022
Co-editor of newsletter for the department of rehabilitation medicine	2018-2021
Laboratory visit Aalborg, Denmark	2019

List of publications

Flux E., van der Krogt, M.M., Cappa, P., Petrarca, M., Desloovere, K., & Harlaar, J. (2020). The Human Body Model versus conventional gait models for kinematic gait analysis in children with cerebral palsy. Human Movement Science; 70:102585. https://doi.org/10.1016/j.humov.2020.102585.

Flux, E., Mooijekind, B., Buizer, A., van der Krogt, M. M., & Bar-On, L. (2023). The influence of wearing an ultrasound device on gait in children with cerebral palsy and typically developing children. Gait & Posture. https://doi.org/10.1016/j.gaitpost.2023.02.007.

Van 't Veld, R.C., **Flux, E.**, van Oorschot, W., Schouten, A.C., van der Krogt, M.M., van der Kooij, H., Vos-van der Hulst, M., Keijsers, N.L.W., & van Asseldonk, E.H.F. (2023). Examining the role of intrinsic and reflexive contributions to ankle joint hyper-resistance treated with botulinum toxin-A. J Neuroeng Rehabil; 20:1–14. https://doi.org/10.1186/s12984-023-01141-8.

Flux, E., van der Krog,t M.M., Harlaar, J., Buizer, A.I., & Sloot, L.H. (2021) Functional assessment of stretch hyperreflexia in children with cerebral palsy using treadmill perturbations. Journal of NeuroEngineering and Rehabilitation; 181;18:1–17. https://doi.org/10.1186/S12984-021-00940-1.

van 't Veld, R.C., **Flux, E.**, Schouten, A.C., van der Krogt, M.M., van der Kooij, H., van Asseldonk, E.H.F. (2021). Reducing the Soleus Stretch Reflex With Conditioning: Exploring Game- and Impedance-Based Biofeedback. Frontiers in Rehabilitation Sciences; 2:1–13. https://doi.org/10.3389/fresc.2021.742030.

van 't Veld, R.C., **Flux, E.**, Schouten, A.C., van der Krogt, M.M., van der Kooij, H., & van Asseldonk, E.H.F. (2021). Reducing the Soleus Stretch Reflex With Conditioning: Exploring Game- and Impedance-Based Biofeedback. Frontiers in Rehabilitation Sciences; 2:1–13. https://doi.org/10.3389/fresc.2021.742030.

Flux, E. Bar-On, L., Buizer, A.I., Harlaar, J., van der Krogt, M.M. (2023). Electromyographic biofeedback-driven gaming to alter calf muscle activation during gait in children with spastic cerebral palsy. Gait & Posture; 102:10-17. https://doi.org/10.1016/j.gaitpost.2023.02.012.

About the author

Eline Flux was born on December 26th, 1990 in Gouda, the Netherlands. She has always been a very curious, optimistic, engaged and active girl. She attended high school at Het Coornhert Gymnasium in Gouda, where she specialized in nature, health and sciences. Besides her studies, she very much enjoyed working at the psychogeriatric department of an elderly home and she spent a lot of time on gymnastics. The biomechanical challenges of gymnastics, combined with the relative high risk of injuries, stimulated her to study Human Movement Sciences.

During her bachelors, she completed two different minors, both health and sports, and attended several courses of the two other minors to comply with her broad interest in the area of human movement sciences. Additionally, she followed a summer school in Human Sciences in Finland. She performed her bachelor research project at Össur Iceland, a company specialized in prosthetics and orthotics, which encouraged her to become a researcher in the field of rehabilitation.

In 2016, she graduated with distinction *cum laude* from the Research Master Human Movement Sciences. Her research project focused on gait analysis in children with cerebral palsy, which she performed at the VU University Medical Center (VUmc) at the Department of Rehabilitation Medicine. She received the Gerrit Jan van Ingen Schenau promising young scientist award to conduct part of her research at Sapienza University in Rome.

During her research master, she additionally completed the educational course of Exposz, Amsterdam with outstanding results, which gave her the tools to teach at the university of applied sciences. After graduation, she began as a lecturer in movement analysis at The Hague University of Applied Sciences alongside a job as a research assistant at the VU University Medical Center.

In 2017 she got offered her PhD position under supervision of Prof.dr.ir. Jaap Harlaar and dr. Marjolein van der Krogt. The PhD project was part of the REFLEXIONING project, a collaboration with TU Enschede, the Sint Maartenskliniek and industrial partners Motek Medical en TMSi, which was embedded in the NEUROCIMT consortium. This was also the moment that she said goodbye to the nursery home, after working at the psychogeriatric department for almost 10 years.

She currently works as a senior researcher at the Amsterdam University of Applied Sciences in the research department “bewegen in en om de school”. Her research focuses on gathering knowledge and developing tools for physical education teachers that will support them to optimally teach each individual in class.

Aside from work, Eline still enjoys gymnastics in many ways: competing, coaching, spectating and judging. Over the years, she became, if possible, even more curious and she can be characterized by her optimistic and problem-solving mindset, she is flexible and creative and always willing to help.

Children with neurological disorders, such as cerebral palsy, often experience difficulties with walking or never develop a customary gait pattern. Such pathological gait pattern seriously affects their mobility and therefore restricts them in daily-life activities. These pathological gait pattens are complex and have a heterogeneous etiology, which challenges optimal treatment. Nevertheless, a main factor in many cases is stretch hyperreflexia, or spasticity. Unfortunately a meaningful and reliable assessment of spasticity is still a clinical problem: Is it possible to replace currently used manual assessments with more objective instrumented or motorized assessments? And is there a relation between the magnitude of stretch reflexes in a passive, controlled setting and in a dynamic functional setting? In this thesis, we took up these questions and explored different methods to assess and contextualize stretch reflexes. Furthermore, we explored the next step: how can stretch hyperreflexia be treated? We have enhanced biofeedback-driven training protocols to modulate stretch reflexes. Moreover, we explored the feasibility of these methods for children with cerebral palsy, both in a passive, controlled setting and in the context of walking. Finally, we present recommendations to further explore biofeedback-driven training to down regulate stretch hyperreflexia.

Amsterdam Movement Sciences

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Amsterdam Movement Sciences conducts scientific research to optimize physical performance in health and disease based on a fundamental understanding of human movement in order to contribute to the fulfillment of a meaningful life.