Spastic equinus deformity in children with Cerebral Palsy – Treatment effects in terms of muscular morphology and function

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Zusammenfassung

Die infantile Zerebralparese (IZP) ist das Resultat einer frühkindlichen Hirnschädigung. Die meisten Betroffenen entwickeln eine spastische Parese, was zu Schwäche, erhöhtem Muskel-Dehnungswiderstand und Kontrakturen führt. Muskel-Sehnen Eigenschaften sind daher wohl deutlich verändert und skelettale Deformitäten sind die Folge. Der Spitzfuß ist eines der häufigsten Defizite und wird oft durch Intervention am Gastrocnemius behandelt. Das übergeordnete Ziel dieser Arbeit war es, nicht-invasive Behandlungsstrategien für diese Pathologie näher zu untersuchen. Außerdem sollten die Zusammenhänge zwischen Muskelstruktur und Funktion beleuchtet werden.

In der ersten Studie wurden die Effekte einer Unterschenkel-Lagerungsorthese auf die Muskelmorphometrie des spastischen Gastrocnemius unter zu Hilfenahme von Ultraschall und 3D Bewegungsanalyse untersucht. Zeitgleich wurde eine gesunde Referenzgruppe ohne Behandlung eingeschlossen. Vor der Behandlung war die Muskel-Sehnen Einheit bei IZP Kindern im Vergleich zur Norm weniger dehnbar, der Muskelbauch und seine Faszikel (~Faserbündel) kürzer, wohingegen die Sehnen deutlich länger erschienen. Durch die Unterschenkelorthesen verbesserte sich die passive Dorsalextension vor allem mit gebeugten Knien. Die Gastrocnemius Muskelbauch- und Sehnenlänge blieben aber relativ unbeeinflusst, wohingegen die Muskeldicke und die Länge der Faszikel weiter abnahmen. Beim Gehen verbesserte sich dennoch die Fußhebung und außerdem wählten die Kinder eine höhere Ganggeschwindigkeit. Lagerungsorthesen können also das Gangbild verbessern, führen gleichzeitig aber zu Atrophie und ggf. zu einem Verlust von seriellen Sarkomeren. Dies lässt sich am ehesten durch die Immobilisierung im Rahmen der Behandlung erklären.

Um eine alternative Therapieform zu finden, wurde in der zweiten Studie die kontraktile Aktivität des Gastrocnemius mittels Ultraschall, Bewegungsanalyse und EMG während des Gehens, Vorwärts-Bergauf, bzw. Rückwärts-Bergab, sowie in der Ebene untersucht. Die Werte von IZP Kinder wurden ebenfalls mit einer Kontrollgruppe verglichen. Das Bergaufgehen steigerte die konzentrische Exkursion der Faszikel, wohingegen das Rückwärts-Bergabgehen zu vermehrter Exzentrik führte. Da sich exzentrisches Training gesunder Muskeln in der Vergangenheit positiv auf Faszikellängenwachstum auswirkte, schlussfolgerten wir, dass Rückwärts-Bergabgehen ggf. auch für Kinder mit IZP vorteilhaft wäre. Während des Gehens in der Ebene zeigte sich zudem, dass Kinder mit IZP nicht nur per-se kürzere Gastrocnemius Faszikel haben, sondern ebenfalls auf verkürzten, relativen Faszikellängen arbeiteten. Da Kinder mit IZP besonders lange Sarkomere haben, könnte die geringe relative Faszikellänge beim Gehen eine nützliche Anpassung sein, um ausreichend aktive Kraft produzieren zu können.

In der dritten Studie wurde daher das Rückwärts-Bergabgehen auf dem Laufband mit statischem Dehnen als traditionelle Therapieform verglichen. Ultraschall, Bewegungsanalyse und handgesteuerte Dynamometrie wurden verwendet, um die Plantarflexorenkraft, die passive Sprunggelenksbeweglichkeit, die Gastrocnemius Morphometrie sowie die Steifigkeit und Dehnbarkeit auf Muskel-Sehnen und Gelenkebene zu untersuchen. Es wurde davon ausgegangen, dass nur das exzentrische Training Muskelwachstum anregt, die Kraft steigert und das Gangbild verbessert. Im direkten Vergleich beider Behandlungen führte das Rückwärts-Bergabgehen zu schnelleren Gehgeschwindigkeiten und mehr Dorsalfexion in der Standphase. Nach dem Dehnen verschlechterte sich die Kniebeugung in der Schwungphase. Dehnen zeigte keinerlei Benefits gegenüber dem Laufbandtraining. Manuell statischem Dehnen sollte daher kein besonderer therapeutischer Stellenwert bei frei-gefähfigen IZP Kindern eingeräumt werden. Rückwärts-Bergabgehen ist dagegen ein effektives Gangtraining. Es setzt vermutlich neuronale und koordinative Reize. Eine Steigerung der Intensität könnte allerdings notwendig sein, um morphologische Muskel-Sehnen Anpassung zu stimulieren.

In der Zusammenschau aller Ergebnisse scheinen positive Änderungen im Gangbild bei IZP Kindern sowohl durch Unterschenkelorthesen, als auch durch Rückwärts-Bergabgehen erreichbar zu sein. Beides führt aber nicht zu Muskelwachstum. Funktionelle Verbesserungen scheinen daher auch stark von neuronal, koordinativen Aspekten abhängig zu sein.

Abstract

Cerebral Palsy (CP) results from an early brain damage. Most children develop spastic paresis, which leads to a lack of muscle force, pathophysiologically increased stretch-resistance and joint contractures. Muscle-tendon properties are considerably altered and promote skeletal deformities. The gastrocnemius muscle is frequently targeted to alleviate a common deficiency known as equinus. The overall objective of this thesis was to investigate several non-invasive treatment strategies for this muscle pathology. In addition, this thesis strived to promote the understanding about gastrocnemius structure-function relationships in CP.

The first study investigated the effects of ankle foot orthotics on spastic gastrocnemius morphometrics as well as on gait by using ultrasound and motion capturing. Untreated controls served as reference. Prior to bracing, the gastrocnemius muscle-tendon unit was less extensible, while the muscle belly and fascicles were shorter and the tendon longer. Bracing increased passive dorsiflexion primarily with the knees flexed. Muscle belly and tendon length showed little change, but fascicle length as well as muscle thickness declined. Nevertheless, children walked faster and foot lift improved. We concluded that braces improved function but may also lead to atrophy and to a loss of serial sarcomeres probably related to the immobilization.

During the second study, we searched for a readily available, substituting therapeutic stimulus and compared the contractile activity of the gastrocnemius on treadmills, namely during flat-forward, forward-uphill and backward-downhill gait using ultrasound, motion capturing and EMG. Results of children with CP were compared to healthy peers. Uphill gait promoted concentric fascicle action, while backward-downhill gait increased eccentric fascicle action. Since eccentric training had been previously shown to increase fascicle length in controls, backward-downhill walking could have been a potentially effective training for the gastrocnemius in CP, too. In addition, apart from having innately shorter fascicles, fascicles of CP children also worked at a shorter relative length. Due to findings of much longer sarcomeres, limited relative fascicle length could hence be an adjustment to produce enough active forces during gait.

During the third study, we compared backward-downhill walking versus static, manual stretching. Ultrasound, motion analysis and handheld dynamometry were used to test plantarflexor strength, passive ankle joint flexibility, as well as gastrocnemius morphometrics, stiffness and strain on muscle-tendon and joint level. We hypothesized that only eccentric exercise was capable of inducing muscle growth, promoting strength and improving overground gait. When comparing both treatments, backward-downhill walking led to larger single stance dorsiflexion and faster achievable walking velocities while stretching aggravated knee flexion in swing. Strength, joint flexibility, as well as stiffness on muscle-tendon and joint level were not altered. Manual static plantarflexor stretching may thus not be emphasized in CP children with high ambulatory function. Backward-downhill walking can be an effective gait treatment**,** probably improving coordination or reducing dynamic stretch sensitivity. Nevertheless, more intense training might be necessary to further alter muscle-tendon properties.

In sum, backward-downhill walking and bracing increased function without promoting or even by harming muscle growth. Thus, movement patterns in CP could strongly depend on habitual or coordinative aspects. Future research could focus on the interplay between muscle-tendon properties and neural coordination.

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1. Introduction and literature review

The following introduction gives an overview about children with Cerebral Palsy (CP) and their major impairments with an emphasis on their plantarflexor muscles and the related equinus pathology (1.1.-1.4). Subsequently, the author would like to review previous findings concerning micro- and macroscopical alterations of muscle tendon tissue in CP ranging from cell to organ level (1.5). In the last part of this section (1.6), a summary about previously documented muscle structure-function relationships in CP and previously detected effects of highly common treatments will be presented. All these aspects may assist the reader in understanding the purpose of this thesis (2) and in interpreting the findings of the three conducted studies which are presented later (3-5).

1.1. Cerebral Palsy - Incidence and etiology

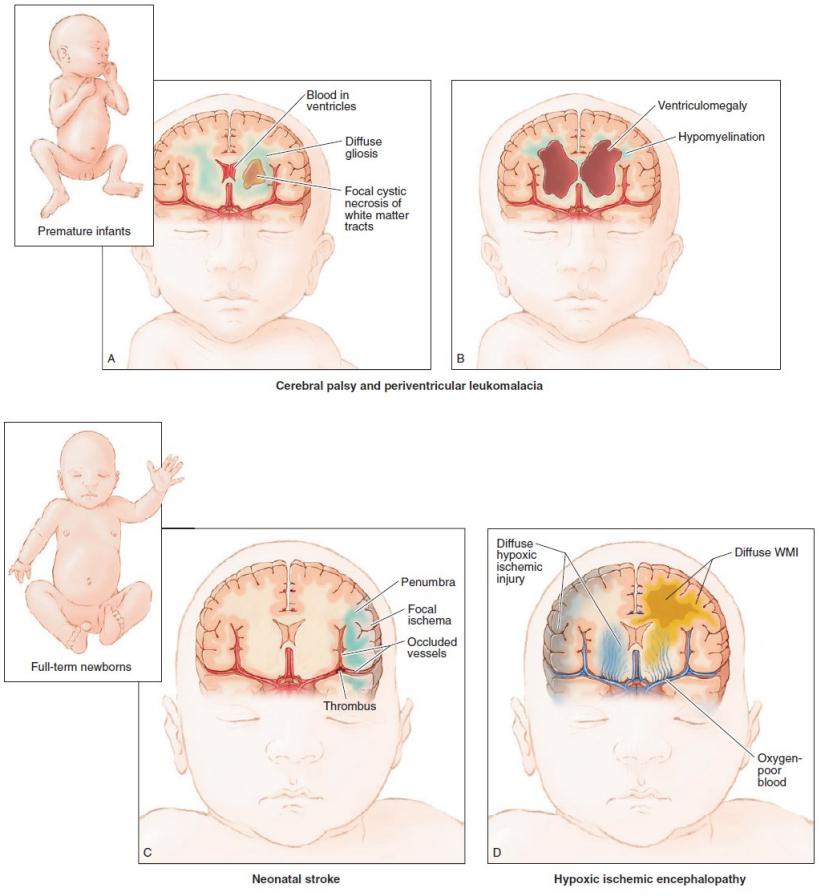

Fig. 1-1 Common brain injuries associated with CP in premature and full-term infants. Extracted from Silbereis et al., 2010, Disease Models & Mechanisms 3, 678-688, p. 679. With permission from the Company of Biologists.

Cerebral Palsy (CP) is the consequence of brain defects (abnormalities or lesions) before or after birth (Fig. 1-1). It affects 2 to 3 children per 1000 birth in Western Europe and is referred to as the most common neuromuscular disability in childhood (Surveillance of Cerebral Palsy in Europe, 2000).

Considering that the German birth statistics (Statistisches Bundesamt, 2017) showed ~738.000 newborns in 2015, around 1500-2200 children were affected by CP only in that year. Worldwide a total prevalence of 17 million people has been reported (Graham et al., 2016).

Eighty percent of the brain insults occur in utero, 10% around the time of delivery and 10% in the post-neonatal period up to 2 years (Wimalasundera and Stevenson, 2016). A particular risk factor is a low gestational age (< 28 weeks) which raises the prevalence to 10 per 1000 (Hoon, 2005). Depending on the period of the insults and the brain development, different regions are typically affected (Krägeloh-Mann and Horber, 2007; Wimalasundera and Stevenson, 2016) (Fig. 1-1). The injury often results from a lack of oxygen supply, infection, stroke or hypotension, with a subsequent inflammatory response (Wimalasundera and Stevenson, 2016). Imaging techniques may help to clarify the nature of the insult, but ~14% of the children have no abnormal magnetic resonance scans (Krägeloh-Mann and Horber, 2007; Reid et al., 2014).

Sixty percent of brain deficiencies affect the periventricular white matter, 20% are grey matter lesions and 10% are brain malformations (Krägeloh-Mann and Horber, 2007). Periventricular white matter is directly next to the two ventricles (the cavities containing the cerebrospinal fluid). It is composed of connecting nerve fibres and myelin. Grey matter is composed of nerve cell bodies. A common reason for white matter damage is periventricular leukomalacia (softening and decay of the white matter) following intraventricular hemorrhage (Fig. 1-1 A) with decreased blood or oxygen supply (Krägeloh-Mann and Horber, 2007). By definition the brain injury itself is non-reversible but non-progressive (Bax et al., 2005). Promisingly, neuroplasticity research also focusses on whether the brain's adaptive potential can be shaped during neuro-rehabilitation in CP patients to provide coping mechanisms (Reid et al., 2015a). Contrarily, the consequences of CP for movement, posture and the musculoskeletal usually deteriorate (Bax et al., 2005).

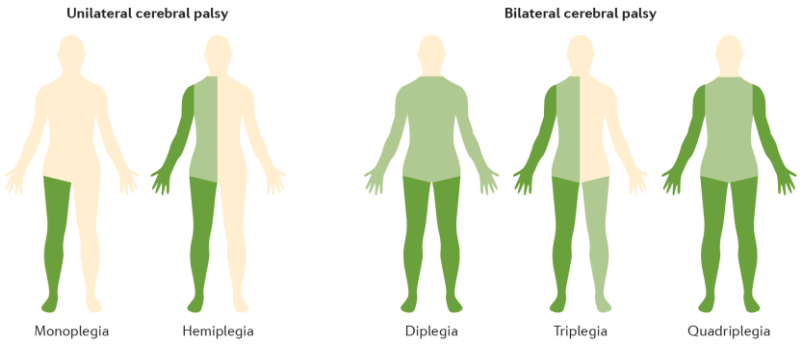

Fig. 1-2 Topographical description in CP. Extracted from Graham et al., 2016, Nat Rev Dis Primers 7, 2:15082, p.4. With permission from Nature Publishing Group.

1.2. Classification schemes

Overall, CP patients appear to be a rather heterogeneous group. A classification according to the topographic limb involvement: uni-, bilateral CP (Fig. 1-2), and the predominant movement disorder: spasticity, dyskinesia or ataxia (Fig. 1-3A), has been suggested (Surveillance of Cerebral Palsy in Europe, 2000). Generally speaking, CP is categorized as damage to the upper motor neuron (Rogers and Wrong, 2017) which means that the primary insult affects descending motor pathways (Fig.1-3 B) from the brain's outer layer to the spinal cord (Purves, 2008).

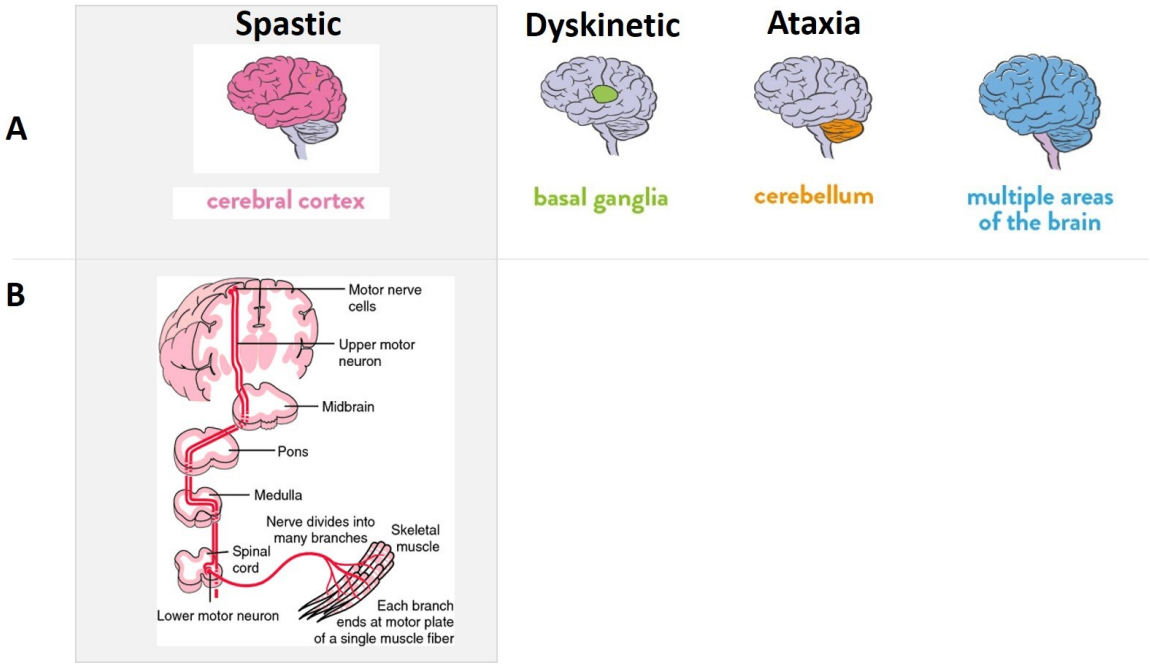

Fig. 1-3 Schematic description of affected brain parts and related disorders. A) Categorization according to affected brain regions and the predominant movement disorders. Note that also multiple areas of the brain might be affected at once. Extracted and adapted from https://www.gillettechildrens.org/get-involved/cerebral-palsy-awareness gallery-infographics-6 (Accessed Jan 31, 2017). With permission from gillettechildrens specialty health care. **B)** Path of Upper and lower motor neuron. Extracted and adapted from Damjanov, I. 2000. Pathology for the health-related professions, Philadelphia; London, Saunders, p. 467. With permission from Elsevier.

In the dyskinetic type, the dominant feature is the presence of involuntary movements whereas the ataxic type primarily displays shaky movements. The former is associated with lesions of the cerebellum (Fig. 1-3A) while the dyskinetic subtype is linked with lesions of the basal-ganglia (Albright, 2009; Purves, 2008). Both brain regions work synergistically to regulate movements (Albright, 2009). The ataxic and dyskinetic disorders are referred to as the extrapyramidal types (Pakula et al., 2009). The third group, which affects 85% of the children with CP, is the spastic subgroup (Surveillance of Cerebral Palsy in Europe, 2000). It is a disorder of the pyramidal tract (~outside the basal-ganglia and cerebellum) which involves nerve fibres traveling through the medullary pyramid, a white matter structure at the brain stem (Purves, 2008). The approximate subtype distribution is 55% for bilateral

spastic CP, 30% for unilateral spastic CP, 7% for dyskinetic, 4% for ataxic, with 4% being unknown (Surveillance of Cerebral Palsy in Europe, 2000).

While patients typically also face disturbances of sensation, cognition, communication or perception (Rosenbaum et al., 2008), the primary pathology of CP is located at the motor system (Verschuren et al., 2011). Consequently, the hallmark is a deficit in motor control (Damiano, 2009). Thus children in all subgroups display delayed motor milestones, e.g. later than usual crawling, sitting or walking. If a child with CP does not walk by the age of 2 years, more than two-third of the children will not achieve the ability to walk with or without support by the age of 6 years (Wu et al., 2004). From age 6 years onwards, there is typically less than 10% further capacity for improvements in their motor potential (Rosenbaum et al., 2002). The gross motor function level is a rough categorization of the children's ambulatory function and distinguishes 5 levels with gradually worse ambulatory skills and increased reliance on assistive devices (Palisano et al., 1997; Palisano et al., 2008). Registries show that around 60% of patients with CP can be classified in level I and II (Reid et al., 2011), so they are able to walk independently without mobility aids such as crutches, walkers or wheelchairs. Nevertheless, in comparison to typically developing peers, their ambulatory mobility as a teenager is reduced (Bjornson et al., 2007). Among other factors, this is likely influenced by increased energy demands (Kerr et al., 2008) or safety concerns, e.g. about stumbling and falling when walking in crowds or over uneven terrain (Palisano et al., 2009).

Patients with CP will usually require life-long care from multiple disciplines, such as from occupational and physical therapists, specialized orthopedics or neurologic physicians and also from technicians producing adaptive equipment or orthotics. Particularly their musculoskeletal disorders affecting the lower limbs are focused on from infancy and beyond. One of the primary aims for therapy in ambulatory patients is to facilitate locomotion in order to increase the patient's activity and enable them to participate in daily activities.

1.3. Major impairments

Calf muscles are thought to considerably contribute to the movement pathology and therefore they are often targeted by invasive and conservative approaches (see section 4). Before getting to their role in gait (section 1.4.), the author would like to provide a short summary about two major features of the movement disorder in CP: paresis and increased resistance of muscles to stretch. Both will be explained by referring to features of the plantarflexor muscles. Increased resistance of muscles to stretch can be neurally or non-neurally mediated which will be outlined in 1.3.2. It is acknowledged that further aspects, such as a lack of selective motor control, which is manifested as synergistic and involuntarily coupled movement patterns, can be considered disabling as well (Chruscikowski et al.,

2016; Ostensjo et al., 2004). Yet, since these aspects are not specifically targeted during the current thesis, they will not be addressed in further detail.

1.3.1. Paresis

Muscle paresis in CP is caused by disrupted voluntary commands which, simply put, results in weakness. A lack of limb strength is generally detrimental since it is a significant negative predictor for declined motor function in CP (Ross and Engsberg, 2007). Plantarflexors are quite severely involved. During instrumented strength tests, plantarflexors of children with uni- or bilateral CP display about 40-70% decreased concentric (Ross and Engsberg, 2002) or isometric strength levels (Downing et al., 2009; Elder et al., 2003; Stackhouse et al., 2005; Wiley and Damiano, 1998) with respect to values of typically developing controls.

From a morphological perspective, the plantarflexors' active isometric force is proportional to their cross-sectional area (Fukunaga et al., 1996). Therefore, reduced size of spastic muscles in CP children (see 2.2.) could have a negative influence on strength. Fukunaga et al. (1996) also suggested that, in general, differences in fibre type play a role for torque production capacity, with slow muscles having a lower specific tension (force per unit area). Indeed, some studies in CP reported that the gastrocnemii (Ito et al., 1996) or that the general triceps surae muscles (Marbini et al., 2002) display a shift towards slower muscle fibre types (see 2.1.). Apart from that, an increased portion of the spastic plantarflexors seems to be taken up by intramuscular fat (Noble et al., 2014a) which reduces the actual amount of contractile tissue within a given muscle volume.

Apart from these structural features, neural deficits promote paresis. When relating the maximal voluntary isometric torque of the plantarflexors in CP to the underlying cross-sectional area, the relative torque production capacity is limited (Elder et al., 2003). Since this was shown for affected and non-affected limbs (Hussain et al., 2014; Elder et al., 2003), recruitment deficits of central origin likely have an impact on muscle force production in CP as well. Hence, from a neuro-physiological perspective, children with CP have a reduced ability to fully activate their plantarflexors (Elder et al., 2003; Rose and McGill, 2005; Stackhouse et al., 2005). It is therefore likely that only a reduced fraction of the muscle is voluntarily activated. Furthermore, Rose and McGill (2005) suggested that patients with CP are unable to recruit higher threshold motor units or drive lower threshold motor units to higher firing rates. In addition, increased antagonistic co-activation during maximal plantarflexor contractions has been noted (Elder et al., 2003; Stackhouse et al., 2005). This was thought to limit agonistic force production. Yet in adults with unilateral CP, antagonistic co-activation of tibialis anterior muscle was no negative predictor of plantarflexor torque production during strength tests (Hussain et al., 2014).

Moreover, Dietz and Sinkjaer (2007) argued that neural dysfunction, e.g. increased muscle tone, may also compensate for paresis although findings in CP are heterogeneous: While increased passive joint resistance seems not to be related to reduced plantarflexor strength (Ross and Engsberg, 2002), weaker children indeed display increased reflexes (Poon and Hui-Chan, 2009). Nevertheless, Poon and Hui-Chan (2009) also suggested that paresis and spasticity are separate dysfunctions of the motor system in CP.

1.3.1.1. Assessments

To evaluate the extent of paresis in orthopedic practice, maximal voluntary plantarflexor strength is typically manual evaluated and subjectively graded from 0 (~no contraction) to 5 (~normal strength) (Medical Research Council, 1981). Alternatively, the number of unilateral heel raise repetitions is counted (Dreher et al., 2012; McNee et al., 2009). For the heel raise test, age-dependent normative values exist (Lunsford and Perry, 1995; Maurer et al., 2007; Yocum et al., 2010), e.g. fewer than 13 repetitions have been considered conspicuous for children (Maurer et al., 2007). Notably, heel raises may reflect primarily strength endurance. Although repeatability for heel raise tests in children with CP older than 6 years seems to be high (van Vulpen et al., 2013), the discriminatory power of such tests is limited since CP children may frequently not be able to perform a single heel raise.

For a more objective analysis, portable hand-held dynamometric devices can be easily implemented in routine diagnostics. For maximal isometric plantarflexor strength tests, an acceptable standard error of the measurement of 2.7 Nm has been reported when averaging 3 repetitions from hand-held dynamometry (van Vulpen et al., 2013). The smallest detectable change was reported to be 25-39% (Taylor et al., 2004; van Vulpen et al., 2013). It is noteworthy to state that using an isokinetic dynamometer has been considered to be difficult in children with CP (Jung et al., 2013). Nevertheless, several studies successfully implemented them for plantarflexor strength diagnostics in children with CP (Brouwer et al., 1998; Stackhouse et al., 2005) and in young adults with CP (Barber et al., 2012). However, due to the associated costs, isokinetic devices are frequently not available in medical treatment centers which are dedicated to pediatric orthopedics. To the best of the author's knowledge, no study directly compared the precision of isokinetic or hand-held devices for plantarflexor strength tests in children with CP. Considerable variability between tests may be attributable to the patients rather than to the equipment.

In summary, active force production in plantarflexors of children with CP is considerably limited. Apart from reduced muscle size or altered compositions (see 2.1 and 2.2.), CP patients face difficulties to maximally activate their muscles. While routine clinical strength tests may have little discriminatory power, hand-held dynamometric devices may be a cost effective alternative for providing objective data on strength.

1.3.2. Pathologically increased muscle stretch-resistance

The origin of the pathologically increased stretch-resistance in CP has been classified into neural and non-neural aspects (Fig. 1-4) (van den Noort et al., 2016).

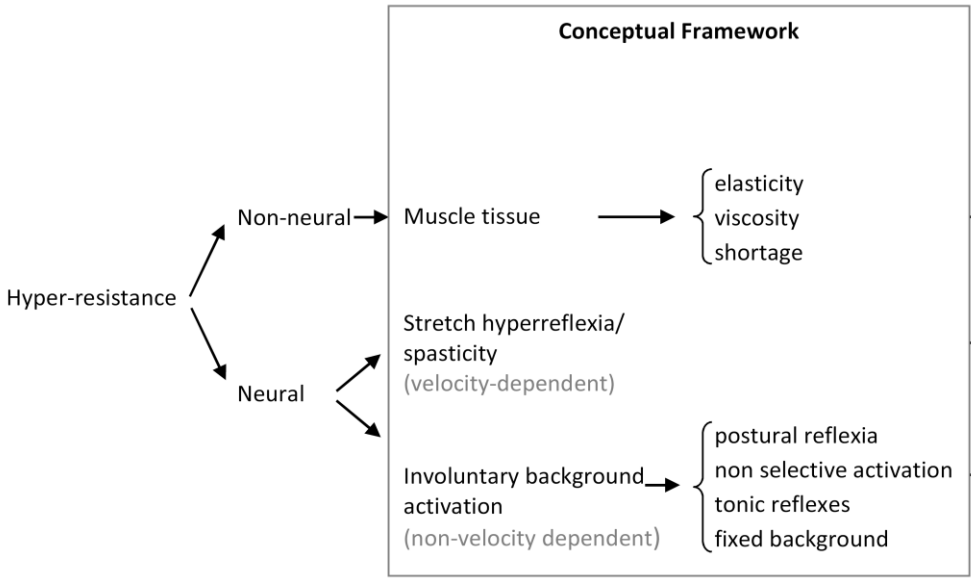

Fig. 1-4 Contributions to pathophysiologically increased muscle stretch-resistance in CP. Extracted from van den Noort et al., 2016, Gait Posture. 49S, 112, p. 112. With permission from Elsevier.

1.3.2.1. Neural origin of muscle stretch-resistance

Spasticity is a ubiquitously used term in the context of CP often criticized for being non-specifically used to describe manually perceived resistance when examining passive joint mobility (Harlaar, 2016). Of note, spasticity should be considered a precise sign rather than a collection of different symptoms (Lin, 2011). It was initially defined as 'a velocity-dependent increase in tonic stretch reflex' (Lance, 1980). Recently a consensus (Fig. 1-4) highlighted that spasticity itself can be one portion of the increased resistance that is felt externally at the joint level, with spasticity itself being velocity dependent hyperreflexia (van den Noort et al., 2016).

Unlike hyperreflexia, there can also be some non-velocity dependent increase in activation of a muscle in CP, referred to as dystonia. It features sustained muscle activity at rest which may be further raised by slow stretch. Thus dystonia may be best placed under involuntary background muscle activation (Fig. 1-4). Consequently, dystonia is also defined as stretch-sensitive tonic muscle contraction (Gracies, 2005b). Dystonia is arousal dependent, e.g. it disappears during sleep, which is not the case for spasticity (Graham et al., 2016). Apart from affecting passive stretch, it can alter limb posturing and, during active motion, dystonia may cause repetitive, tremulous like movements. Dystonia is thus categorized as a disorder of posture and movement (Graham et al., 2016).

The third group of alterations that contributes to increased resistance against stretch is non-neural in origin (see 1.3.2.2.) and is most likely due to alterations in muscle-tendon dimensions and properties.

The major consequence of spasticity is a lack of control over spinal reflexes (Mukherjee and Chakravarty, 2010). Both the input from brain to spinal cord, as well as the interaction between spinal cord and muscle-tendon complexes is altered (Bar-On et al., 2014b; Mukherjee and Chakravarty, 2010). First, for the input from the brain to the spinal cord, 3 descending pathways are mainly responsible (Mukherjee and Chakravarty, 2010): the cortico-spinal, the reticulo-spinal, and the vestibulo-spinal tract. Concerning the cortico-spinal tract (from cortex onto motor neurons in the spinal cord), spasticity is only thought to be caused if the lesions include the premotor and supplementary motor areas (Mukherjee and Chakravarty, 2010). Peacock (2009) and Bar-On et al. (2014b) emphasized that spasticity appears to be a result of damage or abnormal input to the vestibular and reticular nuclei (network of a neurons) or their tracts. Both act to control muscle tone in the lower limbs. Damage of the reticulo-spinal tract decreases its inhibitory influence of spinal motor neurons, resulting in increased muscle tone. The vestibulo-spinal tract is involved in balance and anti-gravity support stimulating extensors. It is connected to the cortex, which inhibits its activity. Thus damage of the vestibulo-spinal tract may lead to flexed limb postures (Bar-On et al., 2014b; Peacock, 2009).

Second, for the interaction between spinal cord and muscle-tendon complex, the main decreased inhibitory spinal mechanisms include decreased reciprocal inhibition (antagonist inhibition by contraction of the agonist) (Leonard et al., 2006) and decreased post activation depression (reflex amplitude depression upon recurrent stimulation) (Achache et al., 2010; Bar-On et al., 2014b).

Concerning the plantarflexors, it was shown that with increasing levels of tibialis anterior contraction, the motor neuron inhibition of soleus (agonistic muscle) is impaired in patients with SCP (Leonard et al., 2006). Additionally, Achache et al. (2010) found impaired post-activation depression in the soleus in young adults with SCP and revealed additional reduced pre-synaptic inhibition of afferent inputs from the muscle spindles. Altered supraspinal drive from higher structures on presynaptic interneurons was thought to be responsible (Achache et al., 2010). A reduction in pre-synaptic inhibition will lead to an increased neurotransmitter release on the motor neuron and reduce the depression of reflexes. Further, missing inhibition from afferent input from golgi-tendon organs delivering information about tendon tension could play a role (Mukherjee and Chakravarty, 2010).

In addition to missing inhibitory spinal mechanisms, exaggerated reflexes might result from hyperactivity of gamma motor neurons (Dietz and Sinkjaer, 2007). They provide the excitation of muscle spindles and are supposed to regulate their tension. This helps provide sensory information about the degree of lengthening or shortening of skeletal muscle fibres (Hammerstad, 2007). A further

aggravating factor for increased reflexes in CP could be hyper-excitability of alpha moto neurons with an amplified and prolonged response to excitation which causes plateau potentials. This is probably related to altered membrane properties (Mukherjee and Chakravarty, 2010).

1.3.2.2. Non-neural origin of muscle stretch-resistance

The second group of alterations that contribute to increased resistance against stretch in children with CP (Fig. 1-4) is likely due to alterations in muscle or tendon dimensions and properties and thus are non-neural in origin.

Firstly, several studies dealt with morphological and mechanical factors affecting passive ankle joint motion in healthy humans: Clearly, the length and compliance of a muscle belly and its tendon can be detrimental for joint flexibility during a stretch maneuver: In adult gastrocnemius muscles, 27-60% of the whole muscle-tendon unit elongation during passive stretch are due to elongation of the muscle fascicles (deMonte et al., 2006; Herbert et al., 2002; Hoang et al., 2007b). These variable percentage values may depend on methodological considerations during testing, e.g. if direct measurements or model-based estimations of the muscle tendon-unit length have been used to calculate tendinous tissue length. Despite being fairly stiff, the tendon (intra-muscular and extra-muscular part) may considerably contribute to the length change of the muscle-tendon unit, e.g. due to tautening of tendon slack and due to its much longer length compared to fascicles. Nevertheless, the relative lengthening demands on muscle fascicles, expressed as percentage change in length (~strain), are much greater than those on the tendon (deMonte et al., 2006; Hoang et al., 2007b). In a refined analysis including information about tendon curvature during dorsiflexing stretch, the actual passive resistive Achilles tendon strains are quite small (2-3%) (deMonte et al., 2006). Still, Kawakami et al. (2008) reported that stiffness of the medial gastrocnemius tendon is one of the limiting factors for ankle joint flexibility. The authors found a negative association between tendon and muscle belly elongations during passive stretch. They reasoned that stiffer tendons lead to larger lengthening of fascicles and may therefore also cause a more severe sensation of muscle stretch (Kawakami et al., 2008).

Apart from that, it is not exactly known to what extent muscle thickness contributes to passive joint flexibility (Weppler and Magnusson, 2010). In middle-aged to old adults, maximum passive dorsiflexion negatively correlates with calf muscle thickness, suggesting that thicker muscle bellies limit passive joint range of motion (Kawakami et al., 2003). Also for elbow-flexors (Chleboun et al., 1997) and hamstrings (Magnusson et al., 1997), negative relationships between measures of muscle size and joint flexibility have been reported in neurologically intact individuals. These associations might be also displayed during maturation. In typically developing children and youth, a decline in passive dorsiflexion is usually noted during maturation (Benard et al., 2011; Weide et al., 2015). Weide et al. (2015) argued that augmented contractile material in parallel could be responsible for the increased

plantarflexor resistance to stretch. Alternatively, since during maturation the gastrocnemius tendon stiffness also increases (Waugh et al., 2012), higher tendon stiffness might also have a negative impact on passive dorsiflexion.

Considering that similar to muscle size, tendon stiffness is highly responsive to mechanical loadings (Bohm et al., 2015), larger muscle size and increased tendon stiffness may frequently occur in concert in trained subjects. Both factors may in principle limit passive dorsiflexion. In addition, intrinsic properties other than muscle mass per-se may be decisive for altered joint-stiffness, since passive plantarflexor muscle stiffness is higher in long distance runners than in untrained men, despite no difference in cross-sectional muscle area (Kubo et al., 2015). So, in typically developing children or in trained adults a decline in dorsiflexion could reflect a history of increased loading instead of degenerative processes.

In patients with CP, however, degenerations due to disuse and immobilization are likely to be the cause of limited dorsiflexion and increased joint stiffness. Amongst others, alterations on microstructural muscle level (see 2.1), e.g. resting filamentary tension, different MHC isoforms, altered Titin or connective tissue properties may generally contribute to increased stretch resistance (Gajdosik, 2001). Frequently cited models for non-neural stretch resistance in children with CP are animal studies with immobilizing interventions (Blanchard et al., 1985; Tardieu et al., 1977; Williams et al., 1988; Williams, 1990; Williams and Goldspink, 1978; Brown et al., 1999). In principle, muscles adapt to a shortened or lengthened immobilization, such that the maximum of force exertion coincides with the immobilized position (Williams and Goldspink, 1978). So, in adult muscle-tendon units, immobilization under tension results in sarcomere addition in series (fibre length increase), while the immobilization in unstrained, shortened positions results in sarcomere loss (fibre length reduction). The latter may be analogous to chronically shortened muscles in CP patients. Obviously, next to length adaptations, cross-sectional atrophy occurs in response to immobilization, which is promoted if a muscle is kept on short length (Dupont Salter et al., 2003; Spector et al., 1982) and also intrinsic properties of muscles may change, e.g. more stiffness per gram of muscle tissue has been observed in immobilized animal muscle (Brown et al., 1999).

In children with CP, the degree of chronic plantarflexor shortening, termed contracture, usually deteriorates with increasing age (Hägglund and Wagner, 2011). Yet few studies specifically documented the 'untreated' progression: McNee et al. (2007) reported a decline of around -2.5° in maximum passive dorsiflexion in 12 weeks. Maas et al. (2014) observed a reduction of -3.8° in 6 weeks. In spastic paretic disorders that do not involve intrauterine development (e.g. stroke or spinal cord injury), degenerative and trophic muscle changes seem to be a rather immediate response after a central lesion while the muscles' inability to relax usually becomes more prominent later on (Gracies,

2005b). Less is known about the temporal cascade in CP but various general pathways for contracture formation have been suggested: The primary speculation in CP children is that muscle length is not able to keep up with bone growth and thus muscle-tendon units are too short and restrict passive joint excursion (Dayanidhi and Lieber, 2014; Graham et al., 2016). This is referred to as the neurogenic hypothesis of inhibited growth (Hof, 2001). Hof (2001) further distinguished muscle length adaptation due to hypertonia. Here it was explained that chronic muscle activity is thought to keep muscle-tendon units in a shortened position which may induce length dependent muscle atrophy (Mathevon et al., 2015). This will supposedly be aggravated by disuse and immobilization (Gracies, 2005a). Generally, CP has been viewed as a mixture between unloading due to disuse or immobilization and chronic overloading due to spasticity (Lieber, 2010, p. 271ff). Notably, in most mildly affected children, the chronic tension due to resting muscle activity will presumably be of rather low load which may argue against overloading. Finally, as reasoned by Gracies (2005b), there could be a kind of viscous cycle between atrophy and stretch sensitivity. For a shorter muscle, any external pull may lead to a greater rate of length change and thus a more direct transmission to muscle spindles (Maier et al., 1972). This may in turn lead to avoidance of such stretch ranges and promote short length immobilization in children with CP.

1.3.2.3. Assessments

During pediatric orthopedic practice, the extent of triceps-surae contracture is traditionally manually examined by ruler-based goniometry. Distinctions are usually based on evaluations with flexed and extended knees, the latter seeming to be more reliable in typically developing children (Evans and Scutter, 2006). For repeated measurements in children with CP, Fosang et al. (2003) reported a standard error of the measurement of ~ 4–6° for maximum passive dorsiflexion within the same examiner.

Next, as with the manual strength tests, a grading of the resistance throughout a manually applied stretch is part of the clinical routine. This is mostly done by using the (modified) Ashworth (Bohannon and Smith, 1987) or (modified) Tardieu Scale (Boyd and Graham, 1999), for example. Both tests rank the resistance on ordinal scales, are rather subjective, and lack reproducibility (Fosang et al., 2003; Mutlu et al., 2008; Yam and Leung, 2006). They have also been criticized for measuring different concepts of muscle stretch resistance: Scholtes et al. (2006) reasoned that Ashworth-Scales grade muscle tone per-se, while Tardieu-Scales grades its velocity dependent increase. The latter appears to be more in-line with the initial definition of spasticity by Lance (1980). Still, neither modified Ashworth scores (de Gooijer-van de Groep et al., 2013; Willerslev-Olsen et al., 2013), nor Tardieu scores (de Gooijer-van de Groep et al., 2013), nor other manual spasticity scorings (Poon and Hui-Chan, 2009) seem to be associated with the instrumentally determined reflexive torque or passive ankle torque measurements during dorsiflexion stretches (Fig. 1-5).

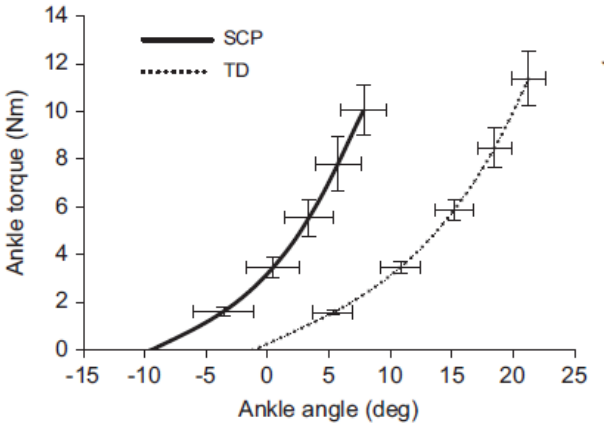

Fig. 1-5 Passive ankle joint moment-angle relationship in CP. Assessments were performed with an isokinetic test device in young adult patients with spastic Cerebral Palsy (SCP) and in typically developing controls (TD) during slow dorsiflexion stretches at 20°/sec. Note the steeper slope of the curve as well as the reduced range of motion in patients with SCP. Extracted from Barber et al., 2011, J Biomech 44, 2496-2500, p. 2499. With permission from Elsevier.

Thus, using instrumented tests, more precise information about the amount and source of increased joint resistance can be gathered. Since fast muscle stretches provoke reflex activity, this was used to study the neural stiffness in plantarflexors in children with CP. Several studies thereby showed that the resistive torque rises abnormally sharply with increasing speed of ankle joint rotation (de Gooijer-van de Groep et al., 2013; Poon and Hui-Chan, 2009; Sloot et al., 2015b; Willerslev-Olsen et al., 2013). Thresholds for reflex excitation of the spastic soleus muscle were reported to be surpassed at median angular speeds of 36°/s, while in typically developing children the reflex response was induced at a median of 101°/s (Willerslev-Olsen et al., 2013). Although velocity-dependent gain of muscle activation is a frequent alteration in CP, increased stretch reflexes only contribute to increased plantarflexor stiffness in about every fifth child (Willerslev-Olsen et al., 2013). On the other hand, nearly every third CP child displays slight position dependent plantarflexor activity during slow dorsiflexion stretches, indicating larger neural sensitivity to increased muscle length (Bar-On et al., 2014a).

Apart from these neural sources, increased joint stiffness during slow and fast stretching in CP patients has been primarily attributed to higher tissue stiffness (Willerslev-Olsen et al., 2013; de Gooijer-van de Groep et al., 2013; Sloot et al., 2015b). During slow stretch maneuvers, the pathological increase in ankle joint stiffness with respect to controls varies from +135 to +340% (Alhusaini et al., 2010; Barber et al., 2011a; de Gooijer-van de Groep et al., 2013; Peng et al., 2011; Ross et al., 2011). Motor-driven isokinetic devices have been frequently used in CP patients for the assessment of such resistive stiffness values (Barber et al., 2011a; de Gooijer-van de Groep et al., 2013; Ross et al., 2011; Willerslev-Olsen et al., 2013). While such set-ups may be considered most standardized, they have been considered impractical in pediatric populations due to limited compliance (Bar-On et al., 2014b).

Therefore, manually geared machines (Peng et al., 2011) or handheld-devices (Alhusaini et al., 2010; Bar-On et al., 2013; Boiteau et al., 1995) have been implemented during the assessment of ankle joint stiffness in CP. These manual instruments have been shown to deliver fairly reproducible measures, e.g. the resistive force encountered at a given ankle angle during slow stretch movements showed an ICC of 0.79 and a coefficient of variation of 13.9% (Boiteau et al., 1995).

In summary, spasticity is a rather loosely used terminology in CP which is often utilized to describe all sources of the perceived resistance during manual muscle stretching. At fast stretch velocities, there is an abnormal gain of muscle activation in children with CP. Instrumented tests further revealed that CP children also display higher resistive torques during very slow stretches. So, largely independent of muscle activation, altered muscle-tendon dimension or properties (see 2.1 and 2.2) are thought to have a negative impact on joint flexibility.

Increased joint resistance and limited dorsiflexion range of motion is usually seen as the pathological representation of contracture formation due to chronic plantarflexor muscle-tendon unit shortening. Although the cascade leading to contracture in CP is not clear, animal experiments suggest that immobilization at short muscle-tendon unit length may promote muscle wasting. Hence this model is also used to explain maladaptations within spastic calf muscles.

1.4. Causes and consequences of equinus gait

The function of plantarflexors during walking is to control tibia advancement, supply propulsion and, in the case of gastrocnemius, accelerate the forward swinging leg (Arnold et al., 2005; Neptune et al., 2001). Further, plantarflexors also contribute to a more upright gait, e.g. the soleus controls tibia advancement and indirectly affects knee extension (Arnold et al., 2005) while both the soleus and the gastrocnemius were shown to provide vertical center of mass accelerations (Steele et al., 2013).

Children with SCP often walk in equinus which means that they contact the ground with the fore- or midfoot and lack dorsiflexion excursion. Experts assume that the gastrocnemius is generally more involved in equinus pathologies (Sees and Miller, 2013). Equinus gait affects 63-64% of the children classified in Gross Motor Function Classification System (GMFCS) Level I and II (Rethlefsen et al., 2016). The lack of dorsiflexion in swing supposedly increases the risk of tripping while the limited base of support during stance may impair balance (Goldstein and Harper, 2001). The consequences are not locally limited to the ankle joint: Concerning the knee, excessive flexion (48-61% prevalence) or hyperextension in stance may be related (20% prevalence). During swing, 41-60% display an additional lack of kneeflexion (Rethlefsen et al., 2016). Additionally, children with equinus are at risk for

developing complex bony foot deformities and 5-24% of children in GMFCS I-II develop valgus feet with a sagged midfoot (Rethlefsen et al., 2016).

Equinus gait usually gets less prominent in older children with CP since young tip-toe walkers, primarily children classified in GMFCS I (Rethlefsen et al., 2016) and bilaterally affected children (Wren et al., 2005) seem to be prone to walk with increased knee flexion later in life (Rethlefsen et al., 2016; Sees and Miller, 2013). Thus, a reduced equinus posture during gait may not indicate that the underlying muscle dysfunction or contracture of the calf has been resolved. Quite the contrary, a progressive reduction of passive dorsiflexion is often over time related to more knee flexion during gait (Maas et al., 2015). This is presumably caused by structural shortness of the bi-articular gastrocnemius. On the other hand, despite a loss in passive dorsiflexion, some CP children can also maintain adequate knee flexion during gait (Bell et al., 2002). Whether children sort of vertically collapse during gait is probably influenced by others factors such as plantarflexor weakness. Notably, also patients with idiopathic gastrocnemius tightness compensate their deficits either at the knee or at the ankle during walking (Chimera et al., 2012; You et al., 2009).

As stated at the beginning of this section, plantarflexors provide propulsion and thus reduced isometric plantarflexor strength of children with CP is associated with less ankle joint power generation while walking (Dallmeijer et al., 2011; Eek et al., 2011). In addition, it had been shown that both reduced ankle range of motion during gait and decreased ankle joint propulsion increases the energy expenditure index (relationship between heart rate and walking speed) during gait of CP children (Pouliot-Laforte et al., 2014).

Searching for the causes of equinus gait, it is assumed that an accentuated strength imbalance between hypertonic plantar- and weak dorsiflexors favors walking in equinus (Hof, 2001). However, the majority of studies report that on average the relative weakness in dorsiflexors is somewhat less pronounced than that of plantarflexors (Downing et al., 2009; Elder et al., 2003; Ross and Engsberg, 2002; Wiley and Damiano, 1998; Hussain et al., 2014) vs. (Poon and Hui-Chan, 2009).

In fact, it appears debatable whether equinus posturing during gait is a cause for functional weakness or an adaptive strategy. On the one hand, computer simulations suggest that toe walking requires more neural muscle activity due to non-optimal conditions concerning the plantarflexors' muscle force-length relationship (Neptune et al., 2007). This may lead to premature fatigue. Experiments on gait of typically developing children also suggest that there is a negative impact of equinus posture on plantarflexor force production: When artificially restricting dorsiflexion during gait (Houx et al., 2013) or when voluntarily walking in equinus (Davids et al., 1999), kinetic measures of ankle joint propulsion decline. On the other hand, instrumented strength tests show that the maximal plantarflexor force generating capacity of CP patients is shifted towards plantarflexion angles (Barber et al., 2012; Brouwer et al., 1998). Therefore equinus posturing during gait could be an adaptive

strategy to provide adequate force output. Eventually, since ankle moments during walking exceed active moment generation during strength tests in children with CP (Dallmeijer et al., 2011; Eek et al., 2011), it was speculated that the loss in passive joint motion should be regarded as an adaptive mechanism in order to rely on passive structures to substitute for missing active strength.

Another cause for equinus gait could be neural dysfunction of spastic plantarflexors. This is quite a controversial topic: Prolonged or abnormally timed plantarflexor activity, as well as co-contraction with dorsiflexors are frequently considered attributes of disturbed neural control. Since young CP patients, often have more pronounced equinus posturing while walking than upon clinical examination, the dysfunction is thought to be dominated by disturbed neural control and not by contracture (Goldstein and Harper, 2001). This is also described as 'dynamic equinus' (Goldstein and Harper, 2001). Yet, this terminology is rather confusing: To distinguish dynamic from fixed equinus, typically a cut-off for passive dorsiflexion is chosen, e.g. max. 5° (Zwick et al., 2004) or neutral ankle alignment (Wren et al., 2010). This may ultimately need to be verified with the patient under anesthesia (Dreher et al., 2012). However the degree of contracture formation is probably difficult to judge. Since CP children show a lack of volumetric muscle growth of the gastrocnemius at a very early age (Barber et al., 2011b) (see 2.2.1) and since dorsiflexion seems to be progressively lost during maturation (Hägglund and Wagner, 2011), a continuum of pathological muscle structural changes appears more reasonable.

Furthermore, mimicry studies pointed out that EMG features during gait, such as premature gastrocnemius activity at the transitions from swing to stance phase, or co-activation of the tibialis anterior and gastrocnemius are representative for toe-walking per-se and not unique to equinus in CP (Davids et al., 1999). Besides, both children with CP and children who walk on their toes for non-neurological reasons display rather similarly altered timing of gastrocnemius and tibialis anterior activity during gait (Rose et al., 1999). Apart from timing issue, there is little knowledge about the role of exaggerated reflexes, however they are thought to be of minor disabling effect (Dietz and Sinkjaer, 2007). For example, concerning children with unilateral CP, Willerslev-Olsen et al. (2014a) showed that exaggerated soleus reflexes did not impede foot lift and further concluded that a reduced central drive to dorsiflexors might have a stronger effect on the landing pattern of the foot. –Still, neural dysfunction of plantarflexors might also be disabling since, for example, increased firing frequencies in the EMG signal of calf muscles of children with CP were reported (Lam et al., 2005; van Gestel et al., 2012). Also, there is preliminary evidence that this reflects muscle weakness (van Gestel et al., 2012). How this relates to equinus posturing is yet unknown.

In summary, a lack of passive dorsiflexion seems to have a detrimental influence on ankle and knee function during gait of children with CP, predisposes for developing foot disorders, and may contribute to a slower and inefficient gait pattern. Apart from structural shortening of the plantarflexor muscle-tendon units, the primary neurological dysfunction seems to be an impaired central drive for force production. Hyper excitability of plantarflexor reflexes during gait has not been directly proven to be dysfunctional. It appears plausible that equinus posturing may be predominantly a consequence of the biomechanical restrictions imposed by the musculoskeletal system and to a lesser extent affected by abnormal muscle activity.

1.5. Pathology on muscle-tendon level in Cerebral Palsy

In the following section, findings from the literature about micro- and macroscopic alterations in muscles of patients with CP will be examined. Findings will be organized from a whole organ level to the cellular level of skeletal muscle tissue (Fig. 1-6 and 1-7).

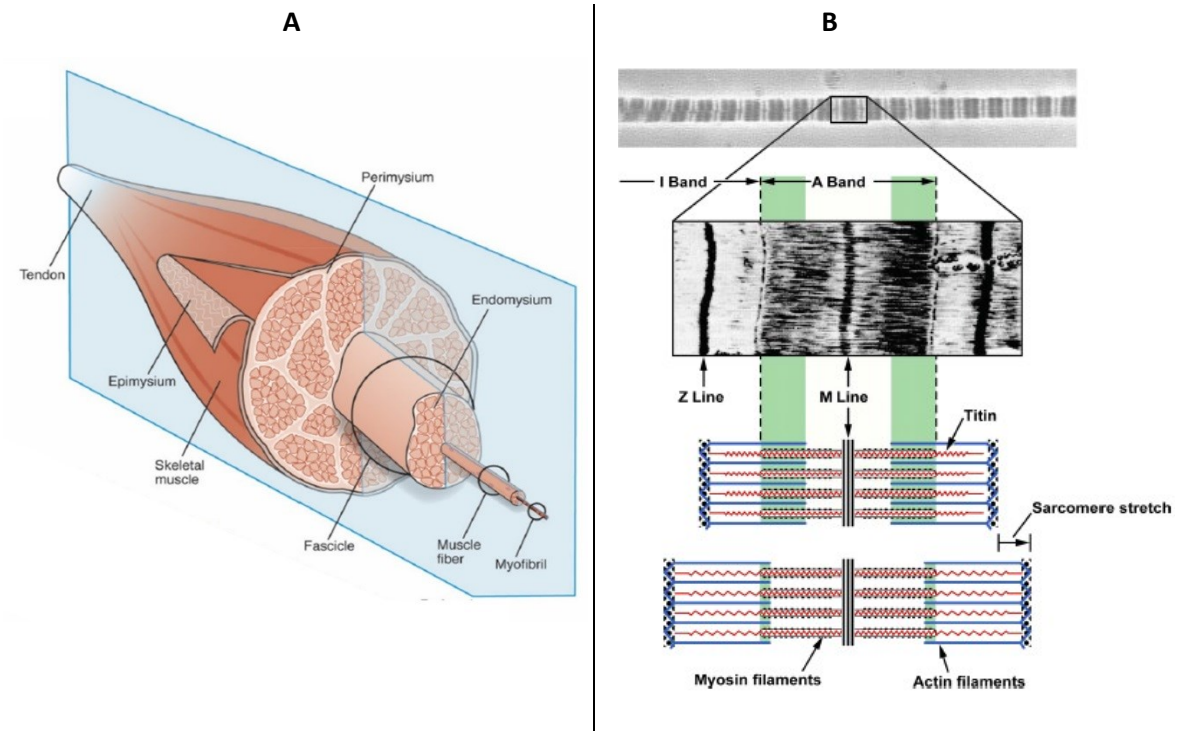

Fig. 1-6 Structural hierarchy of skeletal muscle. A) From whole organ to myofibrils. Note that the connective tissue is organized around groups of myofibres in the perimysium, the structure surrounding single myofibres is referred to as endomysium and the tissue surrounding whole muscle is reflected as epimysium. Extracted from Gillies and Lieber, 2011, Muscle Nerve 44, 318-331, p. 19. With permission from John, Wiley and Sons. B) Sarcomere structure with contractile filaments: actin, myosin, as well as the anchoring protein titin. Extracted from Leonard and Herzog, 2010, Am J Physiol Cell Physiol 299, C14-20, p. 15. With permission from the American Physiological Society.

1.5.1. Microscopic alteration

The presented findings on microstructural alterations are derived invasively from biopsies and may assist in interpreting results on higher structural levels (section 2.2.). To structure this section, findings will be organized from discoveries on smallest to largest scales.

1.5.1.1. Satellite cells

Satellite cells are muscle stem cells located alongside myofibres (Fig. 1-7). They can renew themselves, proliferate and fuse with myofibres to form new muscle tissue and are thereby thought to participate in muscle growth (Dayanidhi and Lieber, 2014).

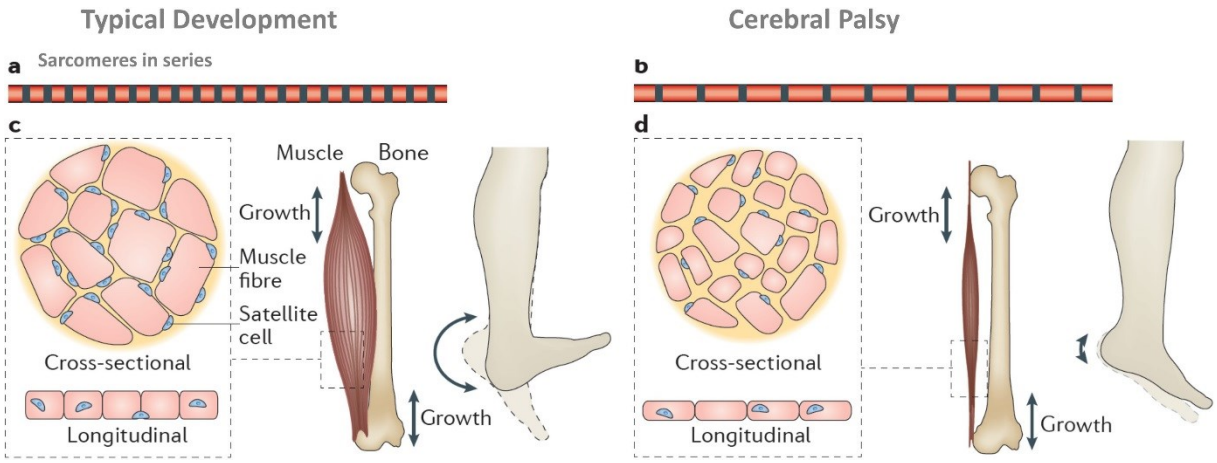

Fig. 1-7 Schematic representation of microscopic changes in muscles of CP children. Left side: Typically developed muscle (a,c). Right side: Alterations with Cerebral Palsy and the consequences on reduced joint range of motion during growth. Longer sarcomere lengths and less sarcomeres in series (b), reduced satellite cell content and decreased fibre cross-sectional area (d). As the bone grows, muscles are probably not able to keep up and joint range of motion decreases. Extracted and adapted from Graham et al., 2016, Nat Rev Dis Primers 7, 2:15082, p.8. With permission from Nature Publishing Group.

Children with CP have a reduced number of satellite cells, as shown for gracilis and semitendinosus muscles (Dayanidhi et al., 2015; Smith et al., 2013). Usually, one presumed factor involved in activation of satellite cells is mechanical muscle stretch. In a recent animal experiment, Kinney et al. (2016) studied chronic stretch casting in a mouse model with artificially reduced satellite cell content (similar to children with CP). The mouse soleus displayed an uncompromised serial sarcomere addition but fibrotic extra-cellular matrix changes and reduction in fibre cross-sectional area were noted. A reduced satellite cell pool in CP muscle may thus in principle not impede adaptations in fascicle length via serial sarcomere addition.

In summary, satellite cell content appears to be reduced in muscles of children with CP which may limit their adaptive potential to grow in size in response to external stimuli.

1.5.1.2. Titin

Titin has been primarily studied in animals (Leonard and Herzog, 2010; Prado et al., 2005). Within sarcomeres, this anchoring protein connects myosin to z-disks (Fig. 1-6) and is involved in both active and passive force production (Leonard and Herzog, 2010). In absence of titin, no passive forces can be produced. Notably, according to Leonard and Herzog (2010), the active forces produced in myofibrils beyond filamental overlap are also based on titin-actin binding which causes shortening of the titin spring length, a mechanism that might protect against stretch-induced muscle injuries. Prado et al. (2005) showed that increased passive fibre stiffness is usually associated with smaller titin mass. Several studies noted differences concerning titin in children with CP. In general, shifts in titin isoforms may compensate for overstretched sarcomeres, potentially making them more compliant (Larkin-Kaiser et al., 2015). For the gracilis muscle, Larkin-Kaiser et al. (2015) found that larger molecular weight of titin was associated with increased sarcomere length. This would be in-line with results of Mathewson et al (2014) showing that titin molecular weight is increased in the triceps surae of CP children. Concerning the gracilis and semitendinosus of children with CP, the titin mass was reported to increase as well (Smith et al., 2011). However, no significant correlation was found between titin weight and passive stiffness of the muscle fibres in CP children (Mathewson et al., 2014).

In summary, there is evidence that titin mass is increased in spastic muscles which is thought to compensate for increased sarcomere length and counteract decreased compliance of muscle tissue.

1.5.1.3. Muscle fibre types

Fibre types can be mainly distinguished according to their dominant myosin-heavy chain content in slow fibres (type I), fast oxidative fibres (type IIa) or fast glycolytic fibres (type IIb), with type IIx distinguished in between sharing intermediate properties of IIa and b (Pette and Staron, 2000). This determines their fatigue resistance and force production capacity, e.g. by means of the contraction velocity. Simply spoken, type I fibres are more enduring, while type II fibres favor a higher strength and power output. As a general rule, increased neuromuscular activity and mechanical loading is supposed to induce shifts from fast-to-slow fibre types, whereas reduced activity and unloading induces changes in the opposite direction (Pette and Staron, 2000).

Concerning the lower extremity of young healthy adults, the gastrocnemius usually contains about half-and-half type I and type II fibres, while the soleus shows a clear dominance for type I fibres (Johnson et al., 1973; Trappe et al., 2001). For children with CP, Ito et al. (1996) reported type-I fibre predominance and a lack of type-IIb fibres in the gastrocnemius. Marbini et al. (2002) also reported increased proportion of type I fibres in the triceps surae. Both groups speculated that a continuous

background stimulation in muscle of CP patients causes a shift towards type I fibresAn increase in slow myosin expression was also found for the gracilis and semitendinosus (Smith et al., 2011). By contrast, when referencing to an adult control group (age difference between groups > 35 years), Mathewson et al. (2014) found shifts from slow to fast fibre types in the gastrocnemius and soleus of CP children. The authors speculated that the typical change from fast to slow fibre types seen during aging might have biased their assessment. Rose et al. (1994) were also unable to provide a clear picture for the gastrocnemius in CP children reporting either a predominance of type I or type II fibres among their subjects.

Concerning the upper extremity, Gantelius et al. (2012) found a higher proportion of MHC IIx and a lower proportion of MHC IIa in spastic wrist flexors. Lower type I fibre rates were only found for wrist extensors. Ponten and Stal (2007) found that the brachi-radialis of young adult SCP patients had more MHC IIx and lower type IIa or type I fibre proportions. When the isoforms of flexor carpi ulnaris of children with CP were compared to adult controls, no differences in fibre type were found (Bruin et al., 2014). Yet a lower proportion of type I fibres in CP children with increasing age was reported (Bruin et al., 2014). Lieber et al. (2003) also observed no difference in myosin composition for several upper limb muscles.

In summary, it is not clear whether MHC isoforms are shifted in a specific fashion in muscles of CP patients. In view of the diversity of findings, it is unclear if CP represents an increased or decreased use model of fibre type adaptations (Lieber, 2010, p. 274 ff.; Graham et al., 2016). Furthermore, no relation between fibre types and function has been established in CP patients

1.5.1.4. Sarcomeres

Sarcomeres are the functional sub-units for muscle contraction and contain thin (actin) and thick (myosin) filaments (Lieber, 2010). According to the sliding filament theory, the degree of filamental overlap (~connected cross-bridges) determines the sarcomere's active force potential (Gordon et al., 1966). Additionally, its passive forces increase with increasing length. Sarcomeres are arranged in parallel and in series and thus they influence the amount fibre force for a given length as well as their shortening distance with respect to time. The latter will affect the force-velocity relationship of skeletal muscle (Hill, 1938). In addition, the serial sarcomere number may also be an indicator of a muscle's passive excursion ability.

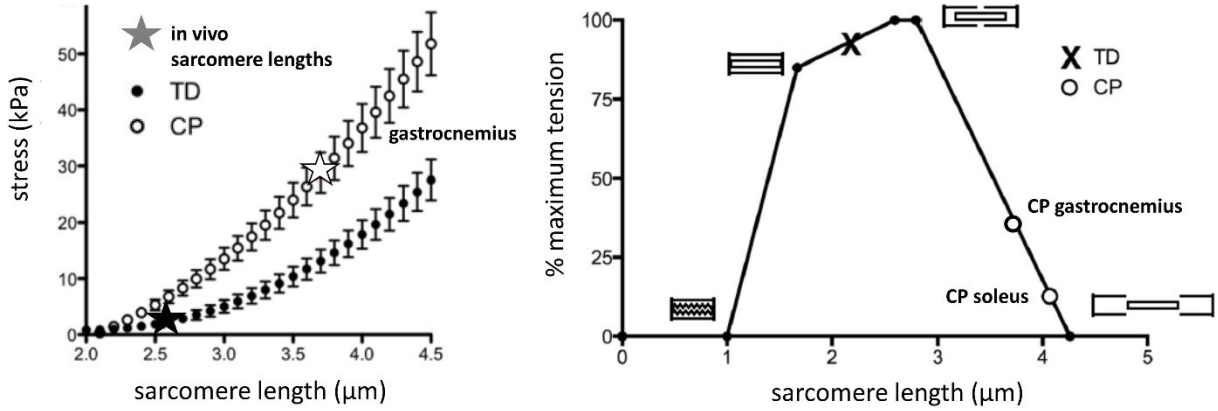

Fig. 1-8 Length tension properties of sarcomeres taken from plantarflexors of CP children. Left side: Passive sarcomere length vs. stress for fibres taken from gastrocnemius muscles. Asterisks show in vivo sarcomere lengths for typically developing controls (TD) and for children with Cerebral Palsy (CP). Extracted and adapted from Mathewson et al., 2014, J Orthop Res 32, 1667-1674. p. 1670. With permission from John, Wiley and Sons. Right side: Theoretical active force production for calf muscles according to a muscle length-tension curve showing that in-vivo sarcomeres of children with CP presumably operate on the descending limb while sarcomeres of TD may operate around the plateau region. Extracted and adapted from Mathewson et al., 2015, J Orthop Res 33, 33-39, p. 34. With permission from John, Wiley and Sons.

In lower limb muscles of children with CP, over-stretched in vivo sarcomeres are considered the most unprecedented change (Graham et al., 2016; Martin Lorenzo et al., 2015). Considerably increased sarcomere length have been found for the gastrocnemius (Mathewson et al., 2014), soleus (Mathewson et al., 2014 and 2015), gracilis (Larkin-Kaiser et al., 2015; Smith et al., 2011) and semitendinosus muscle (Smith et al., 2011). However, there seems to be no difference in actin filament length of sarcomeres in CP (Mathewson et al., 2015). This in an important notion, since actin filament length is generally a major determinant of sarcomere length (Gokhin et al., 2014). Yet interestingly, Gokhin et al. (2014) showed that usually during post-natal growth, actin filament length is considerably reduced for gastrocnemius muscles of mice. In humans, it is traditionally assumed that thin and thick filaments are fixed in length during childhood (van Praagh, 1997). Hence, sarcomeres may not be longer in muscle of children with CP per se but they likely need to operate with little overlap in filamental proteins (Fig. 1-8). As a results of this, they have little potential for active force generation but create high passive forces upon stretch (Fig. 1-8). Further, serial sarcomere number seems to be reduced in muscles of children with CP (Mathewson et al., 2015). Concerning the soleus, a reduction of 40% has been calculated by relating fascicle length measured via brightness mode ultrasonography to sarcomere length measured by laser diffraction (Mathewson et al., 2015).

Two concerns about sarcomere length estimates in CP children worth noting are the lack of aged-matched controls and the degree of joint configuration during biopsies. First, due to ethical issues, adult control muscles are often used as a reference (Bruin et al., 2014; Mathewson et al., 2014). Second, to match the degree of joint configuration between CP children and controls, sarcomere

length may also need to be predicted for more extended joint positions (Smith et al., 2011). Nevertheless, the large deviations from control muscles were considered to be extremely unlikely due to methodological issues (Mathewson et al., 2015). Concerning the functional significance of such findings, more severely limited CP children, e.g. with a higher GMFCS-Level, larger restriction in popliteal angles (Smith et al., 2011) or with more severe hip joint displacement (Larkin-Kaiser et al., 2015) were shown to have larger sarcomere length of hamstrings and adductor muscles. For the plantarflexors, no such functional associations had been reported so far (Mathewson et al., 2014; Mathewson et al., 2015).

Notably, the increase in sarcomere length in CP could also be different between the upper and lower limbs, since similar sarcomere length to controls were found in the upper limb (Bruin et al., 2014). Next, it has also been claimed that sarcomeres of spastic upper limb muscles do not provide insufficient filamental overlap. This is because in maximal extended joint positions, active force generation upon stimulation still appeared to be quite high (Smeulders et al., 2004). Notably, in isolated fibres of spastic upper limb muscles, the completely unloaded resting sarcomere length in children with CP was also found to be lower than in typically developed adult muscles (Friden and Lieber, 2003; Lieber et al., 2003). Still the tensile forces upon sarcomere stretch were much higher and alterations in titin or collagen were thought to be responsible for this (Friden and Lieber, 2003). Moreover, for spastic muscles of the upper extremity, it could be shown that sarcomere slack length was significantly different between fibre and fibre bundle level. Significantly longer sarcomere length were found in fibre bundles (Lieber et al., 2003). Hence, one hypothesis for the overstretched sarcomeres within muscles of children with CP could be that extracellular components (collagen networks) may hold sarcomeres at high in vivo length (Friden and Lieber, 2003).

In summary, increased sarcomere length seems to be involved in the formation of muscle contracture of children with CP. This reduces the serial sarcomere number and therefore also limits the passive muscle extensibility. Increased sarcomere length seems more apparent for the lower limb than for the upper limb and accumulation and altered quality of extra-cellular matrix may be the cause.

1.5.1.5. Muscle fibre size

Measures of a muscle fibre's cross-sectional diameter or cross-sectional area have been used as descriptors of muscle hypertrophy or atrophy. They are supposed to reflect sarcomere arrangement in parallel (Lieber, 2010, p. 183 ff., Edgerton et al., 2002). During immobilization and retraining, there is a direct relation between a muscle's fibre size and its maximal force potential which was shown in animals (Lieber et al., 1997) as well as in humans (Hortobagyi et al., 2000).

For CP patients, Smith et al. (2011) found a decreased fibre size concerning the gracilis and semitendinosus muscle (Fig. 1-9) and Mathewson et al. (2014) also reported that gastrocnemius and soleus fibres tended to be slightly smaller with disorganized fibre shapes

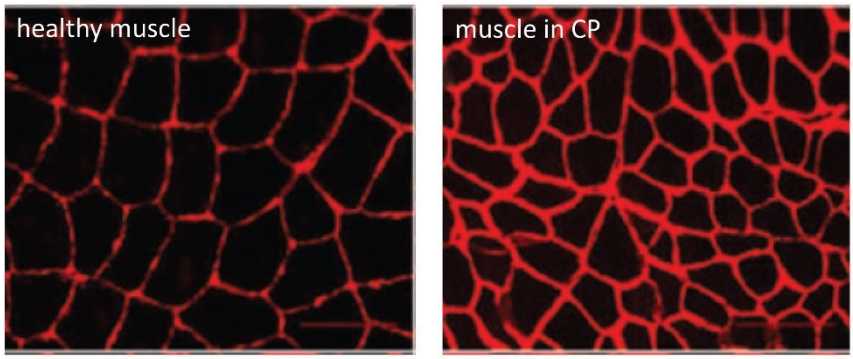

Fig. 1-9 Stained muscle fibre cross sections taken from muscle biopsies. Left side: hamstring muscle of a control child and right side: hamstring muscle of child with CP. Note the disorganized, smaller fibre shapes with more connective tissue stained in red in the muscle of the CP child. Extracted and adapted from Smith et al., 2011, J Physiol, 589, 2625-2639, p.2634. With permission from John Wiley & Sons.

For arm and shoulder muscles, fibres of CP patients were reported to be only one-third the size of normal fibres taken from adult healthy controls (Lieber et al., 2003). Yet some reports show no significant difference in fibre size concerning the flexor carpi-radialis in healthy adults and adult patients with CP (Bruin et al., 2014). These discrepancies might be affected by a high variability in fibre size, as reported for the gastrocnemius, iliacus and hamstring muscles (Rose et al., 1994). This presumably reflects tissue degeneration or modulations in myosin-heavy chain. In addition to that, increased variability in fibre size had been associated with increased energy expenditure during gait and thus increases disability (Rose et al., 1994).

In summary, muscle fibre cross-sections in the lower limb of patients with CP seem to be reduced and fibres feature increased variability in size and shape.

1.5.1.6. Muscle fibre and fibre bundle properties

Few studies examined the passive length tension properties of isolated muscle fibres or fibre bundles in CP. In isolated fibres, increased passive forces at shorter fibre length may reflect less sarcomeres in series or increased sarcomere resting length which causes the sarcomeres to reach their limits of extensibility earlier.

First off, all studies on passive fibre or fibre bundles mechanics in CP suffer from a lack of age matched controls. The closest age match was provided by Smith et al. (2011). They showed that isolated semitendinosus and gracilis fibres of children with CP were not stiffer than control fibres,

whereas fibre bundle stiffness was pathologically increased. This was thought to be related to higher extra-cellular matrix stiffness (Smith et al., 2011). By comparison, Mathewson et al. (2014) also reported increased isolated fibre stiffness for the gastrocnemius and soleus. Moreover, CP children with decreased ambulatory function, classified in GMFCS III-IV, showed greater triceps surae fibre stiffness than children in GMFCS I-II (Mathewson et al., 2014). Contrary to the results of Smith et al. (2011), Mathewson et al. (2014) further showed that fibres and fibre bundles had similar stiffness in CP patients. Hence, Mathewson et al. (2014) speculated that extracellular matrix alterations may differ among different muscle groups. Still, when stretching the muscle tissue to the in-vivo sarcomere length, the stiffness of both fibres and fibre bundles was significantly augmented in children with CP which in turn suggests that increased sarcomere length may play a major role for increased tissue stiffness (Mathewson et al., 2014).

Concerning the upper extremity, Lieber et al. (2003) investigated several arm and shoulder muscles showing that fibres in CP subjects were stiffer than control fibres, while Bruin et al. (2014) found no increase in fibre or fibre bundle stiffness of the flexor carpi ulnaris.

In summary, no clear conclusion about fibre and fibre bundle stiffness can be drawn. Preliminary evidence for the triceps-surae suggests that larger fibre stiffness is related to a decrease in ambulatory function.

1.5.1.7. Connective tissue

Muscles and their composing cells are embedded and connected with extracellular matrix. It has been argued that the extent and quality of a muscle's connective tissue determines its passive extensibility (Gillies and Lieber, 2011; Herbert, 1988).

Since collagen content of the vastus lateralis in CP children correlates with knee extensor muscle tone, it was suggested that thickening of endomysium increases passive muscle stiffness in CP patients (Booth et al., 2001). The semitendinosus was also shown to have significantly increased intramuscular connective concentrations in children with CP (Smith et al., 2011). This was associated with significantly increased fibre bundle stiffness, suggesting a stiffer than normal extra-cellular matrix (Smith et al., 2011). By contrast, Mathewson et al. (2014) showed that the collagen content within the triceps surae was not significantly different between typically developing muscle and spastic muscle tissue and, since fibre bundles were not stiffer than isolated fibres, their results suggested a decreased stiffness of the extra-cellular matrix in CP (Mathewson et al., 2014). However, as previously mentioned, their control samples were more than 4 times older. Thus age could have provided a strong bias since properties of the extra-cellular matrix might be altered during natural aging.

Concerning arm muscles, Bruin et al. (2014) investigated the flexor carpi ulnaris muscle of children with CP. They found that the thickness of the tertiary perimysium (connections of intra- and extra-muscular elements of neural, venous, arterial and lymphatic tissues) in spastic muscles was three times larger than that of a control muscle. They concluded that enhanced myofascial loads may contribute to increased passive stretch resistance and to movement limitations. Similarly, using biopsies of upper limb muscles, Lieber et al. (2003) showed that morphologically, the extra-cellular matrix in muscles of CP children appears disorganized and less dense. Notably, Meza and Lieber (2016) recently suspected that the collagen structure and not the content may be the key to increased stiffness.

In summary, connective tissue accumulations are plausibly involved in contracture formation. However, the evidence concerning the triceps surae is sparse. In addition, it needs to be determined in detail to which extent the mechanical properties of the extra-cellular matrix are different in muscles of CP children.

1.5.2. Macroscopic alterations

In contrast to the aforementioned investigations, the following findings were derived with non-invasive techniques. They have been used quite extensively to study muscle-tendon architecture and properties in CP in the past. Primarily magnet resonance imaging or brightness mode ultrasonography has been applied. Overall, brightness-mode ultrasonography might be more readily available than magnet resonance imaging in treatment centers for children with CP and seems to be easier to use for measuring muscle size on young patients due to reduced acquisition times, reduced financial costs and an absent burden of spatial constrains inside a scanner for the child. However, superficial muscles might be more accurately measured with ultrasound which could also be a reason why the soleus muscle attracted far less attention than the gastrocnemius muscle in patients with CP.

1.5.2.1. Fascicle properties

A **B**

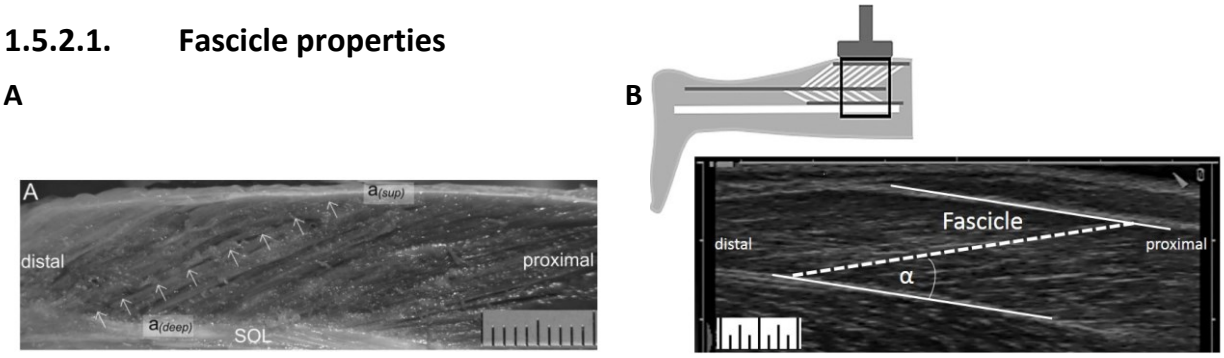

Fig. 1-10 Section of a cadaver medial gastrocnemius and in-vivo ultrasound picture. A) Anatomical section of a cadaver muscle belly. Extracted from Bernard et al., 2009, Muscle Nerve 39, 652-665, p.657. With permission from John, Wiley and Sons. B) 2D in-vivo ultrasound scan of the medial gastrocnemius muscle belly. Ultrasound picture with schematic drawings of a fascicle and its pennation angle from own measurements. Scale unit=1cm. The associated graphic of the shank was extracted and adapted from Kawakami and Fukunaga, 2006, Exerc Sport Sci Rev, 34, 16-21, p. 17. With permission of Wolters Kluwer Health, Inc.

Numerous studies used ultrasonography to evaluate muscle structure by making use of the fact that connective tissue can be discriminated from active muscle tissue (Cronin and Lichtward, 2013). Amongst others, fascicle (~fibre bundle) length and their orientation can be determined (Fig. 1-10). Since muscle fibres may not span the entire width or length of muscles, it had been suggested that fascicle and fibre should not be used as synonyms. Since most fibres seem to be serially connected, fascicles are considered the 'functional unit' for representing fibres (Kumagai et al., 2000). In terms of gastrocnemius fascicles, cadaveric studies suggest that the error between actual fibre bundle length (Fig. 1-10) and fascicle length from 2D ultrasound can be minimized by following specific protocols for ultrasound probe orientation (Benard et al., 2011). In terms of validity, the anatomical accuracy of measurements of fascicle lengths and pennation angles revealed a standard error of 8.7–9.7% (Kwah et al., 2013). Concerning brightness-mode ultrasonography of the medial gastrocnemius, acceptable reproducibility during passive assessments has been established in children with CP (Mohagheghi et al., 2007), as well as in typically developing children (Legerlotz et al., 2010). In CP children, ICC values of 0.81-0.91, 0.85-0.88 and 0.93-0.94 have been established for fascicle length, pennation angle and muscle belly thickness, respectively (Mohagheghi et al., 2007). The average difference between repeated measures was ≤8.1% for fascicle length and ≤8% for muscle belly thickness while it was 2-3° for pennation angles. For healthy controls, Legerlotz et al. (2010) found somewhat larger ICC values and lower coefficients of variation: <6.3% for fascicle length and <3.1% for muscle belly thickness.

As depicted in Fig. 1-11, longer muscle fascicles (with more sarcomeres in series) can be beneficial for various reasons: They may extend the range for active force production (O'Brien, 2016), they are able to produce higher shortening velocities (Blazevich and Sharp, 2005) and they exert higher forces closer to their maximum across a wider range (O'Brien, 2016). Since relative shortening is less, relative contractile velocity is lower in long fascicles and this enables the production of larger forces. Moreover, additional sarcomeres in series may also increase passive muscle extensibility (Butterfield, 2010). They may speculatively also have a protective effect for the muscle by shifting its optimum length to avoid eccentric contractions beyond optimum (Morgan and Proske, 2004). On the other hand, there might also be a tradeoff since longer fascicles can increase the cost for generating force, since more sarcomeres need to be activated (Lichtwark and Wilson, 2008).

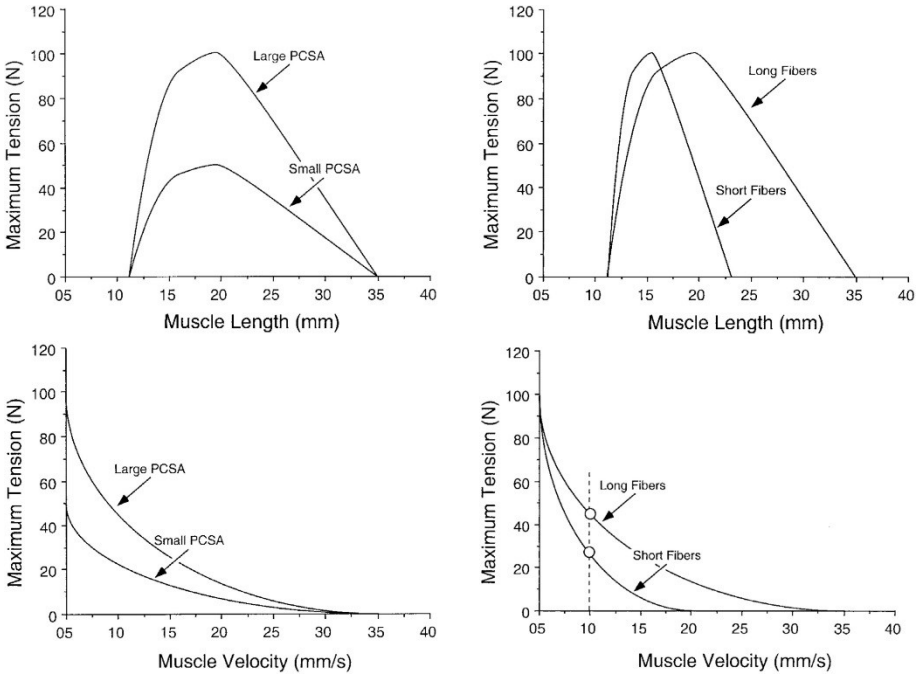

Fig. 1-11 Benefits of larger PCSA and longer muscle fibres on force-length and force-velocity relationship. Schematic illustration. Extracted and adapted from Lieber and Fridén, 2000, Muscle Nerve 23, 1647-1666, p.1659 and p.1660. With permission from John Wiley & Sons.

In healthy adults, longer gastrocnemius fascicles have been positively associated with sprinters and their performance (Abe et al., 2000; Kumagai et al., 2000). Furthermore, Hauraix et al. (2015) experimentally confirmed that, in vivo, the maximum concentric gastrocnemius fascicle force in humans is a function of its shortening velocity.

Contrarily, bed-rest immobilization (Boer et al., 2008), aging (Narici et al., 2003; Stenroth et al., 2012; Thom et al., 2007) as well as neurological insults, e.g. stroke (Gao et al., 2009) have been reported to negatively affect gastrocnemius architecture and reduce fascicle length. Interestingly, in older aged individuals, decreased fascicle length accounts for half of the age related decline of maximal muscle shortening velocity (Thom et al., 2007). In adult stroke survivors, associations between reduced gastrocnemius fascicle length and restrictions in passive joint motion, as well as increased joint stiffness have been found (Gao et al., 2009).

In patients with CP, Barrett and Lichtwark (2010) concluded that there was no consistent evidence that plantarflexor fascicle length is reduced. Lack of standardization may provide a potential bias since most studies did not exactly standardize the joint configurations or the applied ankle moment during their assessments. Since Barrett and Lichtwark's Review (2010), these discrepancies seemed to continue. Concerning the gastrocnemius, two further novel studies in CP children beyond 6 years of age confirm the notion that there is no difference in fascicle length (Barber et al., 2011b; Herskind et al., 2016). Yet, more standardized studies in older children (average age 11-13 years) found that within a common passive ankle range of motion, gastrocnemius fascicles of children with CP are considerably

reduced (-20% to -43%) with respect to typically developing peers (Gao et al., 2011; Kalkman et al., 2016; Matthiasdottir et al., 2014). A plausible explanation for the shorter fascicles may be a loss of serial sarcomeres as speculated by Matthiasdottir et al. (2014). Absolute passive extensibility (~strain) of the gastrocnemius fascicles was reported to be lower in children with CP (Barber et al., 2011a; Kalkman et al., 2016). To the contrary, when considered over a common range of joint motion, the fascicles of CP children according to Matthiasdottir et al. (2014) undergo a bigger relative excursion as they are inherently shorter. Thus the authors speculated that each sarcomere within the gastrocnemius fascicles of children with CP is exposed to a greater mechanical demand upon stretch.

In summary, there is emerging evidence that fascicle length in the gastrocnemius of more mature children and adolescents with CP is reduced.

1.5.2.2. Pennation angle

The angle of the fascicles with respect to line of pull of a muscle is referred to as pennation angle (Fig. 1-10) In the medial gastrocnemius muscle of healthy adults (Kubo et al., 2003; Narici et al., 2003) and in patients with CP (Lee et al., 2015), in-vivo assessed muscle belly thickness or anatomical cross-sectional area and pennation angle of the gastrocnemius share a mild to moderate positive relationship. This shows that greater pennation angles might be indicative of larger muscle size. According to Blazevich and Sharp (2005), a larger pennation angle allows more fascicles (more contractile tissue) to be arranged in parallel.

Accordingly, in healthy subjects, increased pennation angles following training are supposed to reflect radial fibre hypertrophy while muscle atrophy is typically associated with a decreased pennation angles (Blazevich and Sharp, 2005; Franchi et al., 2016). Specifically for the gastrocnemius, resistance trained subjects have thicker muscle bellies in combination with larger pennation angles (Fukutani and Kurihara, 2015). Furthermore, heavy load resistance training of the plantarflexors causes increases in pennation angle (Duclay et al., 2009). Yet in older adults, for example, gains in strength and walking function after resistance training were not linked to increases in gastrocnemius muscle thickness or pennation angle (Raj et al., 2012).

Based upon these results and the atrophic changes in calf muscles of children with CP, it seems logical that pennation angles in the gastrocnemius of children with CP would decrease in comparison to healthy muscle tissue. Yet, Barrett and Lichtwark (2010) summarized that there was no consistent evidence for that. Up to now, decreased pennation angles (Barber et al., 2011b; Malaiya et al., 2007; Shortland et al., 2004), similar pennation angles (Gao et al., 2011; Shortland et al., 2002) or increased pennation angles (Kruse et al., 2016a) have been reported for the gastrocnemius muscle. Notably,

results of Wren et al. (2010) and Shortland et al. (2004) would suggest that in CP patients who had previously been operated on, increases in pennation angle occur in concert with shorter muscle bellies or fascicles, which contrast the hypertrophic changes of heathy muscles seen with training.

In summary, it remains unclear if the pennation angle of the gastrocnemius muscle of children with CP is decreased, as may be suspected from findings on strength loss and atrophy in healthy controls

1.5.2.3. Muscle volumes and size

Overall a muscle's volume is among the determinants of its maximal active force. Concerning the plantarflexors of healthy adults, muscle volumes explain about 32-57% of the variance in isometric strength (Bamman et al., 2000; Baxter and Piazza, 2014; Trappe et al., 2001). Still, measures of the cross-sectional area of the triceps surae are more directly related to isometric force (Bamman et al., 2000). When excluding the effects of different moment arms, 85% of the variance in force at the Achilles tendon is determined by the physiological cross-sectional area of the triceps surae (Fukunaga et al., 1996). When considering the anatomical cross-sectional area (not perpendicular to the fibre direction), the explained variance ceases to 56% (Fukunaga et al., 1996).

For patients with CP, Barrett and Lichtwark (2010) concluded that there is consistent evidence that muscle volumes, cross-sectional areas and thickness are reduced in comparison to control subjects. For the gastrocnemius of patients with CP, several newer studies confirm this (Barber et al., 2012; Barber et al., 2016; Noble et al., 2014b; Oberhofer et al., 2010). In addition, when compared to values of healthy controls, magnetic resonance imaging revealed volumetric atrophy even in high functioning CP children, classified in GMFCS I and II (Noble et al., 2014b). In addition to that, a proximal to distal gradient in volume loss within the lower limbs was found (Noble et al., 2014b). Strikingly, when assessing multiple muscle of the lower limb by means of magnet resonance imaging, the gastrocnemius was one of the most atrophied muscles (Noble et al., 2014b; Oberhofer et al., 2010).

Although 2D ultrasound has been extensively applied to measure muscle architecture in CP patients, in principle, using sequential 2D ultrasound scans, 3D muscle volumes can be reconstructed as well (Barber et al., 2011a; Benard et al., 2011; Barber et al., 2016). Nevertheless, 2D measures may to some extent also serve as proxy for muscle volumes, e.g. gastrocnemius muscle thickness derived from ultrasonography is considerably related to muscle volume from magnet resonance imaging (59-65% of explained variance) (Park et al., 2010).

In summary, there is consistent evidence that volumes and measures of muscle size, e.g. the cross sectional area, are reduced in lower limb muscles of children with CP and the gastrocnemius seems to be quite severely atrophied.

1.5.2.4. Connective tissue and fat

As is the case on a microscopic level, non-contractile tissue infiltration into muscles of CP patients can be also documented on macroscopic scales. Similar findings have been exposed in older people (Hasson et al., 2011) and were interpreted as signs of declined muscle quality and physical inactivity (Marcus et al., 2010).

In CP children, brightness-mode ultrasonography was also used to assess the muscle composition of the gastrocnemius. Pitcher et al. (2015) showed that connective tissue content was generally augmented in CP children and tended to increase with lower mobility status (from GMFCS I to III). It has been further speculated that these findings may reflect intramuscular fat and fibrotic tissue (Pitcher et al., 2015). Using magnetic resonance imaging, Noble et al. (2014a) confirmed that a considerable portion of lower limb muscle volume in CP is taken up by intramuscular fat. Within the spastic gastrocnemius, about twice as much fat as in control muscle was found. Johnson et al. (2009) also observed that fat tissue infiltration in thigh muscles in children with CP is negatively related to the children's physical activity which confirms that sedentary behavior promotes degenerative processes.

In summary, a considerable portion of muscles in CP patients is taken up by connective tissue and fat which seems to get worse with lower mobility status.

1.5.2.5. Tendon properties

Tendons transmit contractile forces by storing and returning strain energy which is also used during ambulation (Alexander, 1991). Increasing plantarflexor muscle power output during gait, for example, requires stiff Achilles tendons to produce optimal efficiency and efficiency is in turn maximized when the required muscle volume for a task can be minimized (Lichtwark and Wilson, 2008). These aspects were partly derived from simulations. Apart from that, magnetic resonance imaging, ultrasonography and dynamometry are frequently applied to study tendon dimensions and properties in humans (Seynnes et al., 2015). Such techniques revealed that tendons can specifically adapt to their loading history. Tendon stiffness may increase following enhanced tendon loadings during training (Arampatzis et al., 2010; Reeves et al., 2003). This may likely help maintain physiological ranges of strain loadings. Sprinters, for example, have stiffer Achilles tendons than endurance runners (Arampatzis et al., 2007b).

Oppositely, with disuse, e.g. after spinal cord injury (Reeves et al., 2003) or with aging (Narici et al., 2005), tendons get more compliant. In the case of the plantarflexors of older subjects, this in turn also alters the excursion of muscle fascicles during walking (Mian et al., 2007).

In patients with CP, multiple studies showed that the gastrocnemius tendon length during rest is longer than usual, e.g. when normalized to the shank length (Theis et al., 2016; Wren et al., 2010), when set in proportion to the whole muscle-tendon unit (Kruse et al., 2016a) or when considered in absolute units (Barber et al., 2012). When focusing on the sole Achilles tendon only, Gao et al. (2011) also found an increased length in CP patients. Gao et al. (2011) hypothesized that longer tendons could be an adaptation to the decreased muscle fascicle length and increased fascicle stiffness of the plantarflexors in CP patients. From a theoretical perspective, Lieber (2010, p.102) reasoned that longer tendons shift the active force length relationship of a short muscle belly to a longer length. More complaint tendons supposedly also broaden the active force length relationship of a muscle-tendon unit. Both could be reasonable adaptive mechanisms in CP patients. Interestingly, in disabled athletes with CP, the gastrocnemius tendon length was reported to be similar to control subjects (Hussain et al., 2013). This may reveal a preserved adaptive potential for tendons in CP. Concerning the Achilles tendon cross-sectional area in CP children, Kruse et al. (2016a), Gao et al. (2011) and Theis et al. (2016) found smaller cross-sectional areas with large statistical effect sizes with respect to values of controls. A smaller cross-sectional tendon area may supposedly lead to larger tissue stress in CP patients when counteracting external loads. On the other hand, active force production by the plantarflexor muscles is also reduced in children with CP (Downing et al., 2009; Elder et al., 2003; Ross and Engsberg, 2002; Stackhouse et al., 2005; Wiley and Damiano, 1998) and the tissue stress on tendons may thus be lower than in healthy controls.

The stiffness of the gastrocnemius tendon in patients with CP has been assessed during active (Barber et al., 2012) and passive conditions (Theis et al., 2016; Kruse et al., 2016a). Barber et al. (2012) found no change in tendon stiffness during isometric strength tests in adults with CP. Theis et al. (2016) assessed the passive resistive stiffness during dorsiflexion stretches in children classified in GMFCS III-IV. They found that tendon stiffness appears to be similar to controls, while Kruse et al. (2016b) reported a decrease in passive resistive stiffness of the gastrocnemius tendon. Notably, both Kruse et al. (2016b) and Theis et al. (2016) reported higher muscle belly than tendon stiffness of the gastrocnemius. Furthermore, both research groups showed that the elastic modulus of the tendon was not significantly altered from control values. Based on these preliminary findings, it seems that material properties of the gastrocnemius tendon are probably not altered in individuals with CP. However, the somewhat variable levels for active plantarflexor force production may complicate and bias this indirect assessment of tendon material properties.

In contrast to that, histological and bio-chemical analysis of hamstrings tendon grafts revealed a tendinopathic-like state in CP patients (Gagliano et al., 2013). It was speculated that increased muscle tone induces persisting strain below injury thresholds. No such tendon biopsies have been reported for the plantarflexors. The findings of increased tendon length do not seem to be unique to plantarflexor muscles since patellar-tendons also seem to be elongated in patients with CP. This was proposed to be related to viscoelastic tendon behavior under chronic load (Seidl et al., 2016), e.g. due to a walking pattern with severely flexed knees.

In summary, CP patients typically have longer and slimmer tendons. Apart from that, no definite conclusion about the mechanical or material properties can be drawn so far.

1.6. Muscle-tendon properties and function in Cerebral Palsy

1.6.1. Relationship to impairments

To stay within the scope of this thesis, this section focusses exclusively on patients with CP. Information about the relationship of muscle-tendon properties and function has been primarily gathered on muscles while tendon properties attracted less attention. As outlined below, measures of muscle size and geometry could be related to impairments on body structure and function level (e.g. during strength or walking tests) and to impairments on activity level (e.g. to mobility), as classified according to the World Health Organization (2001).

First, several studies revealed associations between muscle size and strength in individuals with CP (Bland et al., 2011; Moreau et al., 2010; Moreau et al., 2012; Reid et al., 2015b). Among others, muscle volumes of knee flexors and extensors were related to torque generating capacity during isometric and/or isokinetic strength tests (Reid et al., 2015b). Notably, the associations between muscle volume and strength in CP patients were weaker than in controls, indicating that neural factors (e.g. incomplete muscle activation) may also limit force production. In addition, ultrasound based measures of the vastus lateralis muscle thickness were associated with peak isometric knee extensor torques (Moreau et al., 2010) and the rate of torque development (Moreau et al., 2012)**.** Moreover, for the tibialis anterior, muscle thickness, cross-sectional area, and fascicle length were significantly related to larger isometric strength in CP patients (Bland et al., 2011).

Second, concerning walking or gross motor function tests, a thicker gastrocnemius muscle was associated with lower Timed up and go test times (Yun et al., 2016) and a larger gastrocnemius

pennation angle could be associated with improved gross motor function scores (Lee et al., 2015). Additionally, in children with unilateral CP, interlimb volume ratios of the plantarflexors were correlated with differences in kinetic ankle joint work during push-off derived from 3D gait analysis (Riad et al., 2012). This suggests that larger plantarflexor muscle volume in CP is beneficial for propulsion. Concerning knee extensors, a thicker vastus lateralis and rectus femoris muscle could be linked to faster walking (Yun et al., 2016). Also for the tibialis anterior, larger muscle thickness, cross-sectional area and larger fascicle length were significantly related to faster walking (Bland et al., 2011). Also, a larger cross-sectional area of the tibialis anterior was related to more dorsiflexion during swing-phase of gait (Bland et al., 2011).

Third, concerning activity based measures, the gastrocnemius muscle volume was significantly associated with the children's gross motor function showing that CP children in GFMCS III, without independent walking skills, display smaller muscles than children in level I or II (Herskind et al., 2016). Moreover, larger muscle thickness of the quadriceps femoris derived from brightness-mode ultrasonography was related to better mobility indices (Ohata et al., 2008). Moreau et al. (2010) further showed that rectus femoris fascicle length was significantly positively correlated with indices about sportive and physical functioning. A larger pennation angle of the vastus lateralis was significantly correlated with superior indices for transfers, mobility and general activity.

In summary, several studies reported associations between muscle architectural parameters and measures of function and activity in patients with CP. In particular larger muscle size (e.g. thicker muscles) seemed to be beneficial.

1.6.2. Treatment effects

In the following section, five of the most common treatments for children with CP, namely botulinum neurotoxin injections, orthopedic surgery, orthotics and casts, as well as stretching and resistance training will be discussed. Although many more invasive and non-invasive therapeutic or drug related strategies are in use (Novak et al., 2013; Novak, 2014), they do not primarily target or directly affect muscle-tendon properties. In addition, the author of this thesis also wishes to focus on approaches that have been previously shown to alter muscle-tendon properties in CP. Except for botulinum neurotoxin, which targets neural aspects of spasticity, all other selected treatments either mainly aim to manage contracture or aim to improve strength (Novak et al., 2013). Orthotics and casts have been combined in this section since both rely on prolonged stretch-immobilization as a treatment stimulus. Stretching has been included since it is one of the most frequently applied treatments during physical therapy in CP (Wiart et al., 2008). Yet, in contrast to the other approaches included, manual

stretching has been considered to be rather ineffective from a functional perspective (Novak et al., 2013; Novak, 2014).

The following paragraphs will focus on the background, rationale and work mechanisms of these selected treatments and subsequently will present the already documented consequences on muscle-tendon or joint properties in patients with CP while emphasising the plantarflexors muscles and the ankle joint

1.6.2.1. Botulinum neurotoxin (BoNT)

BoNT is intramuscularly injected and inhibits the release of acetylcholine at the neuromuscular junction. It thus partially paralyzes the muscle (Foran et al., 2003). Within 1 month, the motoneuron begins with co-lateral sprouting, leading to re-innervation (Foran et al., 2003) and 6 weeks is typically seen as the chemically active period (Alhusaini et al., 2011). Finally, within 3 months, the effects should have worn off (Jankovic, 2004). The objective is to decrease neuromuscular reflex activity and hypertonia (Mathevon et al., 2015) and thereby to open a window to increase joint flexibility, e.g. by additional serial casts or orthotics.

The concept assumes that muscles (fibres) in CP children are too short and BoNT will allow them to be stretched out (Gough et al., 2005; Heinen et al., 2010). Yet, it is commonly accepted that the injected muscle will atrophy and it has been feared that this was not completely reversible (Mathevon et al., 2015). Consequently, Barber et al. (2013), for example, speculated that any benefits could be offset by long term weakening. Eventually, due to neutralizing antibodies, the effects may wear-off after multiple injections (Linder-Lucht et al., 2006).

Effects on muscle-tendon and joint properties in CP

All of the following studies included, but were not limited to, gastrocnemius muscle injections in children with CP. They either used multiple injections sides in several lower limb muscles (Eek and Himmelmann, 2016; Williams et al., 2013a; Williams et al., 2013b), exclusive gastrocnemius injections (Park et al., 2014) or combined gastrocnemius-soleus injections (Alhusaini et al., 2011; Barber et al., 2013).

Williams et al. (2013b) reported that the gastrocnemius muscle belly loses 4.5% of its volume within 3-6 weeks of injections, while the non-injected soleus muscle, perhaps compensatory, increased its volume by 4%. The children's walking mobility or isometric strength did not significantly change. No reports regarding concomitant therapies were made. Notably the treated children were not naïve to BoNT treatments, since they already got BoNT in the past. This might have somewhat lowered the atrophic effect. After 3 months, Park et al. (2014) showed that BoNT-injections into the gastrocnemius

led to 11-13% atrophy of the muscle belly thickness and to a -5° decrease in pennation angle while the resting fascicle length increased by 16%. Additionally, Park et al. (2014) observed about a 7-10° gain in maximal passive dorsiflexion. In addition to BoNT treatments, ankle-foot orthotics were recommended as long as possible during the day. Apart from that, Williams et al. (2013a) compared sole BoNT treatments with BoNT and additional strength training. Also the group with sole BoNT treatment displayed increases in plantarflexor muscle volume and gained isometric strength. This shows that BoNT does not necessarily lead to atrophy. However, the total pre-post period was 6 months, making inference about direct consequences of BoNT difficult. Furthermore, in young CP-children allocated to 1 or to 3 calf muscle injections per year combined with orthotic and physiotherapeutic treatment, no gastrocnemius atrophy with respect to baseline was observed and no effect of injection frequency could be shown at 12 months (Barber et al., 2013). Overall, there was a 20% increase in gastrocnemius muscle volume, 7% increase in fascicle length and 11% increase in the physiological cross-sectional area of the medial gastrocnemius muscle in that year. Still, the increase in muscle volume was about two-thirds lower than in aged-matched healthy children. Again, children were recommended to wear additional ankle foot orthotic for 6–8 h per day. Finally, regardless of the effects on the muscle, Barber et al., (2013) found no significant improvement in maximum passive dorsiflexion.

Apart from these studies focusing on the effects of BoNT on muscle morphometrics, a recent investigation found that at 6 months post-injection, plantarflexor strength was able to increase by 9% (Eek and Himmelmann, 2016). This shows that long term weakening with BoNT probably is not a reasonable concern. Still, ankle kinematics during gait were not improved and passive end-range dorsiflexion was not increased (Eek and Himmelmann, 2016). Focusing on passive resistive ankle joint properties, Alhusaini et al., (2011) found that the injection of BoNT did not significantly change joint stiffness or endrange dorsiflexion at 6 weeks.

Taken together, in-line with current recommendations (Heinen et al., 2010), BoNT-treatments were often accompanied by further therapies, e.g. orthotics (Barber et al., 2013; Park et al., 2014) which makes the interpretation of the findings difficult. On the other hand, concomitant treatments are not always specifically reported (Alhusaini et al., 2011; Eek and Himmelmann, 2016). Still, even multiple injections per year may not lead to growth arrest of calf muscles (Barber et al., 2013) and the plantarflexor strength also seems to recover (Eek and Himmelmann, 2016). The fact that passive ankle joint stiffness appeared to be unaltered may reveal that BoNT primarily modulates neural aspects and does not change intrinsic stiffness of the muscle (Alhusaini et al., 2011). Concerning passive joint flexibility, only Park et al. (2014) reported increases in passive dorsiflexion, while no increase joint flexibility was found by some other groups (Alhusaini et al., 2011; Barber et al., 2013; Eek and Himmelmann, 2016).

In summary, BoNT most likely induces muscle atrophy on short-time spans of up to 3 months which is within and shortly above the chemically active period. Nevertheless, the calf muscles of children with CP seem to recover afterwards. However, increases in passive joint flexibility probably largely depend on concomitant treatments.

1.6.2.2. Orthopedic surgery

Lengthening surgeries for plantarflexors are considered an ultima ratio in CP children (Sees and Miller, 2013). Numerous procedures for the triceps-surae can be distinguished (Shore et al., 2010). They are topographically categorized from proximal to distal, ranging from gastrocnemius muscle belly to Achilles tendon lengthenings.

Several insights on such surgeries have been derived from plantarflexors of animals: Transverse sectioning of the rat gastrocnemius aponeurosis, for example, leads to acute rupturing of the intramuscular tissue alongside the section and eventually causes a drop in muscle optimum force, an increase in slack length and lowers the passive resistive forces (Jaspers et al., 1999). Logically, tendon releases also immediately decrease the active muscle force (Jamali et al., 2000). They also drop the tension on the muscle belly which reduces sarcomere length within the gastrocnemius (Baker and Hall-Craggs, 1980). Tenotomies of the triceps surae also lead to marked increases in intra-muscular connective tissue within a few weeks which is probably due to disuse (Jozsa et al., 1990).

Concerning CP children, two general risk factors of surgeries are recurrent equinus and overcorrection, the latter leading to a walking pattern with excessive dorsi- and kneeflexion (Shore et al., 2010). Younger children with CP generally suffer from higher recurrence rates and lengthenings of the Achilles tendon seem to be rather critical for overcorrections (Shore et al., 2010). Hence, early aggressive interventions should be avoided. In addition, plantarflexor surgeries are frequently done in combination with other bony or soft-tissue surgeries. Although remarkable long-term benefits of such procedures on ankle kinematics during gait have been reported (Dreher et al., 2012), they may require extensive post-operative immobilization and retraining (Shore et al., 2010).

Effects on muscle-tendon and joint properties in CP

In a cross-sectional study, CP children who underwent prior gastrocnemius recession or Achilles tendon lengthenings had 7-16% longer distal gastrocnemius tendons and tended to have 30-36% shorter muscle fascicles than CP children who did not receive prior surgeries (Wren et al., 2010). Two further longitudinal studies evaluated the effects of surgeries on calf muscle morphometrics (Fry et al., 2007; Shortland et al., 2004): Both showed remarkably increased passive dorsiflexion after the intervention. This implies that the total length or extensibility of the plantarflexors muscle-tendon

units might have considerably increased. Apart from that, Shortland et al. (2004) reported a 28-32% decrease in fascicle length after surgery. The pennation angle increased at both maximum dorsiflexion and at the resting ankle angle by a mean of 21% and 28%, respectively. The follow-up time was rather variable ranging from 51-610 days. Fry et al. (2007) investigated the Vulpius procedure. During this procedure, the external aponeurosis of the gastrocnemius and usually also the superficial aponeurosis of soleus are sectioned. One year post-op, Fry et al. (2007) observed a reduction in normalized gastrocnemius muscle belly length, however, the absolute muscle volume on average showed a 17% increase, indicating that patients' muscles were able to recover. To the best of the author's knowledge, no study thus far has explored the intramuscular healing process after surgeries. This leaves room for speculations about excessive post-operative scare tissue formation in CP, as shown in animal experiments (Jozsa et al., 1990). As can be seen from the presented findings, surgeries on plantarflexors of children with CP primarily aim to restore dorsiflexion and not to normalize muscle–tendon architecture (Sees and Miller, 2013).

In summary, pathological alterations in muscle-tendon properties, e.g. the mismatch between gastrocnemius muscle belly and tendon length, as well as the reductions in muscle fascicle length might be aggravated by surgeries despite achieving marked increases in ankle joint flexibility.

1.6.2.3. Orthotics and Casts

The major difference between an orthotic and a cast for the lower limb of children with CP is that a cast is not removable on demand and offers little to no flexibility. For equinus deformities, casting usually involves serial cast reapplication at intervals between 2 and 14 days (McNee et al., 2007). In contrast to that, orthotics are constructed from thermoplastic or carbon fibre composites, with or without hinge constructions and are mainly prescribed for 3 purposes: to protect the outcome of surgeries, to prevent musculoskeletal deformities and to improve gait (Davids et al., 2007). Large scale registries show that 4 out of 10 children at least partly wear ankle-foot foot orthotics for maintenance or increase in joint range of motion (Wingstrand et al., 2014).

Orthotics can typically be categorized according to the joints they span. Concerning the lower extremity, orthotics are most frequently grouped into foot orthotics, ankle-foot foot orthotics or knee-ankle-foot orthotics (Specht et al., 2008, p.5). About every second child with CP is treated with ankle foot orthotics (Wingstrand et al., 2014). They are frequently also used as a splint to counteract contractures by applying tensile forces on muscle-tendon units, which is done for several hours a day (Cusick, 1988; McClure et al., 1994). Moreover, overnight use in children with CP is recommended to prevent or delay contractures (National Collaborating Centre for Women's and Children's Health (UK),

2012). Maas et al. (2012) argued to use the term 'orthotic management in rest' instead of splinting, but orthotics for counteracting contracture are frequently also worn during ambulation.

Orthotics and casts intend to increase joint range of motion and basically rely on two stimuli, progressive tensile stress and immobilization. The concept is that immobilization of a muscle in a lengthened position will lead to gains in muscle length with a reduction in equinus posturing. The mechanism is supposed to encompass either biological remodeling of connective tissue or structural muscle growth from addition of sarcomeres in series (McClure et al., 1994). The rationale for this is derived from animal studies. In adult animals, muscle immobilization in a lengthened position led to sarcomerogenesis within muscle fibres (Williams and Goldspink, 1978). Notably though, tendons of growing animals seem to respond more readily than muscles (Blanchard et al., 1985; Tardieu et al., 1977). Thus, when the soleus of young animals is immobilized under tensile stress, growth of tendon length seems to be stimulated first and muscle fibre length actually decreases (Blanchard et al., 1985; Tardieu et al., 1977) since the muscle belly is somewhat kept off-tension. In fact, also for CP children, experts doubt that orthotics or casts will increase the plantarflexors' muscle length. It had been feared that they could actually exacerbate altered morphology by promoting atrophy due to immobilization (Gough, 2007; Miller, 2007).

Effects on muscle-tendon and joint properties in CP

To the best the author's knowledge, no study has provided results about the effects of casts or orthotics on muscle-tendon properties in children with CP without any additional primary treatment. Apart from that, McNee et al. (2007) found that after 12 weeks of full-time below knee serial casting the only significant improvement was an increase in passive dorsiflexion when the knee was flexed while there was no-benefit during gait. Furthermore, Blackmore et al. (2007) concluded that there is little evidence that casting is superior to no casting for treating equinus posturing. In terms of force production, Brouwer et al. (1998) observed a rightward shift in the active plantarflexor length-tension relationships after 3 weeks of continuous below knee casting, indicating that CP children generated more force further into dorsiflexion. Further, the passive end-range dorsiflexion increased. Since all the assessments by Brouwer et al. (1998) were performed with the knee held in flexion, this may provide indirect evidence for biological remodeling within the soleus muscle.

In summary, despite its popularity, the effects of prolonged stretch immobilization by orthotics or casts on muscle-tendon properties of children with CP have not been investigated so far. Notwithstanding the counter-arguments derived from animal studies, the rationale for increased plantarflexor muscle length after treatment with orthotics or casts cannot be definitely disproved.

1.6.2.4. Stretching

Stretching is probably one of the most frequently applied treatments during physical therapy of patients with CP and takes up about 25% of a therapists' time (Wiart et al., 2008). Although various stretching techniques can be discriminated, unless stated otherwise, this section focusses on static stretching. During static stretching, a joint's end-range position is gradually adopted and held for a certain time. This technique seems to be most commonly used in CP children. The major rationale may be considered fairly similar to orthotics and casts, namely to maintain or increase joint range of motion (Wiart et al., 2008). The proposed pathways for gains in passive joint range of motion after stretching generally vary from an increase in muscle length to modified sensations (Weppler and Magnusson, 2010).

When healthy controls statically stretch their plantarflexors, the reported gains on endrange dorsiflexion ranged from about 3-8° after stretching 5-7 days per week for 3-6 weeks. (Blazevich et al., 2014; Gajdosik et al., 2007; Konrad and Tilp, 2014; Mahieu et al., 2007; Nakamura et al., 2012). Findings for alterations in passive ankle joint torque-angle properties are controversial: Some studies linked the increase in joint range of motion to larger tolerated passive moments (Blazevich et al., 2014; Gajdosik et al., 2007), while Gajdosik et al. (2007) reported a general increase in passive joint torques following stretching and Nakamura et al. (2012) and Kubo et al. (2002) reported a decrease in passive joint torques at predesignated ankle joint angles. Finally, Mahieu et al. (2007) reported a decreased joint torque at end-range. Concerning the slope of the passive resistive torque-angle relationships, Blazevich et al. (2014), Konrad and Tilp (2014) and Kubo et al (2002) reported no changes. Hence no definite conclusion can be drawn if and how plantarflexor stretching alters ankle joint stiffness. In terms of active voluntary strength, no significant effects of plantarflexor stretching have been found (Blazevich et al., 2014; Konrad and Tilp, 2014; Kubo et al., 2002).

Concerning muscle-tendon properties of healthy controls, no significant changes in gastrocnemius fascicle length were reported following stretching (Blazevich et al., 2014; Konrad and Tilp, 2014; Nakamura et al., 2012). Nevertheless, Nakamura et al. (2012) and Blazevich et al. (2014) observed increased muscle belly elongation following passive static stretching. Both argued for a decrease in muscle belly stiffness due to alterations in connective tissue properties following stretching. Further, Blazevich et al. (2014) speculated about microscopic changes, e.g. myofilamental or titin alterations. By contrast, Konrad and Tilp (2014) suggested that stretching does not change passive resistive muscle belly stiffness. Concerning the gastrocnemius tendon, active (Blazevich et al., 2014; Kubo et al., 2002; Mahieu et al., 2007) or passive resistive stiffness (Konrad and Tilp, 2014) does not seem to be affected. Eventually, altered stretch tolerance was thought to be a main effect of stretching (Blazevich et al., 2014; Konrad and Tilp, 2014). This might be perhaps caused by different pain perception after treatments.

In immobilization experiments on animals, half an hour of static stretch of the immobilized muscle-tendon unit can be enough to prevent the loss of serial sarcomeres and maintain the joint range of motion (Williams, 1990) while 15 minutes of stretch every other day may help to prevent collagen increases in the muscle typically seen with immobilization (Williams et al., 1988). Therefore, manual stretching may potentially also be beneficial in CP children.

Effects on muscle-tendon and joint properties in CP

Until recently, the overall effectiveness of stretching in CP patients has been strongly doubted (Katalinic et al., 2011; Novak et al., 2013; Pin et al., 2006; Wiart et al., 2008). Katalinic et al. (2011) concluded that stretching may at best have an immediate effect on passive joint range of motion and is not sufficient to induce longitudinal tissue remodeling. Recommendations vary from prescribing alternatives, e.g. orthotics (Pin et al., 2006), allowing the children to stretch and move (Wiart et al., 2008) or cancelling manual stretching during treatment (Novak et al., 2013).

More recently, two groups of researchers specifically focused on the effects of plantarflexor stretching on muscle tendon and joint properties in CP. Firstly, Theis et al. (2015) investigated passive, static stretching (4 x per wk. for 6 wks.) and found a ~31% reduction in passive ankle joint stiffness and a 12% reduction in passive gastrocnemius muscle belly stiffness. In addition, an increase in muscle belly (+23%) and fascicle strain (+13%) was found but fascicle resting length and passive resistive tendon stiffness was not altered.

Secondly, apart from static stretching, a custom-made interactive robotic apparatus was evaluated (Chen et al., 2016; Wu et al., 2011; Zhao et al., 2011) whereby children with CP were treated with a combination of passive cyclic stretching and active-movement training. The active part consisted of voluntarily dorsi- and plantarflexion to play computer games. Zhao et al. (2011) pointed out that after 6 weeks (3 x wk.), fascicle length increased at neutral ankle position for both the soleus (+9%) and gastrocnemius (+3%), with an additional decrease of the pennation angle of -10% and -4%, respectively. Also the length of the Achilles tendon decreased by -6% which appears positive when taking into account the elongated tendons of CP patients (Barber et al., 2012; Kruse et al., 2016a; Theis et al., 2016; Wren et al., 2010). Using this stretching apparatus, Zhao et al. (2011) showed that the stiffness of the gastrocnemius fascicles decreased by 21%, while the tendon stiffness increased by 31%. Two later studies reported increased isometric dorsi- (Chen et al., 2016; Wu et al., 2011) and plantarflexor strength (Chen et al., 2016), decreased passive joint stiffness and increased maximum passive dorsiflexion (Chen et al., 2016; Wu et al., 2011) when training with the same machine. Also functional gains, e.g. an increased walking distance as well as improved balance was reported (Chen et al., 2016; Wu et al., 2011). Obviously, these studies did not solely include stretching as a training stimulus, and the gains in strength were probably attributable to the active exercises. Remarkably, in

contrast to Zhao et al. (2011), who used this robotic stretch trainer, no increases in fascicle length were noted after manual stretching (Theis et al., 2015). This may indicate that, apart from stretching, concomitant strengthening exercises are necessary for inducing changes in fascicle length in CP children.

In summary, there is limited positive evidence that stretching interventions can increase passive dorsiflexion in CP children within 6 weeks and 3-4 weekly sessions. This may also decrease stiffness of the gastrocnemius on the level of the muscle belly and its fascicles.

1.6.2.5. Resistance training

According to the American Academy of Pediatrics, resistance and strength training are used synonymously. They primarily aim to increase force production and may include free weights, weight machines, elastic tubing, or a child's own body weight (McCambridge and Stricker, 2008). Amongst others, the training is specified by frequency, type of resistance, intensity and duration. Recommendations for recreational or athletic purposes in healthy children include progressive training for 2-3 times per week for at least 8 weeks. Usually 2 to 3 sets with 8-15 repetitions are recommended. Although various contraction modes can be distinguished (e.g. concentric, isometric, eccentric, plyometric or isokinetic), no specific recommendation for pediatric training has been made (McCambridge and Stricker, 2008).

For children and adolescence with CP, resistance training is strongly recommended (Verschuren et al., 2016). However, few studies focused on the effects of resistance training on muscle-morphometric. No specific recommendations similar to those stated above for healthy children have been established for CP children. Preliminary evidence suggests that strength training leads to muscle hypertrophy in CP patients (Gillett et al., 2016). Through biochemical interactions, larger muscles may probably also prevent cardio-metabolic health risks later in life (Peterson et al., 2012). More immediately, during childhood, Ross and Engsberg (2007) pointed out that larger lower limb strength in patients with CP is associated with better gross motor function and faster walking speed. So, gains in strength of CP children are supposed to have a positive impact on mobility. Novak et al. (2013) reviewed that strength training was low to moderately effective for improving lower leg strength in CP. Nevertheless, in the past, strength training was also considered contraindicated in CP children due to concerns about increased muscle stiffness and spasticity (Verschuren et al., 2016). These concerns could yet be disproved (Morton et al., 2005; Scholtes et al., 2012). As to the deficits in muscle-tendon properties in CP (2.1. and 2.2.), gains in plantarflexor muscle size appear to be a reasonable target during strength training of CP patients.

Effects on muscle-tendon and joint properties in CP

Several studies focused on the effects of resistance training in children and adolescents with CP. Nonetheless, up to now, only Moreau et al. (2013) specifically investigated the effects on muscle fascicle length: They compared high velocity concentric resistance training versus conventional, slow training on knee extensor muscle morphometrics (24 sessions over 9 wks.). Training at high velocities increased rectus femoris fascicle length by 16%. Strikingly, there was also a 7% decline after training at lower contractile velocities. Moreover, both trainings similarly increased the rectus femoris cross-sectional area, while there was only a significant increase in muscle thickness of the vastus lateralis after slow velocity training. The isometric knee extensor torque increased after the slow training while the peak power increased only after fast velocity training. From a functional point of view, only the training at high velocities led to faster walking speeds. So, muscle strength adaptions and functional gains in CP seem to strongly depend on the provided contractile stimulus during training.

Stackhouse et al. (2007) investigated volitional strength training vs. electrically stimulated training via percutaneous implants to produce supramaximal force levels of the quadriceps femoris and triceps surae. The exercise was composed of isometric leg presses (3 x per wk. for 12 wks). The stimulated group showed superior gains of the quadriceps femoris cross sectional area muscle (+11% vs. +4 %) but neither group showed hypertrophy concerning the triceps surae. The authors speculated that the use of orthotics might have blunted any hypertrophic effect of the training on the triceps surae. Moreover, only the stimulated group increased their walking speed by ~20%.

Lee et al. (2015) compared neurodevelopmental therapy only with neurodevelopmental therapy and additional progressive resistance training (3x per wk. for 6 wks.). The resistance training was composed of sit-to-stand exercises, step-ups and knee rises. They found a significant increase in gastrocnemius muscle thickness (+43%), which was not yet significantly more than neuro-developmental therapy alone (+25%). In addition, the fascicle angle of the gastrocnemius increased by 20% after resistance training, while a decline was noted after neuro-developmental therapy (-12%). For the rectus femoris, the cross-sectional area increased more in the resistance training group (+75% vs. -6%). These morphometric benefits were also reflected by larger self-perceived mobility.

Eventually, McNee et al. (2009) reported a 23% increase in gastrocnemius muscle volume in children with CP after progressive heel raise or theraband training (4 x per wk. for 10 wks.) but no changes in ankle dorsiflexion during gait, walking speed or in walking related performance scores were noted.

In addition to the aforementioned studies, two further randomized controlled trials highlight the difficulty in promoting plantarflexor strength in CP patients with conventional exercises: Dodd et al. (2003) investigated a home-based resistance training protocol (3 x wk. for 6 wks.) using functional

multi-joint body weight exercises and Scholtes et al. (2010) performed a school based group circuit training (3 x wk. for 12 wks.). In both studies no gains in isometric plantarflexor strength were noted.

Moreoever, potential strength gains in the lower limbs of patients with CP are often not transferred to functional improvements. Despite knee extensor and hip abductor strength gains, Scholtes et al. (2012), for example, found no improvement in walking speed or during functional walking tests. In another study, exercises on conventional training machines (2 x wk. for 12 wks.) improved leg press strength in CP patients but no changes in gross motor function, kinematic gait quality, walking speed or functional walking tests were found (Taylor et al., 2013).

Taken together, there is fairly limited evidence that potential gains in muscle strength or architecture will translate to improved function in CP. This ineffectiveness has been quite a matter of controversy. Amongst others, this led to the drastic conclusion that resistance training in CP has no worthwhile functional benefits (Scianni et al., 2009). On the other hand, it was reasoned that, in order to improve walking, specific walking exercises should be incorporated into the training (Boyd and Graham, 1999; Romeiser Logan, 2013). This was supported by a recent review showing that gait training was the most effective intervention to improve walking speed in ambulatory CP children while there was no proof for the efficacy of sole strength exercises on walking speed (Moreau et al., 2016).

1.6.2.6. Rationale for implementing eccentric training

As a general rule of thumb, it had been suggested that the preferential muscle response to training in healthy controls depends on the contraction mode: concentric loadings cause addition of sarcomeres in parallel and eccentric training induces an increase of fascicle length through the addition of sarcomeres in series (Franchi et al., 2016). Concerning the lack of sarcomeres in series and the reductions in fascicle length in children with CP, it seems that eccentric muscle contractions in particular could be beneficial. Proske and Morgan (2001) also reasoned that eccentric training is beneficial for the plantarflexors of patients with equinus gait.

Fundamentals on muscle adaptions to eccentric training have been examined in animals. First, eccentric training appears to be beneficial for growth of muscle fibre length via sarcomerogenesis (Butterfield et al., 2005; Lynn and Morgan, 1994). It thereby induces a rightward shift in the length tension properties of muscle fibres (Butterfield and Herzog, 2006; Lynn et al., 1998). Thus, eccentric training over time enables more force production at larger muscle length. Second, these adaptations are thought to be under control of fibre (or sarcomere dynamics) and only when sarcomeres are trained beyond optimum length during contraction (Morgan and Proske, 2004), eccentric training leads a perturbation in fibre mechanics (Fig. 1-12).

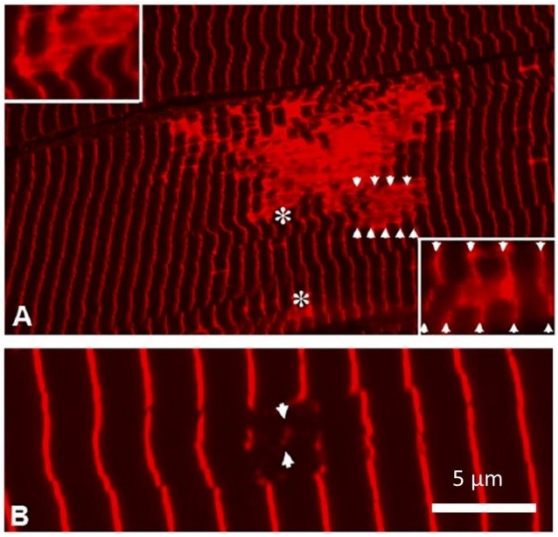

Fig. 1-12 Myofibrillar remodeling following eccentric exercise. Microscopic damage 7-8 days after eccentric exercise while running downstairs. Disrupted sarcomere structure in surgical biopsies of the human soleus muscle. Red stripes mark z-discs (boundaries of sarcomeres) and dark areas mark regions for action and myosin. Extracted and adapted from Carlsson et al., 2007, Neuromuscul Disord., 17, 61-68, p. 64. With permission from Elsevier.

Evidence on the effects of eccentric plantarflexor resistance training in humans is primarily limited to healthy adults (Duclay et al., 2009; Foure et al., 2013; Mahieu et al., 2008). Among others, Duclay et al. (2009) showed that high-intensity eccentric plantarflexor training on conventional strength training machines is able to increase gastrocnemius fascicle length (+7.6%), pennation angle (+6.8%) and actively tested tendon stiffness. In contrast to this, Foure et al (2013) reported no increase in gastrocnemius fascicle length or pennation angle after eccentric plantarflexor training. In contrast to the weight training of Duclay et al. (2009), Foure et al. (2013) and Mahieu et al. (2008) performed eccentric heel drops relying on their participants' own body weight. Although Foure et al. (2013) observed an increase in passive resistive tendon stiffness, they did not observe increases in active strength or muscle size. The Achilles tendon stiffness during isometric muscle contractions also did not change. Notably, Mahieu et al. (2008) reported a decrease in maximal passive resistive ankle torques and an increase in maximum passive dorsiflexion. This indicated that eccentric plantarflexor training could be able to promote joint flexibility which is in-line with findings on the hamstrings (Nelson and Bandy, 2004). The disparity of these results on eccentric plantarflexor training might be partly explained by different training protocols. Whereas Foure et al. (2013) seem to rely on the number of repetitions to increase the training load, Duclay et al. (2009) primarily increased the intensity throughout their training by using larger weights. In addition, the training of Foure et al., (2013) also contained jumping tasks, which might not have provided a sufficiently large eccentric stimulus on the gastrocnemius fascicles.

Next to these fairly conventional exercises, walking backward-downhill has been used in healthy controls to induce eccentric calf muscle loadings (Hoang et al., 2007a; Hoffman et al., 2014; Nottle and Nosaka, 2005a, 2005b). Such gait training induces repeated eccentric strains of gastrocnemius fascicles during the landing phase (Hoffman et al., 2014). It could be shown that after a single bout of prolonged backward downhill walking, blood markers for muscle damage are also upregulated (Nottle and Nosaka, 2005a). In addition, passive resistive tension of the gastrocnemius muscle-tendon unit seems to increase for up to 1-2 days following the training and muscle soreness occurs (Hoang et al., 2007a). An attenuated blood response following the next bout of training may reveal protection from further muscle damage. This is referred to as the repeated-bout effect, a characteristic of eccentric training (Nottle and Nosaka, 2005a). To the best of the author's knowledge, no such training has been evaluated in a longitudinal fashion for CP children.

Eventually, apart from Moreau et al. (2013), training studies in CP children seem to have barely focused on the modalities of muscle contraction and eccentric exercise has not been explored in detail in CP. Only Reid et al. (2010) studied eccentric exercise (3 x wk. for 6 wks.) for elbow flexors of CP children, revealing benefits for concentric and eccentric strength as well as a larger range for active torque exertions after the intervention.

In summary, lower limb muscles of children with CP can grow in response to resistance training within the period of 6-12 weeks and 3-4 weekly sessions. However, gains in strength or in muscle architecture are not necessarily reflected by improved gait. Therefore incorporating walking exercises into training has been advocated. As noted in animals and in healthy controls, differential adaptations within the muscles of patients with CP may depend on the contraction mode during training. Eccentric training in particular may provide a beneficial stimulus for sarcomerogenesis.

2. Purpose of the thesis

As outlined in the previous sections, equinus deformity and the related plantarflexor muscle-tendon pathology of children with CP is neuro-orthopedically targeted with various invasive and non-invasive approaches. One of the final aims of such interventions in ambulatory patients is to improve walking skills in order to increase the children's mobility. Since invasive approaches inherently include much higher risks and can provide enormous psycho-social burdens to the children and their caregivers, avoiding or postponing them should be considered very worthwhile. Thus, the overall objective of this thesis is to focus on non-invasive strategies for calf muscles of children with CP and to gain knowledge about the responsiveness of the related muscle pathology by using ultrasonography. The author would also like to promote our understanding of muscle structure-function relationships during gait.

On the one hand, despite its widespread use in pediatric orthopedics, there is a gap of knowledge about the effects of two highly common treatments on muscle-tendon properties in equinus pathology, namely manual stretching or stretch immobilization with orthotics. Taking into account some findings about promoted muscle atrophy in animals following stretch immobilization, there is a need for investigating possible adverse effects of stretch immobilization on the muscle and on the mobility in CP patients. Manual stretching also has fairly limited scientific evidence and lots of reasonable doubts. On the other hand, it is commonly accepted that 'form follows function' in biological tissue and therefore muscle-tendon pathology in CP may be shaped and alleviated when applying adequate training stimuli. With regard to the current topic, it needs to be shown if and how atrophied plantarflexor muscles and ankle joint contracture in the state of a chronic, neurological disease are able to adapt to exercise and whether this eventually also translates to improved function. Since differential muscle adaptations to training in healthy populations likely depend on the contraction mode, eccentric exercise could provide beneficial stimuli for the plantarflexors causing growth in fascicle length via sarcomerogenesis. Incorporating specific walking exercises into training also seems reasonable. Further, getting deeper insights on plantarflexor working mechanisms during ambulation in CP children will promote our understanding about the link between their muscle pathology and gait patterns. Therefore this thesis was split into three major studies:

The purpose of the first study was to longitudinally investigate the effects of ankle foot orthotics on gastrocnemius muscle morphometrics during passive manual examination, as well as during gait by using 2D ultrasound and 3D motion capturing in children with CP and equinus deformity while referencing to untreated typically developing controls. We thought that after the orthotic treatment, the gastrocnemius muscle-tendon unit would lengthen and passive ankle joint excursion would increase, thus getting closer to reference values form healthy controls. Additionally we wanted to

quantify any atrophic effect on the muscle belly due to immobilization. We expected positive effects of the treatment on gait showing a reduction in equinus posturing.

In order to find an alternative, conservative training stimulus for the calf muscles of children with CP, the second study compared the contractile activity of the medial gastrocnemius on sloped surfaces, namely during forward uphill and backward downhill gait while walking on a treadmill. We used 2D ultrasonography, 3D motion capturing and EMG to explore altered working mechanisms of spastic muscles directly during gait and compared the findings of children with CP to those of typically developing peers. We expected that also during flat forward gait, gastrocnemius fascicles of children with CP would show altered contractile activity and backward downhill gait would be able to induce eccentric fascicle strains in children with CP.

During the third study, we compared eccentric exercise by means of a novel training stimulus, namely backward-downhill gait versus a widely used conventional approach, namely passive and active manual static stretching. 2D ultrasonography, 3D motion capturing, EMG and hand-held dynamometry were used to test plantarflexor strength, passive ankle joint flexibility, as well as gastrocnemius muscle morphometrics, stiffness and strain on muscle-tendon and joint level. We hypothesized that eccentric exercise by backward-downhill treadmill training would be superior to stretching and was capable to improve plantarflexor strength as well as gait. In particular, we aimed to induce gastrocnemius muscle growth with backward-downhill gait. On the other hand, we expected stretching to be an insufficient stimulus for effecting muscle-tendon properties and being incapable of improving walking.

3. First study

Effects of ankle-foot braces on medial gastrocnemius morphometrics and gait in children with cerebral palsy

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3.1. Abstract

Purpose

In children with cerebral palsy (CP), braces are used to counteract progressive joint and muscle contracture and improve function. We examined the effects of positional ankle-foot braces on contracture of the medial gastrocnemius (MG) and gait in children with CP while referencing to typically developing children.

Methods

Seventeen independently ambulant children with CP and calf muscle contracture (age: 10.4±3.0y) and 17 untreated typically developing peers (age: 9.5±2.6y) participated. Children with CP were analyzed before and 16±4 weeks after ankle-foot bracing. MG muscle belly length and thickness, tendon and fascicle length, as well as their extensibility were captured by 2D ultrasound and 3D motion capturing during passive, manually applied stretches. In addition, 3D gait analysis was conducted.

Results

Prior to bracing, the MG muscle-tendon unit in children with CP was 22% less extensible. At matched amounts of muscle-tendon unit stretch, the muscle belly and fascicles in CP were 7% and 14% shorter while the tendon was 11% longer. Spastic fascicles displayed 32% less extensibility than controls. Brace wear increased passive dorsiflexion primarily with the knees flexed. During gait, children walked faster and foot lift in swing improved. MG muscle belly and tendon length showed little change, but fascicles further shortened (-11%) and muscle thickness (-8%) decreased.

Conclusions

Use of ankle-foot braces improves function but may lead to a loss of sarcomeres in series which could explain the shortened fascicles. To potentially induce gastrocnemius muscle growth, braces may also need to extend the knee or complementary training may be necessary to offset the immobilizing effects of braces.

Keywords: Cerebral Palsy; ankle-foot bracing; ultrasound; gastrocnemius; muscle contracture

Abbreviations:

CP: Cerebral palsy
MG: Medial Gastrocnemius
TD: Typically developing peers
MTU: Muscle-tendon unit
MAS: Modified Ashworth Scale
MTJ: Muscle-tendon junction
FA: Fascicle angle
L_{MTU}: Muscle-tendon unit length
L_{FASC}: Fascicle length
L_{MB}: Muscle belly length
L_{TEND}: Tendon length
GMFCS: Gross Motor Function Classification System
PRoM: Passive Range of Motion
3DGA: 3D gait analysis

3.2. Introduction

Symptomology of spastic Cerebral Palsy (CP) includes, but is not limited to, muscular weakness, overactivity and contracture [1]. Muscular contractures are thought to some degree reflect muscle tissue that fails to keep up with bone growth [2]. Plantarflexors are typically seriously affected. Apart from altered neural control, they are intrinsically very stiff [3] resulting in equinus, the most common musculoskeletal impairment in CP [4]. Equinus gait compromises balance and is fatiguing, since it requires more activity of the triceps surae [5]. During childhood the loss in passive dorsiflexion is progressive [6]. Thus, also muscle contracture of the triceps surae seems to deteriorate. On a long term painful bony foot deformities can result. Temporary immobilization of the stretched calf using casts or braces with or without botulinum toxin injections is a popular treatment [7,8]. Braces are commonly applied in non-rigid deformities. By holding the joint near its end-range, progressive contracture should be counteracted and spastic muscles are assumed to untighten and grow at a more equal rate to the bone. Eventually, also the gait pattern should improve. Yet, it is unclear how bracing actually affects the muscle morphometrics in spastic equinus deformity.

Muscle morphometrics in CP

Ultrasound scans provide a non-invasive means to gain information about a muscle's architecture. It could already be shown that plantarflexor morphometrics in CP are altered with respect to typically developing peers (TD) [9-14]. In case of the medial gastrocnemius (MG), one of the spastic leg muscles displaying largest volumetric atrophy [15], there is evidence for reduced muscle belly length (L_{MB}), cross-sectional area and muscle belly thickness (MT) [9]. However, Achilles tendon length (L_{TEND}) appears to be longer [10] while MG fascicle (bundle of skeletal muscle fibres) length (L_{FASC}) seems shorter than in TD [11, 12]. Concerning the latter, L_{FASC}, some inconsistencies have been reported [13, 14]. These discrepancies may be partly explained by difficulties in standardizing the musculoskeletal conditions, e.g. the degree of muscle stretch, during the assessment. On a microscopic level, spastic muscle fibres were also found to contain very long sarcomeres which was interpreted as an inability to add sarcomeres in series with growth [16,17].

Potential response to bracing

Casts and orthotics are currently favourable for contracture management [18]. They should keep the plantarflexor's muscle-tendon units [MTU] in a stretched position. This is assumed to over-time increase L_{MB} with a concomittant reduction in pathological equinus posture. Manual stretching of spastic MG can indeed transiently increase its L_{MB}, L_{FASC}, as well as L_{TEND} [19], but the long-term effectiveness of manual stretch remains doubtful [18]. Cyclic stretches by an external, machine-driven

device in combination with active training stimulated the MG fascicles to grow longer and become less stiff [20] positively demonstrating the MG's adaptive potential. By contrast, most braces induce static, low load stretch over prolonged periods and also immobilize the muscle. Knowledge about the morphometric effects of chronic muscle stretch is primarily derived from healthy animals. When muscle from adult animal is immobilized in a lengthened position, sarcomeres have been shown to be added in series [21]. Muscle fibres in CP may thus grow longer in response to bracing. Yet, the fibres' cross-section could also atrophy because of the immobilizing effect [22]. In juvenile, developing animals, experiments point out that primarily the tendon and not the muscle fibres lengthens in response to stretched immobilization [21,23,24]. Stimulated tendon growths could in fact reduce the stretch effects on muscle fibres and eventually induce sarcomere loss [23]. Such a scenario could theoretically decrease the MG muscle belly thickness [MT]. Because of cross-sectional atrophy and the pinnated fibre arrangement, also the L_{MB} could decrease. In fact, it has been doubted that stretch-immobilization can promote muscle growth in children with CP [25].

The main aim of this study therefore was to longitudinally re-evaluate MG morphometrics in children with CP after a period of ankle-foot bracing. To define the status quo prior to bracing, L_{MB}, MT, L_{FASC} and fascicle angle [FA] as well as L_{TEND} in children with CP was contrasted with TD using ultrasound during passive, manually applied stretches. However, total extensibility (~strain) of the muscle, fascicle and tendon was compared as well. We hypothesized that children with CP and equinus have shorter and thinner MG muscle bellies, shorter L_{FASC} but longer L_{TEND} than TD and that extensibility of the MTU and its components is reduced. After ankle-foot bracing, we expected that passive dorsiflexion would improve, L_{MB}, L_{FASC} and L_T of the spastic MG will be lengthened and extensibility of the MTU and its components will increase. Our second aim was to compare the functional effects of bracing using 3D gait analysis. We expected dorsiflexion to improve during stance and swing, positively affecting foot positioning at ground contact. Thereby walking speed and step length should be increased.

3.3. Methods

3.3.1. Participants

To be included children with CP had to be classified as GMFCS I or II and display non-rigid equinus. Non-rigid equinus was defined as tone on modified Ashworth Scale (MAS) <4 [26] and a passive range of motion (PRoM) lack smaller than -10° dorsiflexion (with flexed or extended knees). Further exclusion criteria were passive PRoM lack greater than a -10° of knee flexion from neutral, crouch gait, leg length discrepancies more than 2cm, any previous surgery to the leg, botulinum toxin injections within 1 year or bracing within 3 months. We thereby consecutively included 17 (9/8 male and female; 9/8 uni- and bilaterally involved, 7/10 GMFCS I and II) children with CP (age range: 5y 11mo–15y 6mo) from our

outpatient department. As a reference group, 17 TD were included (6/11 male and female, age range: 6y 0mo–15y 4mo). Only the (more) involved side was analyzed in children with CP based on passive dorsiflexion. For TD one leg was randomly chosen. Institutional ethics approval was granted and all subjects and their parents gave informed written consent.

3.3.2. Bracing

An articulated ankle-foot orthotic brace was individually manufactured out of glass and carbon fibre reinforced plastics (Fig.3-1). The lower leg shell is an S-type calf-construction with condylar support. It is fixed below the tibial tuberosity with a Velcro strap. The foot shell is a circular foot support. Both parts are linked by a constraint metal ankle hinge aligned in max. passive dorsiflexion while keeping the knee extended without perceiving intolerable discomfort. The subtalar joint was locked by a circular frame, the heel was fixed with a removable heel cap.

Plantarflexion movement was blocked, the dorsiflexion RoM was 5-10°. Every 4-6 weeks the brace was reviewed and the metal ankle hinge was realigned if possible. If plantigrade position could be achieved and if tolerated, gas springs (~ 2-3 Nm) were integrated to provide a constant dorsiflexion push during night-wear (7 of 17 children). This resistance could be voluntary attenuated upon mild plantarflexor contraction and all 17 children were intended to wear this brace during sleep. If passive dorsiflexion was less than -5° from plantigrade, they were additionally prescribed day-time use to extent duration of brace wear. 12 of 17 children met the criteria for day-time use. 3 of those were not compliant with day wear, so that a total of 9 (of 17) wore the brace during day and night. 8 of 17 wore the brace only at night and foot-orthotics during the day intended to prevent foot deformities due to mid-foot or subtalar instability.

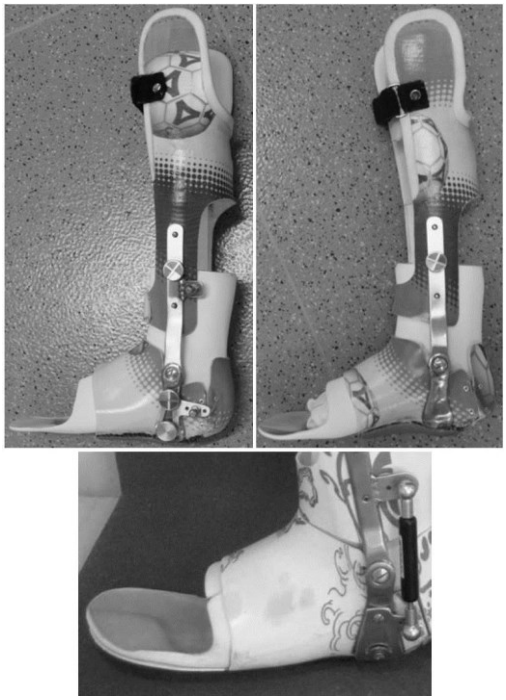

Fig. 3-1 Medial and lateral view of the ankle–foot brace with removable heel cap fixation, subtalar circular locking mechanism and optional posterior gas-spring for further dorsiflexion push.

3.3.3. Set-up and data collection

All children with CP were analyzed before and after bracing. Measurements were performed in the movement laboratory on the day of their outpatient appointments. TD were analysed on a single occasion. Apart from ultrasound scans, all participants were clinically manually examined by the same evaluator and underwent an instrumented 3D gait analysis (3DGA). PRoM for knee extension, popliteal angle (opposite hip flexed) and dorsiflexion with the knee flexed were measured using ruler-based goniometry. Plantarflexor tone was graded on modified Ashworth Scale (MAS) [26]. Passive dorsiflexion with the knee extended was instrumentally measured using motion capture data during MG ultrasound scans.

For 3DGA, a Vicon Nexus system with 8 MX-Cameras was used to capture barefoot gait at self-selected speed along a 12 m walkway. Markers were placed according a modified Plug-In gait Model [27]. Marker data were sampled at 200 Hz and force plate at 1000 Hz via two force plates (AMTI). Gait analysis was repeated until 5 clean strikes on the force plates from each foot could be obtained.

For the ultrasound scans, children were comfortably seated (hip semi-flexed) in a chair (Fig.3-2). Retro-reflective markers of the 3D motion capture system remained on the leg (Fig.3-2) to track knee alignment and ankle motion during the scan. A 7.5 MHz, 8 cm width, linear array probe (Sonoline Adara; Siemens, Munich, Germany) was attached with a carbon cast which was equipped with a cluster of four markers. The probe was attached at two locations: over the muscle-tendon junction (MTJ) and

over the mid-belly (halfway between popliteal crease and MTJ). The image plane was aligned with the fascicles according to a standardized protocol [28]. The leg was passively lifted and the knee extended as feasible. The same examiner manually moved the ankle slowly and continuously from flexion to extension and back. Prior to data collection the ankle was preconditioned with three cycles. Then, three to five dorsiflexion stretches were captured while the children could view the ultrasound screen and were encouraged to relax. If muscle contraction was manually sensed as sudden resistance, or whenever contraction was visually apparent, trials were repeated.

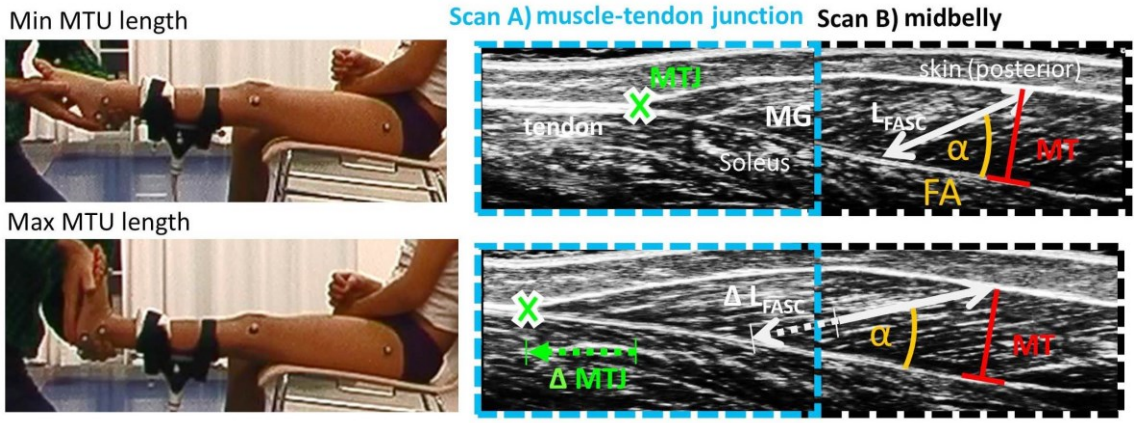

Fig. 3-2 Experimental setup. Left side: Child positioning with custom made carbon cast for probe fixation and markers of the motion capture system. Right side: Superimposed ultrasound scans of the medial gastrocnemius muscle-tendon junction (MTJ) and (B) its midbelly portion with representation of morphometric assessment. L_{FASC}: fascicle length, FA: fascicle angle, MT: muscle thickness.

3.3.4. Data analysis

To compare spatio-temporal gait, velocity and step length were extracted and normalized as described by [29] to account for growth. Besides, peak values for ankle dorsiflexion during stance and swing and knee extension during stance were analyzed. The foot landing pattern was characterized by the foot to floor angle at ground contact. To quantify ankle kinetics, the peak moments during the 1st and 2nd half of stance were selected, as well as the peak power during push-off.

For the ultrasound scans, the position of the markers and the ultrasound movies were continuously captured using Vicon Nexus software (Vicon, Oxford, UK) on 8 cameras with a sampling rate of 200 and 25 Hz, respectively. Subsequent analysis was done in MatLab software (MathWorks, Natick, USA). The MTJ in the Ultrasound movies was manually framewise located (Fig. 2). Concerning the fascicles, 3-5 different mid-belly fascicles were separately localized (straight-line between upper and deeper aponeurosis along hyperechoic [bright] collagenous tissue) and an automated tracking algorithm was used to continuously track their elongation during stretch [30]. MT was measured at min und max stretch, only. MT was defined as the distance between the upper and deeper aponeurosis, perpendicular to the deep aponeurosis [10], located halfway between popliteal crease and MTJ. FA

was calculated as: $\alpha=\arcsin(MT/L_{FASC})$. The distal L_{TEND} was defined as a straight-line from the heel marker to the MTJ. Since the entire Gastrocnemius MTU could not be tracked directly, L_{MTU} was calculated using previously established equations relying on motion capture data concerning tibia length, knee and ankle angles, as well as on individual anthropometrics [31]. L_{MB} was calculated as $L_{MB}=L_{MTU}-L_{TEND}$. [10]. L_{FASC} was represented by the average of all fascicles. For each trial, MTU stretches (from minimum to maximum length) were separated. To represent the average L_{FASC}, L_{MB} and L_{TEND} lengthening across the L_{MTU} stretch for each individual, data of each stretch was split into 10 equally spaced steps. Finally, the averages at these query points were taken before a third-order polynomial was fitted. For L_{MTU}, L_{MB}, L_{FASC} and L_{TEND}, minimum and maximum values were extracted. Besides, L_{MB}, L_{FASC} and L_{TEND} were analyzed at similar degrees of MTU stretch. Since there was no common overlap in L_{MTU} between all participants, the midrange L_{MTU} (halfway between minimum and maximum stretch) was calculated first for each individual with CP before bracing. To standardize comparisons, the average midrange L_{MTU} from children with CP was used for TD. To compare morphometrics before and after bracing midrange L_{MTU} could be individually exactly matched. All parameters were normalized to shank length as defined from the malleolus to the knee marker. Their extensibility was calculated as % change between minimum and maximum length.

3.3.5. Statistics

Shapiro-Wilk tests were used to test normality. At baseline, children with CP were compared with TD. Statistical group differences were evaluated with independent *t*-tests. To compare children with CP before and after bracing, paired *t*-test were performed. Mean differences and 95% confidence intervals were calculated. Alpha-level was set two-sided at 0.05. Standardized effect sizes were expressed as Cohen's d. Threshold values were 0.2, 0.5 and 0.8 for small, medium and large effects. Unless indicated differently, values are presented as mean (±1 SD).

3.4. Results

3.4.1. Participant characteristics and clinical exam

Values for TD and CP and the test statistics are summarized in Table 3-1. There were no significant differences in age, height, shank length or mass ($p>0.279$). Children with CP demonstrated significantly shorter popliteal angles ($p<0.001$). Average passive dorsiflexion in CP with knees flexed (8° [11°]) and extended (2° [10°]) was considerably reduced with respect to (TD 29° [8°] and 15° [5°]), all $p<0.01$. At

Follow-up, on average 16 (4) weeks (range: 12-24 weeks) apart, children with CP significantly grew and gained in mass, height and shank length. During clinical examination passive dorsiflexion improved with the knees in flexion (6° [11°], p=0.048) and extension (4°[8°], p=0.076), while significance was only noted with flexed knees.

Table 3-1 Anthropometrics , clinical exam and parameters of gait of typically developing (TD) and children with cerebral palsy (CP) before and change (post-pre) after bracing.

	TD	CP		CP post bracing		
	Mean (SD)	Mean (SD)	ES	Mean Δ	CI	ES
Anthropometrics						
Age (months)	114 (31)	125 (36)	0.2	3.7††	[3.2, 4.2]	2.3
Height (cm)	137.7 (15.3)	140.0 (17.8)	0.1	1.5††	[1.0, 2.0]	1.6
Shank length (cm)	33.4 (4.5)	33.4(4.8)	0.0	0.6†	[0.0, 1.2]	0.5
Mass (kg)	32.7 (13.0)	38.6 (18.1)	0.4	1.3††	[0.6, 2.0]	1.0
PRoM (°)						
Popliteal angle	8 (10)	36 (10)**	2.3	-4	[-1, 10]	0.4
Knee extension	6 (4)	3 (6)	0.6	0	[-2, 2]	0.1
Dorsiflexion (knees flexed)	29 (8)	8 (11)**	2.3	6†	[0, 11]	0.5
Dorsiflexion (knees extended)	15 (5)	2 (10)**	1.7	4	[0, 8]	0.5
Plantarflexion (knees extended)	39 (5)	42 (8)	0.4	1	[-1, 4]	0.3
MAS (0-4)						
Plantarflexor tone (knees flexed)	0.0(0.0)	1.7 (0.9)**	2.7	0.1	[-0.3, 0.5]	0.2
Plantarflexor tone (knees extended)	0.0(0.0)	2.3 (1.1)**	3.1	0.2	[-0.6, 0.2]	0.2

PRoM: Passive range of motion, MAS: muscle tone on Modified Asworth Scale, SD: Standard Deviation, ES: Effect Size (Cohen's d), CI: Confidence Interval. *Significant differences between TD and CP with p< 0.05 (** p< 0.01). †Significant differences between pre and post bracing in CP with p< 0.05 (†† p< 0.01).

3.4.2. Morphometrics

In children with CP, mean knee flexion angle during scans was 9° (5°) in CP vs. 5° (4°) in TD (p=0.015). Thus similar LMTU were reached at different ankle angles due to altered knee alignments (Fig.3). The midrange LMTU from CP (109.4% shank length) corresponded to 23° (8°) and 25° (6°) plantarflexion in CP and TD.

Average L_{MTU}-L_{FASC}, L_{MTU}-L_{TEND} and L_{MTU}-L_{MB} relationships are plotted in Fig. 3-3. Detailed statistics can be found in Table 3-2. Prior to bracing, the total MTU extensibility in CP with respect to controls was reduced by 22% (p=0.002). As illustrated in Fig. 3-4, this was accompanied by less fascicle (-32%) and tendon (-34%) extensibility (both p≤0.014). L_{MB} and L_{FASC} were significantly shorter throughout the stretch (all p0.035). L_{TEND} was significantly longer at minimum and midrange LMTU stretch (both p≤0.039). At midrange, L_{MB} and L_{FASC} were 7% (p=0.016) and 14% (p=0.032) shorter while LTEND was

11% (p=0.013) longer. MT was thinner, most pronounced (12%) during min MTU stretch (p=0.027) and FA appeared to be comparable between TD and CP children.

After bracing, the L_{MTU}, L_{MB} and L_{TEND} did not significantly change (all p≥0.272) but L_{FASC} was significantly shorter throughout the stretch (all p≤0.035). At matched midrange L_{MTU} stretch, 11% of L_{FASC} were lost with respect to baseline. Simultaneously, MT decreased by 8%, reaching significance at max MTU stretch (p=0.018), while FA showed minor change. No significant changes in fascicle and muscle extensibility were noted (p>0.104), whereas tendon extensibility increased by 20% (p=0.017).

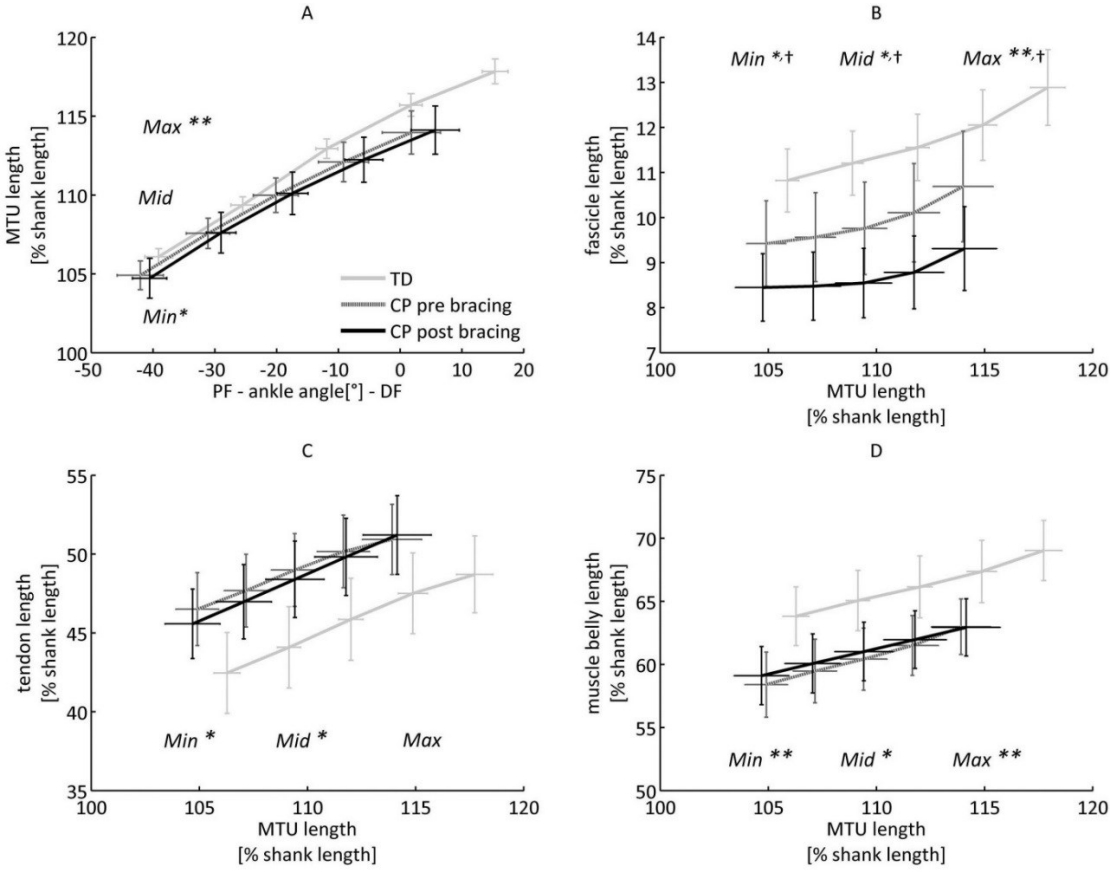

Fig. 3-3 Normalized muscle morphometrics during stretch. Data are group mean (1 SEM). *Significant differences between TD and CP with p<0.05 (**p<0.01) and †significant differences between pre and post bracing in CP with p<0.05 (†† p<0.01) tested at minimum (Min), matched midrange (Mid) and maximum (Max) muscle-tendon unit length.

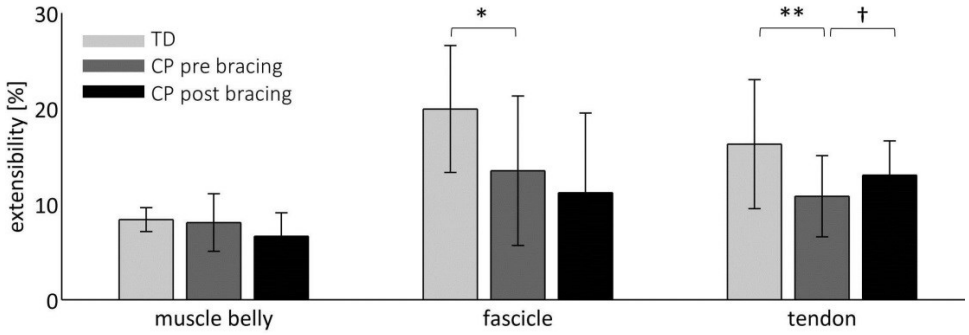

Fig. 3-4 Extensibility of the muscle belly, fascicle and tendon. Data are group mean (1 SD). * Significant differences between TD and CP with p< 0.05 (**p< 0.01). †Significant differences between pre and post bracing in CP with p< 0.05**.**

Table 3-2 Normalized muscle morphometrics of typically developing (TD) and children with spastic cerebral palsy (CP), as well as changes after bracing (post-pre) in CP

		TD	CP		CP post bracing		
	degree of MTU stretch	Mean (SD)	Mean (SD)	ES	mean Δ	95% CI	ES
MTU length	min	106.1 (1.2)	104.9 (2.1) *	0.7	-0.2	[-1.6, 1.2]	0.1
[% shank]	matched mid	109.4 (0.0)	109.4 (2.3)	-	-	-	-
	max	117.8 (1.8)	114.0(3.1)**	1.6	0.2	[-1.7, 2.0]	0.2
Muscle belly length	min	63.8 (5.2)	58.4 (5.8)**	1.0	0.7	[-0.7, 2.0]	0.3
[% shank]	matched mid	65.2 (5.5)	60.4 (5.5)*	0.9	0.5	[-0.7, 1.7]	0.2
	max	69.1 (5.4)	63.0 (4.9)**	1.2	0.1	[-1.4, -1.4]	0.0
Muscle belly thickness	min	4.4 (0.5)	3.9(0.8) *	0.8	-0.3	[-0.6, 0.0]	0.5
[% shank]	max	4.2 (0.5)	3.7(0.8)	0.7	-0.3†	[-0.6, -0.1]	0.6
Fascicle length	min	10.8 (1.6)	9.4 (2.1)*	0.7	-1.0†	[-2.0, -0.1]	0.6
[% shank]	matched mid	11.3 (1.6)	9.8 (2.3)*	0.7	-1.1†	[-2.2, -0.1]	0.6
	max	12.9 (1.9)	10.7 (2.7)**	0.8	-1.5†	[-2.5, -0.2]	0.6
Fascicle angle [°]	min	24.3 (4.1)	25.0 (4.9)	0.2	1.0	[-1.5, 3.6]	0.2
	max	19.2(3.2)	21.4(5.7)	0.5	0.7	[-2.0, 3.3]	0.1
Tendon length	min	42.4 (5.7)	46.4 (5.0)*	0.7	-0.9	[-2.5, 0.8]	0.3
[% shank]	matched mid	44.2 (5.5)	49.0 (5.2)*	0.9	-0.5	[-1.7, 0.7]	0.2
	max	49.0 (5.4)	51.3 (5.1)	0.4	0.1	[-1.8, 1.9]	0.0

SD: Standard Deviation, ES: Effect Size (Cohen's d), CI: Confidence Interval, matched mid: parameters at midrange MTU length that refers to 50% MTU stretch in CP. * Significant differences between TD and CP at p< 0.05 (** p< 0.01). †Significant differences between pre and post bracing in CP at p< 0.05 (†† p< 0.01).

3.4.3. 3D gait analysis

Results are shown in Table 3-3. Before bracing, children with CP walked 16% slower while taking 13% shorter steps than TD and landed with a significantly steeper foot contact (all p≤0.001). Average constraints in knee extension (-3°) and dorsiflexion (-4°) in stance did not reach significance (p≥0.102). Obstructions in dorsiflexion were more pronounced during swing (-5°, p=0.004). Ankle moments in early stance were pathologically increased, whereas ankle moments and power used for propulsion were considerably diminished (all p≤0.002). After bracing, walking speed significantly increased by 8% (p=0.014) while children tend to take longer steps (3%, p=0.068). Children landed with a significantly better foot to floor angle (p=0.006) and the average pattern changed towards heel-toe gait. Dorsiflexion gains in stance failed to reach significance (+2°, p= 0.073) but showed significant increases in swing (+2°, p=0.045). The pathologically increased ankle moment during early stance develops towards reference values (p<0.001). While propulsive ankle moments were reduced (p=0.013), power was not significantly changed (p=0.550).

Table 3-3 Results of 3DGA of typically developing (TD) and children with cerebral palsy (CP) before and change (post-pre) after bracing.

3DGA	TD	CP		CP post bracing		
	Mean (SD)	Mean (SD)	ES	Mean Δ	CI	ES
vel (non. dim.)	0.47 (0.06)	0.40(0.08)**	1.1	0.03†	[0.01,0.06]	0.5
step length (non. dim.)	82.2 (7.3)	71.2(11.5)**	1.1	2.2†	[-0.2, 4.5]	0.4
landing angle (°) - foot to floor	11(6)	1(7)**	1.5	2†	[1, 4]	0.6
knee flexion (°) – midstance	6(4)	9(9)	0.5	1	[-1, 3]	0.1
dorsiflexion (°) – late stance	12 (4)	8(8)	0.5	2	[0, 4]	0.4
dorsiflexion (°) – swing	6(3)	1(6)**	0.9	2†	[0, 4]	0.4
ankle moment (Nm/kg) – early stance	0.6 (0.1)	0.9(0.2)**	1.3	-0.1††	[-0.2, -0.1]	0.8
ankle moment (Nm/kg) – late stance	1.3 (0.2)	1.1(0.2)**	1.0	-0.1†	[-0.2, 0.0]	0.5
ankle power (W/kg) – late stance	3.9 (0.9)	2.0(0.7)**	2.4	0.1	[-0.1, 0.2]	0.1

3.5. Discussion

This study set out to provide information about gastrocnemius muscle morphometrics in children with CP before and after a period of ankle foot bracing while referencing to untreated typically developing (TD) peers. Our assumption was that the spastic medial gastrocnemius (MG) would change towards TD and lengthen after a period of ankle-foot bracing. However, no significant gains of muscle belly (L_{MB}) or tendon (L_{TEND}) length occurred and fascicle length (L_{FASC}) further shortened while muscle bulk decreased. Nonetheless, on a joint level, significant increases in passive dorsiflexion were noted.

This primarily affected dorsiflexion with knees flexed but the majority of the children also gained dorsiflexion when assessed with extended knees. During gait, children walked faster and in particular dorsiflexion during swing, as well as the foot landing pattern improved.

Muscle morphometrics prior to bracing

Before bracing, shortening of the spastic MG L_{MB} and L_{FASC} was pronounced in CP and maximum values during stretch only approached minimum values of TD. This displays considerable atrophy among relatively high functioning, independently ambulant children and youth (GMFCS I and II) with CP. As described before, muscle thickness (MT) was less [9] and fascicle angle (FA) appeared to be similar [13,14]. Shorter L_{FASC} *and* L_{MB} also agree with recent investigations [11,12]. Besides, our data confirms that MG fascicles are less extensible than usual [32]. Therefore, it appears reasonable to assume that spastic MG fibres may lack sarcomeres in series or that they might contain longer, already drawn-out sarcomeres, as had been shown for forearm or hamstring muscle [16,17]. Potentially, more connective tissue could also impede the actual extensibility of the fibres [17]. Whether these alterations are caused by altered muscle growth or result as consequence of decreased loading and reduced physical activity remains a subject of controversy [33]. In contrast to L_{MB} and L_{FASC}, at midrange stretch L_{TEND} was longer than usual [10]. Even though L_{TEND} at similar degrees of muscle-tendon unit (MTU) stretch was longer, its total extensibility seemed to be compromised. In summary, if clinicians or therapists want to improve MG muscle pathology in children with CP, growth of muscle belly in length and thickness, as well as longer L_{FASC} appear to be desirable goals. Longer L_{FASC} with more sarcomeres in series could in principle promote function by increasing the muscle's contractile velocity and enable a muscle to exert force over a larger joint RoM [34].

Muscle morphometrics after bracing

Conversely, after bracing, additional 11% in L_{FASC} were lost at matched amounts of MTU stretch and MT decreased by 8%. This is the first study to provide information about spastic calf morphometrics after brace wear. Previous investigations on the longitudinal change of calf muscle morphometrics were also done on invasive treatments with worse outcome: Despite improving passive dorsiflexion, gastrocnemius recessions induced shortening of L_{MB} [35] and 32% shortening of L_{FASC} [36]. Botulinum toxin injections caused reductions in MT of ~12% [37]. While we observed shorter L_{FASC}, the L_{MB} modifications seemed negligible. Due to its pinnated fibre arrangement, loss in L_{FASC} may not be reflected by loss in L_{MB}, if, as observed, the MT reduces, too. Although we did not instrumentally assess the force producing capability of the MG, these architectural deteriorations suggest that the muscle would have gotten weaker.

Potential causes for morphometric changes

A potential cause for shorter L_{FASC}, and thus progressive muscle contracture, could be that muscle tissue indeed failed to keep up with bone growth [2]. For normalization, L_{FASC} was set in relation to the shank length [11]. In TD children MG L_{FASC} usually grows in proportion with the tibia [38]. During the current intervention, the shank of children with CP grew by 1.8%, while unscaled L_{FASC} dropped by 0.3cm. Hence, these atrophic changes do not solely expose a lack of scaling to bone growth.

Another reason for shorter L_{FASC} could be adaptations of the tendon. Although no significant changes in fascicle and muscle extensibility were noted, the extensibility of the tendon increased. Still, we did not observe changes in L_{TEND} defined as a straight-line from the heel marker to the MTJ. However, in absence of instrumented measures for the applied tension during passive stretch, this measure ignores slack. Consequently, we generally underestimate L_{TEND} at small degrees of MTU stretch and overestimate its extensibility. Slack is usually surpassed shortly beyond neutral ankle alignment in TD [32]. In our data, only 2-3% of tendon extensibility in TD children would be noted above that point which confirms previous reports about intrinsic tendon tissue strain [39]. Moreover, during max MTU stretch, when all slack is taken up, no changes in L_{TEND} of children with CP were noted which could suggest that no major changes in L_{TEND} occurred. Assuming that the tendon could have initially gotten more compliant during bracing, such as observed in growing animals [23,24], the spastic fascicles were unstrained which can trigger loss of sarcomeres [21,22] and would fit the reductions in L_{FASC}. Although intrinsic tendon properties are cumbersome to measure more detailed information is necessary to clarify this.

Worth mentioning, the MG tendon also integrates the run-out from the deep MG aponeurosis and the Achilles tendon to which both gastrocnemius and soleus merge. With the soleus fascicles also attaching distal to the MG's muscle-tendon junction (MTJ) the increase in MG tendon extensibility could as well reflect a more compliant soleus. This also explains the significant increase of passive dorsiflexion with the knee held in flexion. Most ultrasound research in CP is currently done on gastrocnemius morphometrics probably owed to its superficial position. Clearly, more information about the soleus architecture in equinus needs be gathered.

Eventually, the current outcome could be attributed to the bracing regime. Overall, the MG is highly susceptible to disuse atrophy [40]. Since ankle motion in the brace was also largely restricted, a possible reason for the loss in L_{FASC} and decreased MT could be decreased muscle ecxcursion which is important in regulating sarcomere number in growing animals [41]. Besides, as all children likely slept with bent knees, the ankle-foot brace may have not provided sufficient stretch on the bi-articular gastrocnemius. Surely, a knee-ankle-foot brace would be logic to target gastrocnemius contracture. Sees and Miller [42] recently emphasized this, elsewise suspecting contracture of the gastrocnemius to worsen. Our results reinforce this. As a final point, considering that prior to bracing, average

dorsiflexion with knees extended in the current study group was 2°, use of augmented force into further dorsiflexion seemed critical. Such an extensive bracing treatment is very demanding and may cause insufficient compliance. That may be one reason why below knee casting or night time AFOs are often being used [7,8].

Functional benefits of bracing

Next to improving passive dorsiflexion with flexed knees, this 'positional' bracing also prevented deterioration in passive dorsiflexion with the knee in extension in 76% of the children (13 of 17). The average dorsiflexion gain of 4° with extended knees marginally failed to reach significance. In the past, also below knee serial casts have been shown to only increase passive dorsiflexion with flexed knees [8]. Based on the progressive loss of passive dorsiflexion during CP childhood [6], these results appear to be a beneficial outcome for children with CP! More importantly, from a functional perspective, the children walked faster and their ankle kinematics improved primarily in swing. Positioning the foot better for landing can be vital to avoid tripping and to prevent mid-foot break deformities. Reduced ankle moments during early stance further may also display less pathological dynamic joint stiffness after bracing. However, the reduced moments for push-off may be a side-effect but are in accordance with reduced muscle thickness.

We think that these functional gains outweigh the atrophic effects on muscle morphometrics. Restoring dorsiflexion and normalizing muscle morphometrics may not necessarily occur in concert. By concurrently improving morphometrics a larger or potentially more sustainable change in function may be achieved. Coordinative (neural) aspects may of course also modulate the direct relations between morphometrics and function. These aspects should be goals of future interventions. Most likely this may include activities, such as calf strength training [43] or instrumented cyclic stretching [20].

Considerations for ultrasound scans

Ultrasound scans are frequently used to study muscle architecture in CP. To perform valid comparisons between TD and CP, morphometrics should be assessed at similar muscle states. Some studies extracted MG parameters at resting or neutral ankle position without detailed info on knee alignment or at PRoM limits only [13, 14, 37]. Recently, a common ankle angle was suggested [12]. Still, even at similar ankle alignment, our data shows that the MTU can be considerably shorter in CP. Albeit referencing to L_{MTU}, we found no common overlap. Possibly, we could have done so when allowing for semi-flexed knees [12] but the MG would then be slightly off-tension. By using a calculation of the entire MTU path we accounted for different knee angles during testing, but the issue of standardization may depend on patient positioning. MG scans are mostly done when lying prone

[10, 11, 14, 20, 38], but also when lying supine [19] or when sitting [12]. The latter probably poses most difficulties to achieve straight knees in case of short hamstrings. With the current set-up, it seemed best choice to compare TD at the average midrange L_{MTU} of the children with CP.

3.6. Limitations

First, we investigated a convenience sample and can only speculate about untreated natural progression of contracture. The treatment duration was somewhat variable depending on the childrens' outpatient attendance and not every child received full-time bracing. Longer treatment duration explained 15% of the loss in L_{FASC} assessed by simple linear regression (R^2). Besides, effects on L_{FASC} were not different between the bracing protocols (p=0.580). In future the separate impact of day and night-time orthotics should be quantified. Eventually, the applied tension during stretch was not instrumentally standardized. Nevertheless, it seems very unlikely that shorter L_{FASC} after bracing could be attributed to consistently reduced manually applied tension, since L_{FASC} was shorter during the entire MTU stretch.

3.7. Conclusions

To the best of our knowledge, this is the first study about calf morphometrics in CP after a non-invasive orthotic treatment. Prior to bracing, the children with CP had shorter and thinner MG muscle bellies, shorter fascicles but longer distal tendons than controls. Positional ankle-foot braces significantly improved passive dorsiflexion with the knees flexed and improved the gait pattern of the children but failed to improve MG morphometrics. Further shortened fascicles and thinner muscle bellies are likely due to the fact that bracing potentially decreased the MG excursion or kept the bi-articular muscle off-tension. Theoretically, braces may need to extend the knee if MG morphometric pathology should be targeted. Promoting dorsiflexion and normalizing muscle morphometrics seems difficult to be accomplished in concert using traditional orthopaedic means such as surgery, botulinum toxin injections or brace wear in isolation. Consequently, there is a need for concomitant treatments that promote muscle growth.

Conflict of interest

All authors declare that they have no conflict of interest.

3.8. References

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4. Second study

Contractile behavior of the medial gastrocnemius in children with bilateral spastic cerebral palsy during forward, uphill and backward-downhill gait

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4.1. Abstract

Background

Plantarflexor tightness due to muscle degenerations has been frequently documented in children with spastic cerebral palsy but the contractile behavior of muscles during ambulation is largely unclear. Especially the adaptability of gastrocnemius muscle contraction on sloped surface could be relevant during therapy.

Methods

Medial gastrocnemius contractions were measured during flat-forward, uphill (+12% incline) and backward-downhill (-12% decline) treadmill gait in 15 children with bilateral cerebral palsy, walking in crouch, and 17 typically developing controls (age: 7-16 years) by means of ultrasound and motion analysis. Tracked fascicle and calculated series elastic element length during gait were normalized on seated rest length. Additionally electromyography of the medial gastrocnemius, soleus and tibialis anterior was collected.

Findings

During forward gait spastic gastrocnemii reached 10% shorter relative fascicle length, 5% shorter series elastic element length and showed 37% less concentric fascicle excursion than controls. No difference in eccentric fascicle excursion existed. Uphill gait increased concentric fascicle excursion in children with cerebral palsy and controls (by 23% and 41%) and tibialis anterior activity during swing (by 33% and 48%). Backward downhill gait more than doubled (+112%) eccentric fascicle excursion in cerebral palsy patients.

Interpretation

Apart from having innately shorter fascicles at rest, flat-forward walking showed that spastic gastrocnemius fascicles work at shorter relative length than those of controls. Uphill gait may be useful to concentrically train push-off skills and foot lift. During backward-downhill gait the gastrocnemius functions as a brake and displays more eccentric excursion which could potentially stimulate sarcomere-genesis in series with repeated training.

Keywords: Cerebral palsy; Ultrasound; Gastrocnemius; Fascicles; Sloped walking; Muscle architecture

Abbreviations:

SCP Spastic cerebral palsy
TD Typically developing
SEE Series elastic element

4.2. Introduction

Weak (Barber et al., 2012; Dallmeijer et al., 2011) and stiff plantarflexors (Barber et al., 2011) are major constraints in spastic cerebral palsy (SCP). Both can have neural origins since impaired voluntary drive reduces active strength (Stackhouse et al., 2005) and involuntary, velocity or posture dependent muscle activity increases passive stretch resistance (Bar-On et al., 2014). Apart from that, structural degenerations could have a negative impact because the triceps-surae shows large volumetric loss and increased intramuscular connective tissue (Noble et al., 2014; Pitcher et al., 2015). While gastrocnemius fascicles (bundles of fibres) seem to be shorter in SCP-patients than in typically developing (TD) (Hösl et al., 2015; Matthiasdottir et al., 2014; Barber et al., 2011), biopsies revealed longer sarcomeres (Mathewson et al., 2014). The shorter fascicles hence contain less sarcomeres in series and this may contribute to reduced passive fascicle extensibility (Hösl et al., 2015; Barber et al., 2011) and a smaller range for active force exertion (Barber et al., 2012).

The relationship between muscle structure and ambulatory dysfunction in SCP are still largely unknown. Typically plantarflexor weakness is associated with less propulsion during gait (Dallmeijer et al., 2011). Concerning fascicles, less sarcomeres in series could compromise shortening excursion and velocity (Butterfield, 2010) and active force exertion may be shifted towards plantarflexion. As a consequence, short gastrocnemii may force to walk in equinus. However, the bi-articular gastrocnemii can also promote crouch gait (Maas et al., 2015). Besides it had been speculated that the spastic gastrocnemius was unable to resist tensile forces and experiences increased eccentric loadings (Fry et al., 2006). These loadings were vaguely supposed to harm fibre growth (Gough and Shortland, 2012) or promote fibrosis (Pitcher et al., 2015).

Insights might be gained by monitoring gastrocnemius contractions during gait. So far, such approaches are based on simulations (Steele et al., 2013; Neptune et al., 2007) or on TD mimicking spastic gait (Fry et al., 2006). In toe-walking, the gastrocnemius seems to work isometrically to concentrically and operates on short-length (Fry et al., 2006; Neptune et al., 2007). Brightness-mode ultrasonography has been used as an acceptable methodology to directly assess the contractile behavior of the muscle-tendon unit (Aggeloussis et al., 2010), for example in adults (Ishikawa and Komi, 2008; Cronin and Finni, 2013), in children (Fry et al., 2006) or in elderly (Mian et al., 2007). It has been found that gastrocnemius' fascicles and muscle-tendon unit do not necessarily lengthen or shorten simultaneously (Ishikawa and Komi, 2008). Consequently, inference about fascicle action from muscle-tendon unit length calculations appears inconclusive. Fascicle contraction changes in elderly (Mian et al., 2007), during running (Ishikawa and Komi, 2008) and is modified on inclines or declines (Lichtwark and Wilson, 2006; Hoffman et al., 2014). So contractile behavior depends on the investigated sub-group and is generally modifiable.

Such information could aid to understand pathological gait patterns in SCP. In addition, it might help to develop exercises against gastrocnemius deficits since different types of dynamic muscle contraction can cause particular muscle adaptations. In TD, eccentric training might be favorable to induce longitudinal growth of fascicles (Franchi et al., 2014). Treadmills are often used to practice level walking in SCP but modifications, e.g. concerning the slope or walking direction, may modulate the type and extent of the contractile activity and thereby target the muscular deficit: Forward-uphill training reduces passive stiffness of spastic plantarflexors (Willerslev-Olsen et al., 2014, 2015) but the mechanisms remain somewhat unclear while backward-downhill may provide eccentric calf loadings, as shown in TD (Hoffman et al., 2014, Hoang et al., 2007a). Investigating SCP-patients during these two tasks hence could be relevant for promoting non-invasive therapies.

The main purpose of this study was to analyze the contraction of the medial gastrocnemius in SCP-patients and TD during level, uphill and backward-downhill gait with ultrasound, motion analysis and EMG. Due to the shortened fascicles and the findings from mimicry and simulation studies, we expected that the spastic gastrocnemii show less fascicle lengthening than TD and that fascicles reach shorter length during level gait. Due to reports in healthy adults, we anticipated that uphill gait induces larger fascicle operating length and more concentric fascicle shortening in TD and SCP while backward-downhill gait causes larger eccentric fascicle lengthening and a shift of the fascicle operating regions towards shorter length.

4.3. Methods

4.3.1. Participants

Children with SCP had to be classified as GMFCS-Level I or II (Palisano et al., 1997) and display bilateral involvement. Exclusion criteria were any leg surgeries at all or botulinum toxin injections within 12 months. Only data of the more involved side (less passive dorsiflexion) was included. 15 children with SCP (4 females) and 17 TD (8 females) between 7–16 years took part. 11 SCP children were classified as GMFCS I, 4 as GMFCS II. For TD the right leg was analyzed. Experiments received medical ethics approval by the Technische Universität München and informed written consent was obtained.

4.3.2. Protocol

Participants were physically examined and performed a 3D gait analysis on a treadmill (Atlantis, Heinz Kettler, Ense-Parsit, Germany). Subjects wore a harness (h/p/cosmos, Nussdorf-Traunstein, Germany) without weight support which was connected to a safety frame (Mobil Konzept Loadmaster

80, RMT RehaMed Technology, Dietzenbach, Germany). All trials were done barefoot on even surface (flat-forward), on +12% inclined surface (uphill) and on -12% declined surface (backward-downhill) (Fig. 4-1).

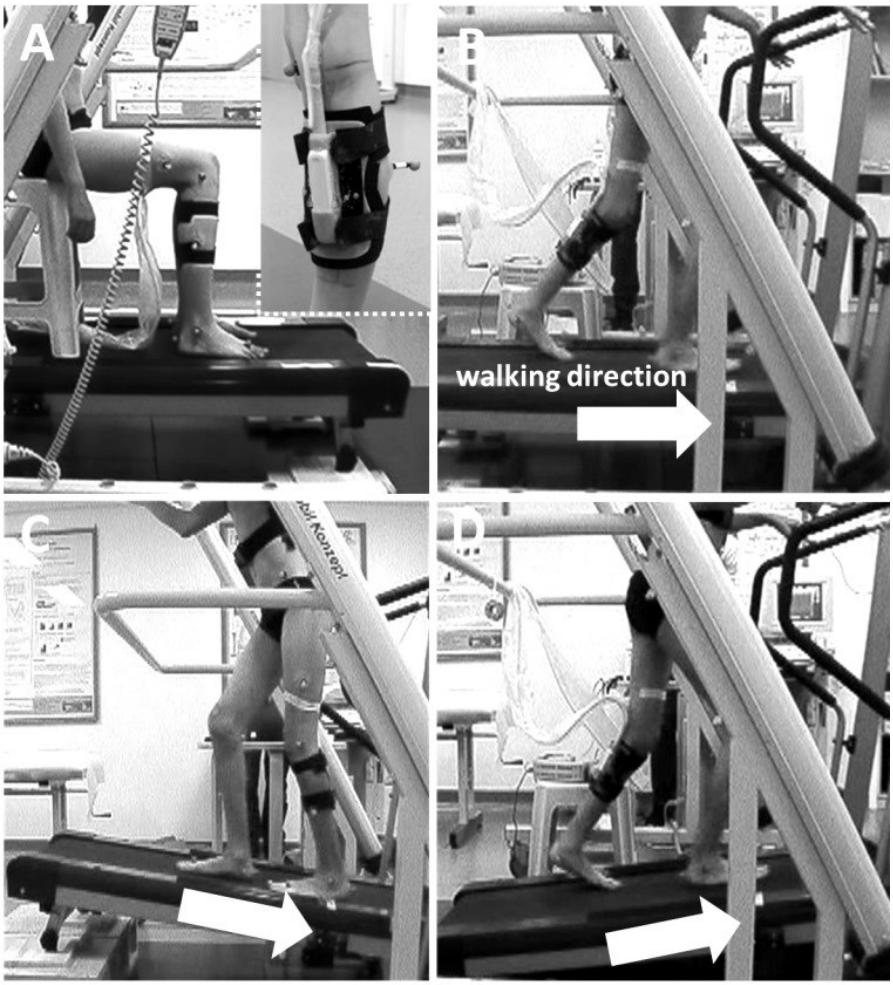

Fig. 4-1 Test conditions: A) seated rest measurement and ultrasound probe placement, B-D) treadmill walking for B) flat-forward, C) -12% backward-downhill slope and D) +12% uphill slope.

This protocol was applied twice because probe fixation did not allow for simultaneous measurements of ultrasound and EMG. The order of the slopes was randomized and half of the participants started with EMG. During each condition data was captured during 10 sec., starting when the subjects felt comfortable. Prior to walking, a 5 sec. long seated rest measurement was done, with knees 90° flexed and ankles in neutral (Fig. 1A). On the treadmill, 5-10 min. habituation time was provided during which preferred forward speed was determined with the subject blinded to the panel (Dal et al., 2010). The investigator increased the speed in 0.1 km/h increments until the subject reported to walk comfortable. Then, 1-1.5 km/h was added, followed by a stepwise decrease of 0.1 km/h to re-establish comfortable walking. This procedure was repeated three times and speeds were averaged. The uphill and backward-downhill speed was reduced to 85% and 50% of flat-forward speed to provide settings that should be also applicable for prolonged exercise. Values were chosen after studying the literature (Willerslev-Olsen et al, 2014; Joseph et al., 2016) and pilot testing. Since some

SCP-patients were not able to walk without handrail, all participants were constrained to touch a lateral rail. During backward-downhill walking subjects grasped a rail at chest height.

4.3.3. Physical exam

Passive range-of-motion for knee extension, dorsiflexion and popliteal angle were measured using ruler-based goniometry. Plantarflexor tone was graded on modified Ashworth Scale (Bohannon and Smith, 1987). Peak isometric plantarflexor force (N/kg bodyweight) was assessed by hand-held dynamometry using an uniaxial Force sensor (Mobi, Sakaimed, Tokyo, Japan) during 5 MVCs (3 sec. contraction, 1 min. rest). Subjects were seated (hips semi-flexed, knees extended). After discarding the lowest and highest value, 3 trials were averaged.

4.3.4. Gait analysis

A Nexus system (Vicon Inc., Oxford, UK) with 8 MX-Cameras was used to capture lower limb kinematics using a modified Plug-In gait Model (Stief et al., 2013) at 200 Hz. One additional marker was placed at the medial calcaneus, leveled with the heel marker. Gait events were derived as described by Zeni et al. (2008). All subsequent analysis was done in MatLab (MathWorks, Natick, USA). Non-dimensional walking speed was calculated (Hof, 1996) and sagittal joint angles were determined. Ankle angles were calculated using the foot markers without the toe to avoid bias by midfoot-bending. Peak values for dorsi-, knee- and hip flexion in stance and swing were calculated. Furthermore the sole angle (foot to belt) and knee flexion at initial contact were extracted.

4.3.5. Electromyography

Activity of the medial gastrocnemius, soleus and tibialis anterior was captured wireless with a DTS System (Noraxon, Scottsdale, USA). Surface electrodes (Blue Sensor N, Ambu, Ballerup, Denmark) were placed on the muscle bellies and signals were sampled at 1000 Hz. All strides during the 10 sec. were analyzed separately. Signals were off-line filtered as described by Panizzolo et al. (2013) and mean rest activity was subtracted from walking signals before normalizing each signal on max. activity of all forward trials. For the medial gastrocnemius and soleus, mean activity during stance and for the tibialis anterior, mean activity from end of single stance to touch-down was calculated.

4.3.6. Ultrasonography

An Echoblaster 128 ultrasound (Telemed, Vilnius, Lithuania) was used to image medial gastrocnemius fascicles at 60·Hz with a linear probe at 8·MHz and a field of view of 60·mm. The probe was held in place with a plastic cast covered with neoprene (Fig. 1A). Measurements of fascicle length were made at a mid-belly position (half-way between muscle-tendon-junction and popliteal crease)

and the scanner was aligned according to Benard et al. (2009). The ultrasound was synchronized with the motion capture data via a pulse that was fed to the EMG System. Ultrasound videos of 6 strides were analyzed separately during gait and static measurements were performed during seated rest. Fascicle length was measured with a tracking algorithm (Gillett et al., 2013) and subsequent manual frame-wise inspection. Pennation angle (α) was determined with respect to the deep aponeurosis (Mian et al., 2007) and muscle-tendon unit (MTU) length was calculated from knee and ankle angles (Orendurff et al., 2002). The length of the series elastic element (SEE) was determined by $L_{SEE} = L_{MTU} - L_{FASCICLE} * \cos\alpha$ (Fukunaga et al., 2001). Thickness of the muscle belly during rest was measured, too (Hösl et al., 2015). During gait, all morphometric variables were normalized on resting length and all rest values were normalized on shank length. Gait data were interpolated to 100 points across each stride and an average for each participant and condition was determined. Outcome parameters were max. values during stance as well as the amount of lengthening (throughout loading response and single stance) and shortening excursions (throughout single stance and push-off) of fascicles and SEE.

4.3.7. Statistics

Participant characteristics, physical exam results and morphometrics during rest were compared with unpaired t-tests. Using the Shapiro-Wilk test, requirements for normality in some walking data sets were not achieved. So differences between conditions (flat-forward vs. uphill vs. backward-downhill) were tested separately for SCP-children and TD with repeated measure or Friedman ANOVAs where indicated. Paired t- or Wilcoxon tests were used for post-hoc comparisons. Differences between groups (SCP vs. TD) on flat-forward walking were tested directly with unpaired t- or Mann–Whitney U test where appropriate. Alpha-level was set to 0.05 and effect sizes were expressed as Cohen's d for significant results.

4.4. Results

4.4.1. Anthropometrics and physical exam

There were no significant differences in age between groups (Table 4-1), but SCP-patients were 8% smaller in height, 18% lighter and had 10% and 8% shorter legs and shanks (all $P \leq 0.031$). They displayed significant reductions in passive dorsiflexion (-14°), as well as 23° of popliteal angle restrictions. Max. isometric plantarflexor force was 28% less in children with SCP (all $P \leq 0.003$).

Table 4-1 Anthropometrics, physical exam and muscle morphometrics during rest

	SCP (n=15)		TD (n=17)			
Anthropometrics	Mean	(SD)	Mean	(SD)	*P*	ES
Age [years]	11.0	(2.8)	12.2	(2.3)	0.219	0.4
Height [cm]	142.6	(14.5)	154.6	(11.9)	0.016	0.9
Mass [kg]	35.8	(8.6)	43.9	(9.9)	0.019	0.9
BMI [kgm^{-2}]	17.4	(2.1)	18.1	(2.0)	0.289	0.4
Leg length [cm]	73.7	(9.0)	81.8	(7.4)	0.009	1.0
Shank length [cm]	32.7	(3.9)	35.6	(3.4)	0.031	0.8
Physical exam						
Passive knee extension [°]	4	(5)	6	(3)	0.077	0.6
Poplitealangle- (opposite hip flexed) [°]	34	(10)	11	(12)	<0.001	2.1
Passive Dorsiflexion - 0° knee flex. [°]	1	(8)	15	(4)	<0.001	2.1
Passive Dorsiflexion - 90° knee flex. [°]	17	(11)	27	(5)	0.001	1.3
Instrumented Plantarflexor force [N/kg]	3.9	(1.4)	5.4	(1.1)	0.003	1.2
Plantarflexor tone - knees extended [MAS]	2.2	(0.8)	0.0	(0.0)	<0.001	4.0
Medial Gastrocnemius morphometrics (seated rest)						
Fascicle length [mm]	27.4	(9.4)	36.8	(5.2)	0.001	1.3
Fascicle length [% shank]	8.5	(2.8)	10.3	(1.1)	0.014	0.9
Series elastic element length [% shank]	89.1	(2.8)	87.8	(1.2)	0.081	0.6
Pennation angle [°]	26.0	(5.7)	24.9	(3.4)	0.504	0.2
Thickness [% shank]	3.5	(0.9)	4.3	(0.5)	0.003	1.1

MAS: Modified Asworth Scale [1-4], SD: Standard Deviation, ES: Effect Size (Cohen's d). TD (Typically Developing) and children with SCP (Spastic Cerebral Palsy)

4.4.2. Morphometrics during rest

During rest children with SCP had 17% shorter fascicles, 19% thinner muscle bellies (both P≤0.014, d≥0.9) and tended to have a 1.5% longer SEE (P=0.081).

4.4.3. Walking speed

Absolut flat-forward speed was lower in SCP-patients, 1.07 (0.16) m/s vs. 1.23 (0.08) m/s (P <0.001, d=1.2). Non-dimensional speed was also lower: 0.40 (0.06) in SCP vs. 0.44 (0.03) in TD, but differences were not significant (P=0.071). SCP children took shorter steps 0.53 (0.08) m vs. 0.65 (0.05) m (P<0.001, d=1.8) at a higher cadence 2.03 (0.18) steps/sec vs. 1.89 (0.15) steps/sec (P=0.031, d=0.8). Belt speed on slopes were preset but 2 (of 15) SCP-patients only managed to walk backward-downhill at 34% and 38% of flat-forward speed.

4.4.4. Joint kinematics

Group means (1 standard deviation) for all conditions are shown in Fig. 4-2. Raw values can be found in the table 4-2. For TD the ANOVA indicated significant difference between conditions for all parameters (P<0.001). For children with SCP significant main effects were found concerning the sole angle, dorsiflexion in stance, knee flexion at initial contact and hip flexion in stance and swing (P≤0.007).

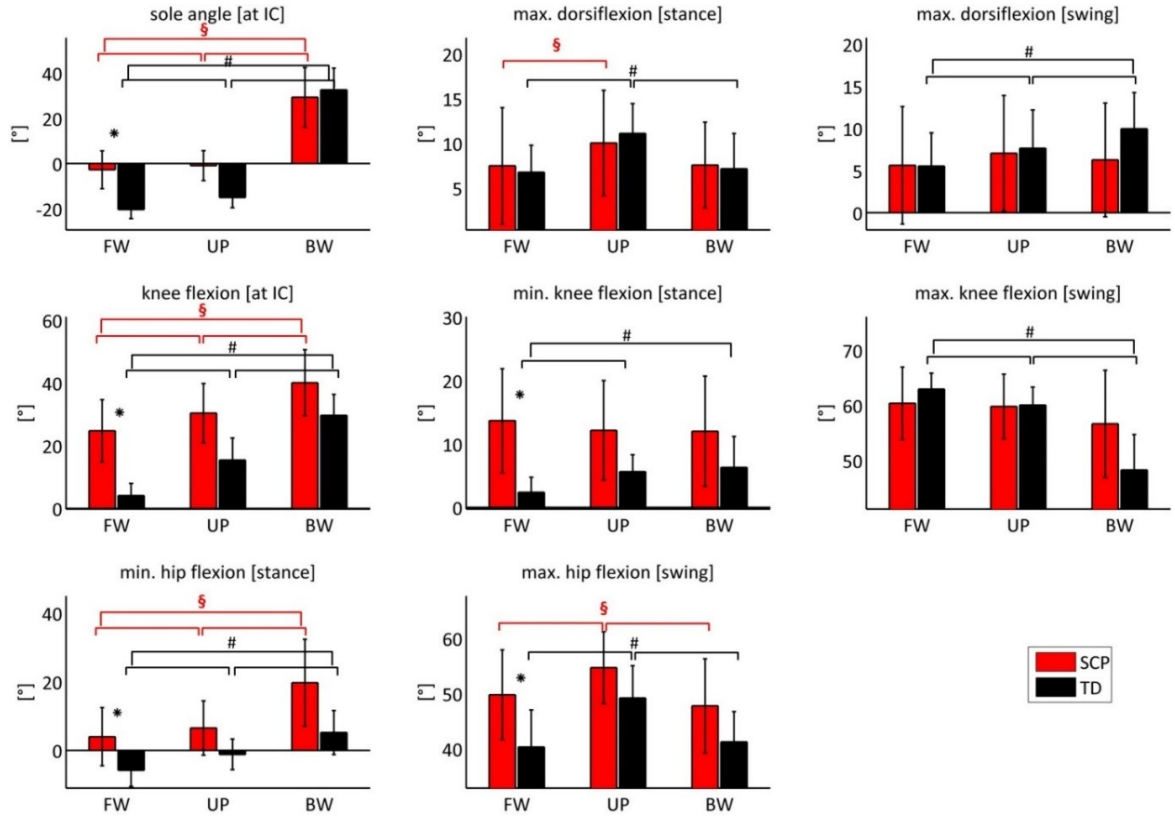

Fig. 4-2 Sagittal joint kinematics of the foot, ankle, knee and hip. FW: flat-forward; UP: uphill, BW: backward-downhill, IC: Initial contact. * significant differences between TD (Typically Developing) and children with SCP (Spastic Cerebral Palsy) during FW; § significant differences between conditions for SCP; # significant differences between conditions for TD, p<0.05.

Flat-forward walking

During flat-forward gait, SCP-patients showed 10° more hip flexion in stance and swing (d=1.5 and 1.3) and more knee flexion in stance (12° concerning min. knee flexion, d=1.9). They also landed with 17° flatter sole angle (d=2.8). All corresponding tests showed P≤0.001. No group differences in dorsiflexion were noted in stance and swing (P≥0.518) or in knee flexion in swing (P=0.078).

Uphill walking

With respect to flat-forward gait, both SCP and TD-children landed with 2° and 5° flatter sole angles (P=0.019, d=0.6 for SCP and P<0.001, d=1.6 for TD) and with 5° and 9° more knee flexion (both P<0.001. d=1.1 for SCP and d=1.0 for TD). The following tests remained at P≤0.001: Both groups increased their

dorsiflexion in stance (3°, d=1.4 and 4°, d=2.0) while TD also increased dorsiflexion in swing (2°, d=1.0). The hip of SCP and TD-children was more flexed in stance (3°, d=1.2 and 5°, d=1.6) and in swing (5°, d=1.5 and 9°, d=2.2). Only in TD, more knee flexion in stance (4°, d=1.4) and less knee flexion in swing (3°, d=1.6) was noted.

Backward-downhill walking

Both groups landed with toes first. With respect to uphill gait they further increased knee flexion at ground contact (10° in SCP and 15° in TD), as well as hip flexion in stance (16° in SCP and 11° in TD). The Cohen's d was 0.8-2.0 (all P≤0.011). Only TD further increased dorsiflexion in swing (2° from uphill, P=0.003, d=0.9) while knee flexion in swing further decreased (12° from uphill, P<0.001, d=2.0). Similar to uphill walking, TD showed more knee flexion in stance than forward (P<0.001, d=1.2). But dorsiflexion of TD in stance decreased (P=0.003, d=0.9) and was similar to forward gait.

4.4.5. Electromyography

Fig. 4-3 shows the traces of muscle activity and morphometrics and Fig. 4-4 visualizes the outcome parameters. For TD the ANOVA indicated significant differences between conditions for all muscles (all P≤0.007). For SCP-children significant main effects were found for soleus and medial gastrocnemius activity (both P<0.001).

Flat-forward walking

During flat-forward gait, activity of the medial gastrocnemius (+31%), soleus (+32%) and tibialis anterior (+58%) was larger in SCP-children than in TD (all P≤0.031, d=0.9-1.3).

Uphill walking

Only in TD-children, medial gastrocnemius (+23%, d=1.2) and soleus activity (+29%, d=0.8) was significantly increased (both P<0.001), while in SCP-patients rather similar medial gastrocnemius and soleus activity was noted with respect to flat-forward gait (P>0.855). Tibialis anterior activity increased by 48% in TD (P=0.001, d=0.9) with respect to flat-forward gait. Despite the absent main effect, also 33% more tibialis anterior activity in SCP had been found with respect to flat-forward walking (P=0.048, d=0.5).

Backward-downhill walking

The soleus and medial gastrocnemius activity dropped by 37% and 44% in SCP-children (both P<0.001, d=1.1 and 1.5) and by 31% (P=0.003, d=1.0) and 23% (P=0.038, d=0.8) in TD with respect to

flat-forward gait. Consequently, plantarflexor muscle activity was also significantly less than uphill. Tibialis anterior activity was not different from flat-forward or uphill in both groups (all P≥0.080).

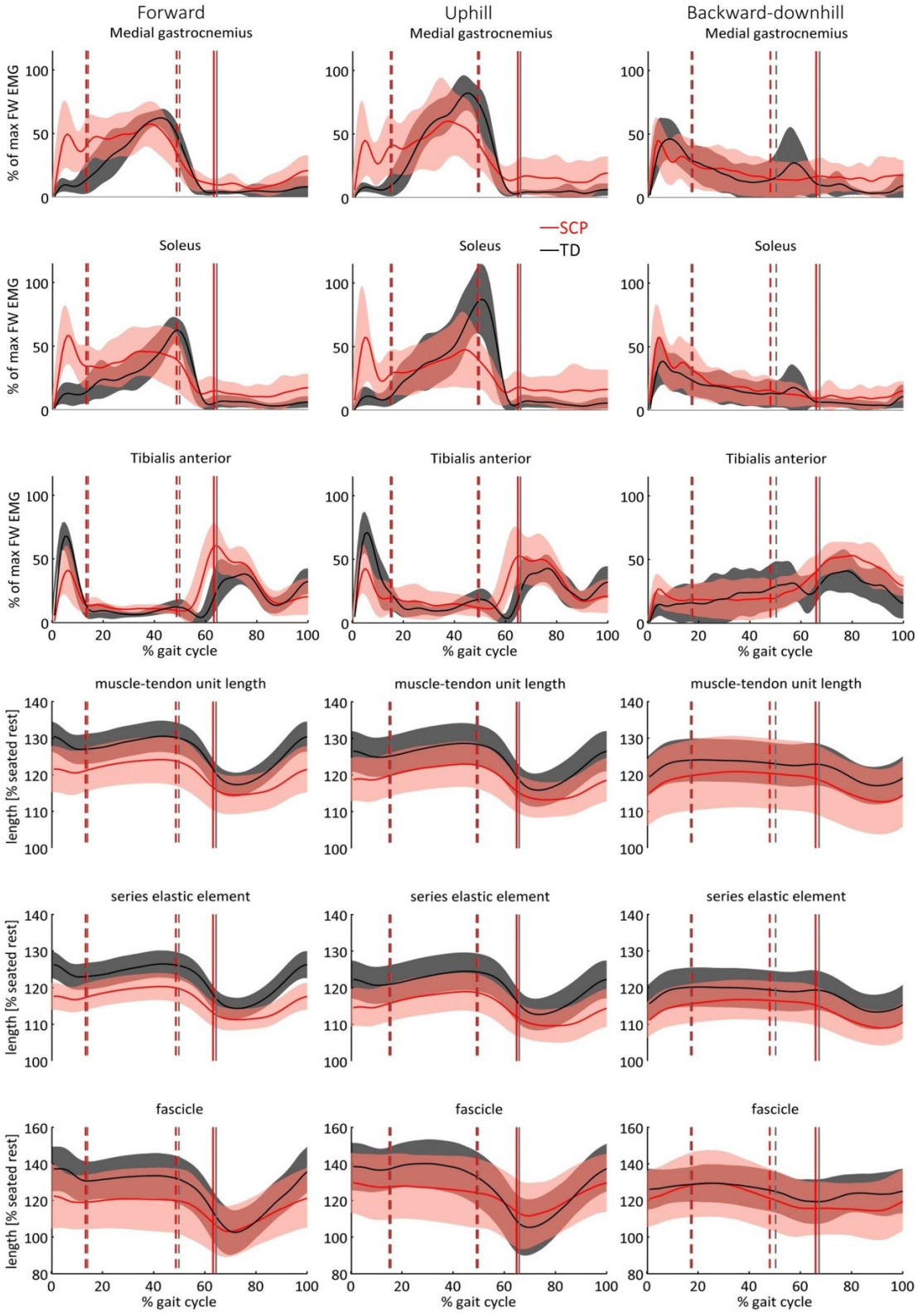

Fig. 4-3 Group average traces for shank muscle activity and medial gastrocnemius morphometrics across the gait cycle. Vertical dashed lines separate double from single support and vertical solid lines stance from swing. Shaded bands show group means ± 1 standard deviation.

Table 4-2 Overview of the outcome parameters concerning joint angles, muscle morphometrics and muscle activity

		SCP (n=15) Mean (SD)			TD (n=17) Mean (SD)		
		FW	UP	BW	FW	UP	BW
Joint angles [°]							
Foot & Ankle	Sole angle [at IC]	-3 (8)	-1 (7)	29 (13)	-20 (4)	-15 (5)	33 (10)
	Max. dorsiflexion [stance]	7 (7)	10 (6)	8 (5)	7 (3)	11 (3)	7 (4)
	Max. Dorsiflexion [swing]	6 (7)	7 (7)	6 (7)	6 (4)	8 (5)	10 (4)
Knee	Flexion [at IC]	25 (10)	30 (9)	40 (11)	4 (4)	15 (7)	30 (7)
	Min. flexion [stance]	14 (8)	12 (8)	12 (9)	2 (2)	6 (3)	6 (5)
	Max. flexion [swing]	60 (7)	60 (6)	57 (10)	63 (3)	60 (3)	48 (6)
Hip	Min. flexion [stance]	4 (8)	7 (8)	20 (13)	-6 (5)	-1 (4)	5 (6)
	Max. flexion [swing]	50 (8)	55 (6)	48 (9)	40 (7)	49 (6)	41 (5)
Medial Gastrocnemius morphometrics [% seated rest length]							
Fascicle	Δ Lengthening [stance]	4.8 (4.4)	4.4 (4.1)	10.2 (7.7)	5.1 (3.4)	4.9 (3.4)	7.4 (8.2)
	Δ Shortening [stance]	15.1 (5.3)	18.5 (8.2)	15.7 (7.1)	23.8 (6.6)	33.4 (9.3)	14.1 (8.4)
	Max. length [stance]	125.6 (17.9)	132.7 (17.9)	130.5 (18.0)	139.0 (11.8)	141.5 (13.5)	132.4 (10.1)
Series elastic element	Δ Lengthening [stance]	4.0 (1.5)	4.8 (1.3)	5.7 (1.9)	3.6 (0.8)	3.9 (0.9)	4.6 (1.0)
	Δ Shortening [stance]	7.3 (2.0)	7.0 (2.1)	1.3 (0.5)	9.6 (2.2)	9.0 (1.2)	1.4 (0.4)
	Max. length [stance]	120.5 (6.1)	119.0 (5.7)	116.8 (9.3)	126.7 (3.7)	124.5 (5.2)	120.2 (5.4)
Shank muscle activity [% max. FW EMG]							
	Soleus [stance]	35.6 (11.7)	33.2 (13.7)	22.4 (11.4)	27.0 (7.2)	34.8 (11.2)	18.7 (9.6)
	Medial gastrocnemius [stance]	38.9 (8.3)	38.9 (14.0)	21.7 (14.1)	29.7 (6.0)	36.5 (5.3)	22.9 (11.2)
	Tibialis anterior [swing]	13.4 (5.7)	17.8 (10.6)	15.2 (9.8)	8.5 (4.3)	12.6 (4.6)	11.5 (5.8)

Mean (1 Standard Deviation), SCP: Spastic Cerebral Palsy, TD: Typically developing, FW: flat-forward, UP: uphill, BW: backward-downhill.

4.4.6. Morphometrics during gait

In TD-children the ANOVA indicated significant differences between conditions for all parameters (all P<0.001) despite fascicle lengthening (P=0.351). For SCP-children, significant differences between conditions were found for fascicle lengthening (P=0.041), SEE lengthening and shortening (P=0.008 and P<0.001) and for max. SEE length (P=0.028).

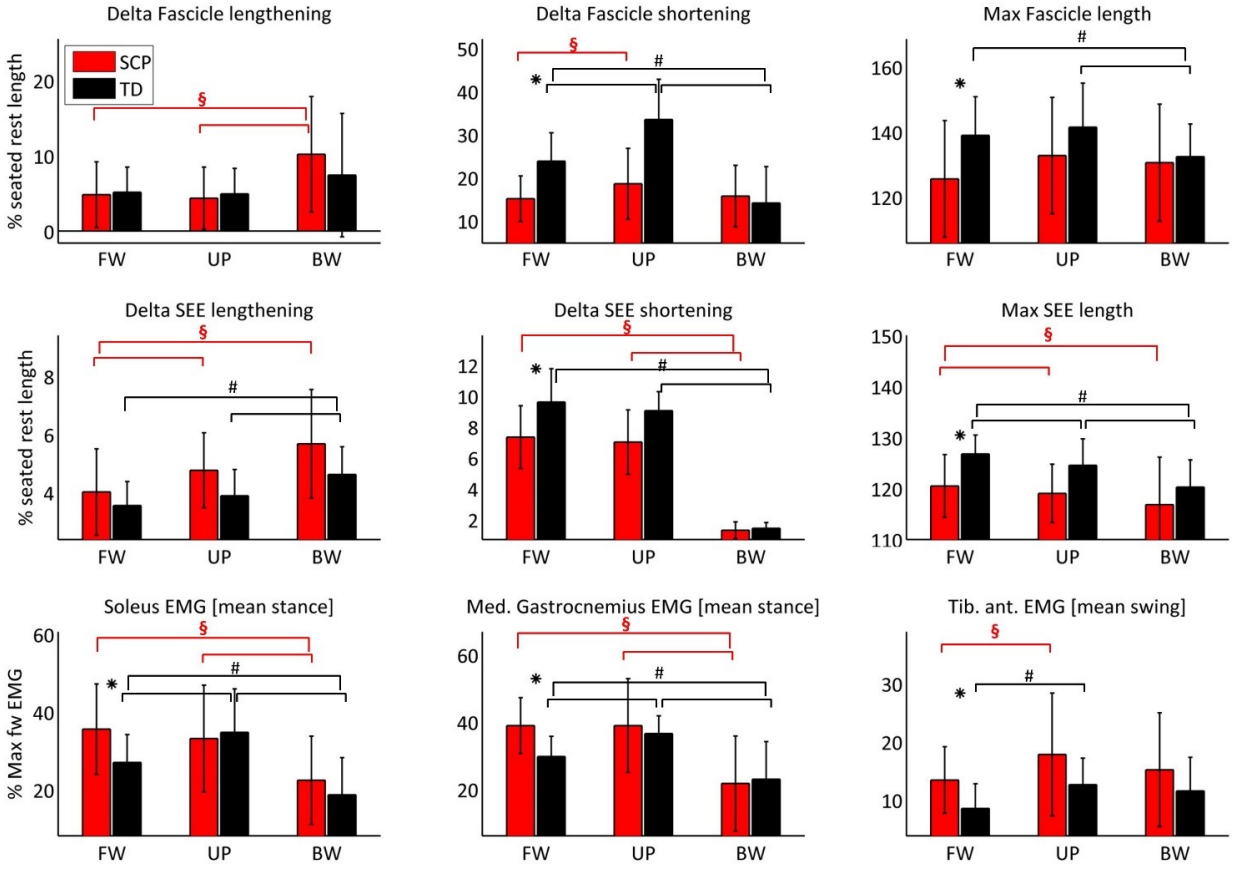

Fig. 4-4 Fascicle and series-elastic element (SEE) lengthening and shortening excursions and maximal length during stance, as well as shank muscle activity. FW: flat-forward; UP: uphill; BW: backward-downhill. *significant differences between TD (Typically Developing) and children with SCP (Spastic Cerebral Palsy) during FW; § significant differences between conditions for SCP; # significant differences between conditions for TD, p<0.05.

Flat-forward walking

There was 37% less concentric fascicle (P=0.001, d=1.4) and 24% less concentric SEE excursion (P=0.002, d=1.1) in SCP-patients with respect to TD. Spastic fascicles (-10%, P=0.038, d=0.9) and SEE (-5%, P=0.004, d=1.3) also reached significantly shorter max. length. No significant group differences in eccentric fascicle (P=0.571) or SEE excursion (P=0.345) were found.

Uphill walking

During uphill walking significantly more concentric fascicle excursion than during flat-forward gait was noted in TD (+40%, P<0.001, d=1.2). Also in SCP-children an increase of 23% (P=0.034, d=0.6)

occurred but the ANOVA failed to indicate a significant main effect. Only in SCP-children, this was accompanied by 19% increase in eccentric SEE excursion (P=0.001, d=1.0). The SEE reached significantly shorter max. length than in flat-forward gait in SCP-children (-1%, P=0.048, d=0.6) and in TD (-2%, P=0.038, d=0.5) .

Backward-downhill walking

Only in SCP-children, eccentric fascicle contraction increased with respect to flat-forward and uphill walking (+112% and +132%, both P≤0.017, d=0.7-0.9). In TD, fascicle shortening was significantly reduced with respect to flat-forward (-41%, P=0.002, d=0.9) and thus also from uphill gait. In TD max. fascicle length was shorter than forward (-5%, P=0.002, d=0.9) and uphill (-6%, P=0.001, d=1.0). In both groups this was accompanied by larger lengthening excursions of the SEE with respect to the flat-forward condition, +42% in SCP (P=0.010, d=0.7) and +28% in TD-children (P=0.001, d=1.0). Only in TD this SEE lengthening was significantly larger than uphill (+18%, P=0.006, d=0.8). At the end of stance, SEE shortening was diminished in both groups with respect to the other conditions (Fig. 4-4). The max. SEE length in SCP-children was 3% shorter than forward (P=0.030, d=0.6). The reductions with respect to uphill-gait were not significant (P=0.156). In TD the max. SEE length was 3% shorter than uphill (P<0.001, d=1.2) and thus also shorter than forward.

4.5. Discussion

We analyzed SCP-children and TD with specific focus on adaptations of the medial gastrocnemius contractile behavior during flat-forward, uphill and backward-downhill gait. Apart from having innately shorter fascicles at rest, SCP-children also reached shorter relative fascicle length than TD during flat-forward walking and showed less concentric excursion during push-off. Uphill gait increased concentric excursion of the fascicles in both groups while backward-downhill gait induced larger eccentric fascicle excursions in SCP-children.

During flat-forward gait, participants with SCP walked with considerably flexed knees which is inefficient and puts great burden on knee extensors. Deterioration of passive dorsiflexion (assessed with extended knees) is a negative factor for such a crouch gait pattern (Maas et al., 2015). On the one hand, short and inextensible gastrocnemius fascicles could constrain knee extension to permit similar dorsiflexion during gait. When comparing the fascicle operating regions (Fig. 4-5), the spastic fascicles only somewhat reached the regions of healthy fascicles. On the other hand, healthy gastrocnemius fascicles usually work near the descending limb of their length-tension curve during gait (Hoffman et al., 2014) and since spastic fascicles worked at shorter length, they may be used more towards the ascending limb. In this region, fascicles are ordinarily able to produce less active force.

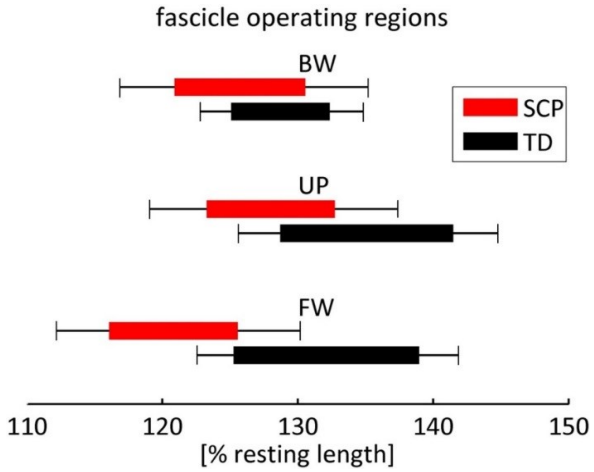

Fig. 4-5 Fascicle operating regions during stance phase of gait. Error bars indicate the standard error of the group mean minimum or maximum fascicle length. FW: flat-forward; UP: uphill, BW: backward-downhill. Vertical height of the bars is arbitrary. TD (Typically Developing) and children with SCP (Spastic Cerebral Palsy).

So, limited relative fascicle length during gait may be a pathological sign. This assumption relies on a fascicle force length relationship that assumes equal inherent sarcomere length between groups. However, due to findings of much longer spastic sarcomeres (Mathewson et al., 2014), contractile filament overlap within sarcomeres may worsen at more extended fascicle length. Hence, limited relative fascicle length could be an adjustment to use spastic sarcomeres in a configuration where they are able to produce enough active forces, otherwise contractile filaments could be stretched apart beyond overlap.

The observed lack of concentric fascicle shortening could be affected by less sarcomeres in series and may contribute to the typical reductions in ankle joint power (Dallmeijer et al., 2011). Reduced pull from muscle shortening contraction could in turn be the reason why the SEE reached shorter relative length in SCP-children. In addition, no difference in eccentric fascicle excursion between SCP-patients and TD existed. Although such loads may not be responsible for the genesis of contracture or fibrosis (Pitcher et al., 2015), SCP-children with less isometric plantarflexor force experienced more fascicle lengthening excursion (Pearson's r=-0.57, P=0.026) and therefore the gastrocnemius fascicles may indeed face difficulties to resist tensile forces (Fry et al., 2006).

Since muscles can adapt to altered use, it appeared interesting to modulate the gastrocnemius contractile behavior with sloped treadmilll walking. In general, gait adaptations concerning joint angles and muscle activity appeared to be more diverse and more pronounced in TD. This could be due to coordinative or musculoskeletal restrictions in SCP-children. Nevertheless, uphill gait increased concentric excursion of the medial gastrocnemius fascicles but no significant change in max. fascicle length or eccentric fascicle excursion occurred. Decreased passive stiffness of spastic plantarflexors after uphill training (Willerslev-Olsen et al., 2015) could accordingly be a response to increased

concentric loads. Interestingly, uphill training also strengthens dorsiflexors and increases toe-clearance during flat-forward gait (Willerslev-Olsen et al., 2014). Even though tibialis anterior activity increased in our sample of SCP-children, the clearest kinematic adaptations during uphill walking happened in hip flexion (Fig. 2) and SCP-children faced difficulties to increase dorsiflexion in swing. This was possibly constrained by less voluntary tibialis anterior recruitment (~smaller EMG increase than in TD) or impeded by the plantarflexor contractures. Likewise the plantarflexor EMG in SCP-children did not increase in stance. Altogether SCP-patients may substantially rely on hipflexors during uphill training.

Walking backward-downhill forced to strike the ground with the toes which appears counterintuitive in SCP-children. However, it couples knee extension with dorsiflexion motion during weight acceptance and thereby induced larger medial gastrocnemius fascicle lengthening excursions in SCP. This was not the case for TD who might have been able to provide more isometric force to withstand lengthening. Besides the eccentric excursion values were rather variable in TD. Although all subjects had experience in treadmill walking, backward-downhill gait may be less variable and stabilize with further training.

Backward-flat gait has been successfully applied for coordinative gait training in SCP-children (Kim et al., 2013) but no effects of backward-downhill training on calf muscle pathology have been reported. Generally, effects of eccentric plantarflexor training appear to be promising: For TD- adults, eccentric training can increase passive ankle joint flexibility, plantarflexor strength (Mahieu et al., 2008), medial gastrocnemius fascicle length and tendon stiffness (Duclay et al., 2009). These benefits apparently contrast conceptions about the harmfulness of eccentric loads in SCP (Fry et al., 2006, Pitcher et al., 2015, Gough and Shortland, 2012). When using backward-downhill gait during therapy, the medial gastrocnemius fascicles in SCP are unlikely to sustain macroscopic damage since they seem to be trained at moderate length (not significantly different from forward or uphill) and their eccentric excursion seems to be of low amplitude. As a precaution, such training should be gradually adjusted.

It is difficult to deduce something about tendinous loads but neither backward-downhill, nor uphill gait induced larger max. SEE length in any group (Fig. 4). Taking into account that large contraction induced deformation is necessary for improving tendinous stiffness (Bohm et al., 2015), the potential benefits of sloped gait modifications may not target the gastrocnemius tendon.

4.6. Limitations

First off, the external validity of treadmill gait has been subject of controversy but no difference in medial gastrocnemius fascicle behavior between treadmill and overground gait had been observed in

TD-adults (Cronin and Finni, 2013). Still preferred treadmill speed is slower than overground (Dal et al., 2010, van der Krogt et al., 2015). Additionally, for joint kinetics of SCP-children, a power-shift from the ankle to the hip has been noted on treadmills (van der Krogt et al., 2015). This could explain some general differences in concentric fascicle excursion between SCP-children and TD. Noteworthy, we deliberately reduced the speed on slopes and constrained all subjects to touch handrails since those settings appear realistic during regular treadmill walking therapy (Kim et al., 2013, Willerslev-Olsen et al., 2014, Chrysagis et al., 2012).

Apart from that, a seated position was used for normalization of morphometrics. At this joint configuration, the gastrocnemius muscle has usually fallen slack (Hoang et al., 2007b). Despite the fact that fascicle operating regions of TD-children, expressed with respect to rest length (Fig. 4-5), appear comparable with data of TD-adults (Hoffman et al., 2014), slack length could be instrumentally assessed when investigating muscle-tendon behavior during gait in future studies.

4.7. Conclusions

Medial gastrocnemius fascicles appear to be used on very short relative length during spastic gait with similar eccentric and less concentric excursion compared to controls. Flexed knees in crouch gait could be related to structural shortness of gastrocnemius fascicles. Uphill gait increases concentric gastrocnemius fascicle action and tibialis anterior activity and may be useful to train push-off and foot lift. During backward-downhill gait, the medial gastrocnemius functioned as a brake and displayed more eccentric excursion in SCP-children which could potentially stimulate sarcomere-genesis in series with repeated training. Both training modes may offer advantages, but none of them may promote tendon stiffness since the SEE was longest during forward gait.

Conflict of interest

None of the authors has any commercial of other interests that create a conflict of interest for the work presented here.

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5. Third study

Effects of backward-downhill treadmill training versus manual static plantarflexor stretching on muscle-joint pathology and function in children with spastic Cerebral Palsy

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Submitted

5.1. Abstract

Background:

Patients with spastic Cerebral Palsy are prone to equinus deformities, likely affected by short and inextensible plantarflexor muscles. Manual stretching is a popular treatment but its effectiveness concerning joint mobility, muscle-tendon morphometrics and walking function is debated. Eccentric exercise by backward-downhill treadmill training could be a therapeutic alternative.

Methods:

10 independent ambulators with spastic Cerebral Palsy (12 [SD 4] years old, 2 uni- and 8 bilaterally affected) participated in a randomized crossover-study. One group started with manual static stretching, the other one with backward-downhill treadmill training. Each treatment lasted 9 weeks (3 sessions per week). Pre and post treatments, 3D gait was assessed during comfortable and during fastest possible walking. Ultrasonography and dynamometry were used to test plantarflexor strength, passive joint flexibility, as well as Gastrocnemius morphometrics, stiffness and strain on muscle-tendon and joint level.

Findings:

When comparing both treatments, backward-downhill treadmill training lead to larger single stance dorsiflexion at comfortable walking speed (+2.9°, P=0.041) and faster maximally achievable walking velocities (+0.10 m/s, P=0.017). Stretching reduced knee flexion in swing, particularly at faster walking velocities (-5.4°, P=0.003). Strength, ankle joint flexibility, as well as stiffness on muscle-tendon and joint level were not altered, despite similar increases in passive muscle and fascicle strain with both treatments (P≤0.023).

Interpretation:

Manual static plantarflexor stretching may not be emphasized in Cerebral Palsy patients with high ambulatory status. BDTT can be an effective gait treatment, probably improving coordination or reducing dynamic stretch sensitivity. More intense BDTT might be necessary to further alter muscle-tendon properties.

Keywords: Cerebral Palsy; ankle-foot bracing; ultrasound; gastrocnemius; muscle contracture

Abbreviations:

SCP Spastic cerebral palsy

BDTT Backward downhill treadmill training

5.2. Introduction

Children with spastic Cerebral Palsy (SCP) are constrained by muscle weakness and prone to developing contractures and restricted joint mobility. The ankle-joint typically shows progressive loss in passive dorsiflexion during maturation (Hägglund and Wagner, 2011) and appears to be stiffer than in typically developing peers (Alhusaini et al., 2010; Barber et al., 2011). Next to a pathological gain of muscle activity (Bar-On et al., 2014a), one mechanism responsible for an increase in joint stiffness is the mechanical reduction of muscle extensibility. Therefore, reduced muscle belly length (Fry et al., 2004; Malaiya et al., 2007), reduced fascicle length (Barber et al., 2011; Hösl et al., 2015; Matthiasdottir et al., 2014), increased in-vivo sarcomere length and a lack of sarcomeres in series (Mathewson et al., 2014) or increased intramuscular connective tissue (Noble et al., 2014) may contribute to unusually high passive ankle joint stiffness.

Together with the prevailing weakness, the lack in joint flexibility impairs walking. Reduced dorsiflexion decreases metabolic efficiency (Ballaz et al., 2010), while less plantarflexor strength may reduce propulsive joint power (Eek et al., 2011): Moreover, stretch reflex thresholds of the bi-articular Gastrocnemius during passive testing have been shown to impede stretch velocities of the muscle-tendon unit during gait (Bar-On et al., 2014b). This could compromise maximal achievable walking velocities.

Many interventions in SCP aim to decrease joint stiffness and increase joint excursion. Manual stretching is very popular but its effectiveness is debated (Novak et al., 2013; Wiart et al., 2008). Generally, its effects have been explained by a mechanical response of the muscle or a modification in a person's sensation (Weppler and Magnusson, 2010). In healthy subjects, plantarflexor stretching can increase passive dorsiflexion (Blazevich et al., 2014; Konrad and Tilp, 2014; Nakamura et al., 2012) but gains in passive dorsiflexion are not necessarily reflected during gait (Johanson et al., 2009). In non-independently ambulant SCP-children, manual stretching can increase passive dorsiflexion and decrease joint and muscle stiffness (Theis et al., 2015), but no effects on strength or on gait have been reported.

To counteract weakness, resistance training has been recommended and there is preliminary evidence for positive effects on muscle growth and strength in SCP children (Gillett et al., 2016). However, strength gains may not necessarily improve gait (Scholtes et al., 2012). Therefore, combining strength and walking exercises appears promising. In addition, the muscles' contractile modes during training may play a pivotal role in stimulating muscle growth: For healthy humans, in particular eccentric training increases fascicle length (Franchi et al., 2014) and seems capable to improve lower limb flexibility (O'Sullivan et al., 2012). More specifically, eccentric plantarflexor training can increase passive dorsiflexion, decrease resistive torques (Mahieu et al., 2008) and increase active isometric strength (Duclay et al., 2009). In SCP-patients, higher isometric plantarflexor strength is related to

larger ankle joint push-off power during gait (Eek et al., 2011). Recently, it has been found that backward-downhill treadmill training [BDTT] provides eccentric Gastrocnemius fascicle loadings in SCP-patients (Hösl et al., 2016). While flat-backward treadmill training in SCP-patients improves forward-walking speed (Kim et al., 2013), no longitudinal results of BDTT on gait, strength or ankle joint stiffness as well as on muscle morphometrics have been reported.

In summary, eccentric exercise by backward-downhill treadmill training could be an alternative to stretching that may be also capable to improve strength and gait. Therefore we compared the effects both interventions. With respect to muscle joint-properties, we expected that BDTT will increase Gastrocnemius muscle and fascicle length, promote strength and joint extensibility and decrease passive joint stiffness. Stretching will increase passive dorsiflexion and decrease passive stiffness while showing no signs of muscle growth. In terms of gait, BDTT will enable subjects to walk faster, due to increased plantarflexor strength and increased ankle joint push-off power. We expected that gains in passive dorsiflexion with stretching will not translate to improved dorsiflexion during gait.

5.3. Methods

5.3.1. Participants

10 participants with SCP (1 female) could be included from a school for disabled children (4/6 in GMFCS Level I/II). Two were uni- and 8 bilaterally involved. The mean age of the participants was 12 (SD 4, range: 5-19) years, their body weight was 41 (SD 17) kg and their height was 142 (SD 17) cm. 70% of participants walked with increased knee flexion (> 1SD from a control collective), 50% on tip-toes and non-had recurvatum. None of them received any Botulinum Toxin injection within 24 months. Two subjects had bony surgeries to the femur more than 24 months apart. None had any surgery to the lower leg and no orthotics were worn during the study period.

5.3.2. Design

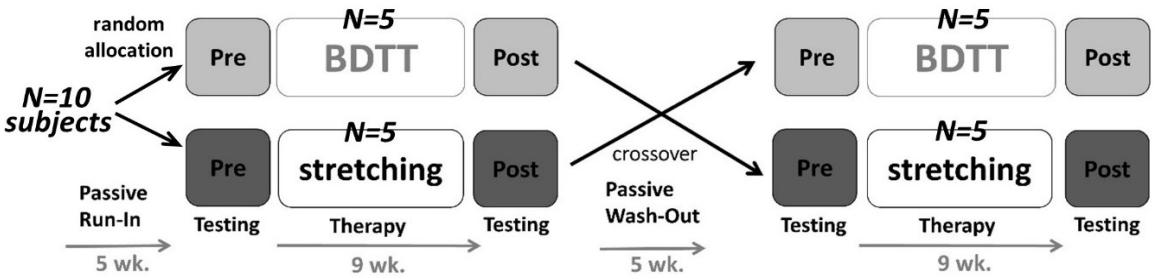

Fig. 5-1 Study design. 10 subjects were included in a randomized two-treatment, two-period 2x2 crossover-design (AB/BA) with 9 week long treatment periods.

A two-treatment, two-period crossover-design (AB/BA) was used (Fig. 5-1). Participants were randomized to two groups (each N=5) with a component of minimization for age and GMFCS Level. One group started with stretching, the other one with BDTT. There were two 9 week treatment periods (3 sessions per week), preceded and intersected by a passive run-in and wash-out of 5 weeks. Each period started and ended with an assessment of 3D gait, muscle-joint biomechanics and functional ambulatory mobility. The protocol was ethically approved by the German Physiotherapy Association (2014-08) and children and parents gave informed written consent.

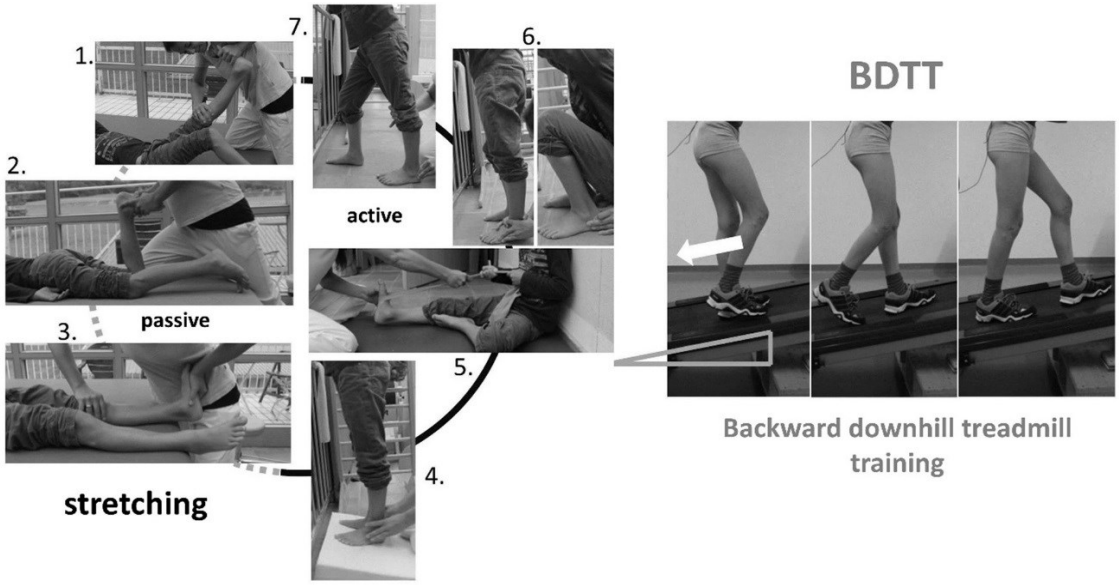

Fig. 5-2 Treatment interventions: Exercises for manual static calf stretching (1-3 passively executed by therapist, 4-7 actively executed with guidance). Landing phase during backward-downhill treadmill training (BDTT) on declined treadmill belt surface, arrows indicate direction of walking and limb motion. 10 subjects were included in a randomized two-treatment, two-period 2x2 crossover-design (AB/BA) with 9 week long treatment periods.

5.3.3. Static calf stretching

The program consisted of 7 exercises (Fig. 5-2, details in supplements) referring to a video kit (Morrel and Lau, 2009). Four exercises were self-initiated, 3 were passively executed. Despite the bilateral maneuvers (Fig. 5-2), exercises were alternately executed with 5 repetitions per leg and end-range positions were held for 20 sec. (Theis et al., 2013). In unilaterally affected children only the hemiparetic side was treated.

5.3.4. Backward-downhill treadmill training [BDTT]

An Atlantis treadmill (Heinz Kettler, Ense-Parsit, Germany) was used. Subjects were equipped with a ceiling-mounted safety harness (h/p/cosmos, Nussdorf-Traunstein, Germany) and wore their own sport shoes. During the first session, the belt was declined at -10.8% and comfortable backward

beltspeed was determined to be 0.47 (SD 0.11) m/s. To do so, the beltspeed was set to 50% of comfortable forward walking velocity and if necessary reduced. From week 2-6 beltspeed was weekly increased by ~10%. During week 4-6, the decline slope was weekly raised by -1.6%. During the last 2 weeks, the participants had to carry weight belts of 5% and 10% bodyweight to increase the load on the calf during landing. The beltspeed during the final week was 0.64 (SD 0.25) m/s at a decline of -15.6%. 23 min total walking time was set as a goal for each session, which could be split into 2-4 bouts of continuous walking (max. 11.5 min), interspersed by seated resting. Subjects were encouraged to take large steps, maintain an erected posture and limit hand-rail support.

5.3.5. Assessments

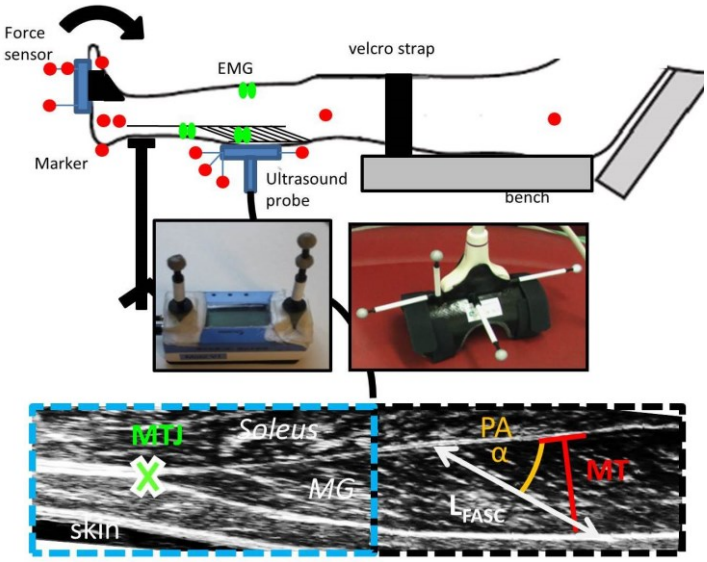

Fig. 5-3 Set-up for the assessment of muscle-joint properties with hand-held dynamometer and ultrasound probe within custom-made carbon cast for fixation. Lower part: Superimposed ultrasound scans of the medial Gastrocnemius (MG) with representation of morphometric assessments. MTJ: Muscle-tendon junction, LFASC: fascicle length, PA: Pennation angle, MT: muscle thickness.

All tests were performed after 1 day of rest to exclude temporary tissue deformation. A Vicon system with 8 MX-Cameras (Vicon Inc., Oxford, UK) was used to capture overground barefoot walking at comfortable and at 'as fast as possible' speed. Markers were placed according to the Plug-In gait Model and sampled at 200 Hz. Force data was captured at 1000 Hz via two force plates (AMTI, Watertown, USA). Three to five clean strikes on the force plates could be obtained. All affected legs were investigated. To quantify ambulatory mobility a Timed Up-and-Go test (Williams et al., 2005), as well as the Gross-Motor-Function-Measure-66 D & E were used (Palisano et al., 2000).

Due to time constrains, muscle and joint properties have been investigated only in the more affected leg (based on passive dorsiflexion). Children sat on a weight-bench at 60° hip flexion and extended knees (Fig. 5-3). Markers from gait analysis remained on the leg and one additional marker was attached at the lateral calcaneus to limit potential bias by midfoot-bending (Hösl et al., 2015). A

hand-held force sensor (Mobie, Sakaimed, Tokyo, Japan) was equipped with 3 markers and attached underneath the foot so that its tip was aligned with the metatarsal heads. A continues force signal and the surface EMG of the medial Gastrocnemius, Soleus and Tibialis anterior (Blue Sensor N electrodes, Ambu, Denmark) was captured with a DTS System (Noraxon, Scottsdale, USA). Analog signals were captured at 1000Hz, marker data at 200 Hz. To analyze morphometrics, a 7.5 MHz, 8 cm width, linear ultrasound probe (Sonoline Adara, Siemens, Munich, Germany) was attached with a carbon cast and a cluster of four markers. The probe was attached at two locations: once over the medial Gastrocnemius muscle-tendon junction (MTJ) and once over the mid-belly (Hösl et al., 2015) and testing was performed twice. Ultrasound videos were captured at 25Hz. To assess muscle-joint mechanics, the examiner manually moved the ankle slowly and continuously from flexion to maximal extension and back. Each stretch lasted a verbal 3 sec. count (Boiteau et al., 1995). The ankle was preconditioned with 3 stretches and then 10 oscillations were captured. For plantarflexor strength tests, 5 maximum voluntary contractions were carried out. Each time the ankle was positioned as close as possible to neutral as a starting point. The 'make test' was used, in which the child maximally pushed for 3 sec. (1 min rest phase).

5.3.6. Data analysis

For gait analysis, walking velocity (m/s), step length (cm) and cadence (steps/min) were calculated. For gait kinematics, mean knee- and dorsiflexion during single stance (°), peak knee- and dorsiflexion (°) and mean toe clearance (cm) during swing phase and peak hip extension and flexion were chosen. For kinetics, positive peak ankle plantarflexion moment (Nm/kg) and power (W/kg) was extracted. Gastrocnemius muscle-tendon unit (MTU) length was calculated (cm) from shank segment length and proximal and distal MTU portions were calculated using regression equations (Orendurff et al., 2002). We calculated peak Gastrocnemius MTU stretch velocity during swing to determine the dynamic stretch tolerance. (Bar-On et al., 2014b).

For instrumented muscle-joint biomechanical assessment, marker and force data were bi-directionally low-pass filtered with a 3rd order Butterworth filter at 8Hz and 5Hz, respectively. The EMG was rectified and filtered bi-directionally with a 4th order 30Hz high- and 10 Hz low-pass Butterworth filter. Ankle angles were calculated with the foot represented by a line connecting the heel and the midpoint of the forefoot and calcaneus marker. The applied force was assumed to be directed perpendicular to the sole, located at the force sensor's mid and the lever arm was taken as perpendicular distance to the bi-malleolar axis. The moment was gravity corrected for the foot. Inertial components were neglected. From each stretch, the moment-angle relations and the EMG signals were time-normalized to 100 points. The cumulative EMG integral of all muscles for each oscillation was summed up and from the 10 oscillations, the ensemble average of 3 stretches with minimal EMG

interference were used. Peak dorsiflexion and peak moment were extracted. Joint stiffness was calculated as the slope of the moment–angle curve, between 20-80% of the individual common torque range for all occasions (Theis et al., 2015). We also calculated the ankle angle at the end of that range. For active strength, the maximum value during a 250ms moving average window was used (Downing et al., 2009) and all trials were averaged. The coefficient of variation was 11.4%.

For the ultrasound, 2D coordinates of the muscle-tendon junction (Fig.5-3) were manually digitized using tracker software (Open Source Physics Project, http://physlets.org/tracker/). Its 3D location within the laboratory was calculated by using the probe to cast marker location. Tendon length was defined as linear distance from the heel marker to the muscle-tendon junction, muscle belly length from muscle-tendon junction to the knee joint center. Fascicle length was measured with a tracking algorithm (Cronin et al., 2011) and tracks of 5 fascicles were averaged. Pennation angle (α) was determined with respect to the deep aponeurosis and extracted at rest. Resting lengths of the muscle and fascicles were defined at maximal plantarflexion (Theis et al., 2015). Tendon resting length was measured when the muscle-tendon junction started to move distally using a threshold of 0.5 mm which indicated force transmission through the tendon. Peak strain was calculated as %-change in length relative to rest. Midbelly thickness was defined perpendicular to the deep aponeurosis. For simplicity, the force (moment) was assumed to be homogeneous throughout the MTU. Resistive muscle and tendon stiffness were determined in the same range as joint stiffness as length change relative to joint torque (Nm/cm).

5.3.7. Statistics

For each treatment both intervention periods were pooled. To test the difference in treatment effects between stretching and BDTT for statistical significance, an analysis of covariance (ANCOVA) was applied (Metcalfe, 2010). The dependent variables were the post-values and the covariate were the baseline values with treatment as between-subjects factor. Significantly different treatment effects were expressed as baseline adjusted mean group differences and 95% confidence intervals within the text, effect sizes are displayed as partial η2. Subsequently, pre-post changes for each therapy were also separately tested for stretching and BDTT using paired t-test. Alpha was set to 0.05.

5.4. Results

Compliance for stretching and BDTT was 96.3% and 95.9%. No more than 2 of 27 sessions were missed by any subject. By comparing the pre-intervention values of each outcome between stretching and BDTT, no significant imbalance in any parameter was observed (P≥0.390).

5.4.1. Gait-analysis

Comfortable walking condition

Results are displayed in Figure 5-4 and Table 5-1. There was a significantly different treatment effect in dorsiflexion in single stance (2.9°, CI [0.1, 5.6], P=0.041) and in knee flexion in swing (3.4°, CI [0.2, 6.7], P=0.041). In detail, no significant change in dorsiflexion (+1.3 [SD 4.7]°, P=0.262) or knee flexion were noted after BDTT (0.2 [SD 5.2]°, P=0.873) but stretching showed a decrease in dorsiflexion (-1.7 [SD 3.7]°, P=0.064) in stance and knee flexion in swing (-3.0 [SD 5.0]°, P=0.021).

Fastest possible walking condition

The fastest possible velocity displayed a significantly different treatment effect (0.10 m/s, CI [0.02, 0.19], P=0.017). Participants walked significantly faster after BDTT (+0.06 [SD 0.12] m/s, P=0.035) and no significant change was observed after stretching (-0.04 [SD 0.14] m/s, P=0.229). This was accompanied by a significantly different treatment effect in cadences (12.3 steps/min, CI [4.2, 20.3], P=0.004). We observed higher cadences after BDTT (+5.9 [SD 12.7] steps/min, P=0.064) and after stretching, a significant decline was noted (-7.4 [SD 11.0] steps/min, P=0.011). With respect to joint kinematics, there was a significantly different treatment effect concerning knee flexion in swing (5.4°, CI [2.0, 8.8], P=0.003). Knee flexion in swing was significantly less after stretching (-5.2 [SD 6.0]°, P=0.002) and after BDTT values remained unaltered (+0.5 [SD 5.7]°, P=0.701). Regarding Gastrocnemius MTU lengthening velocities in swing phase, a significantly different treatment effect was noted (4.1 cm/sec., CI [0.6, 7.6], P=0.023). Values on average decreased after stretching (-2.2 [SD 5.2] cm/sec., P=0.091) and increased after BDTT (+2.1 [SD 5.0] cm/sec., P=0.096). Apart from that, separate pre-post comparisons showed that only after stretching knee flexion in stance was significantly less (-4.0 [SD 7.0]°, P=0.027).

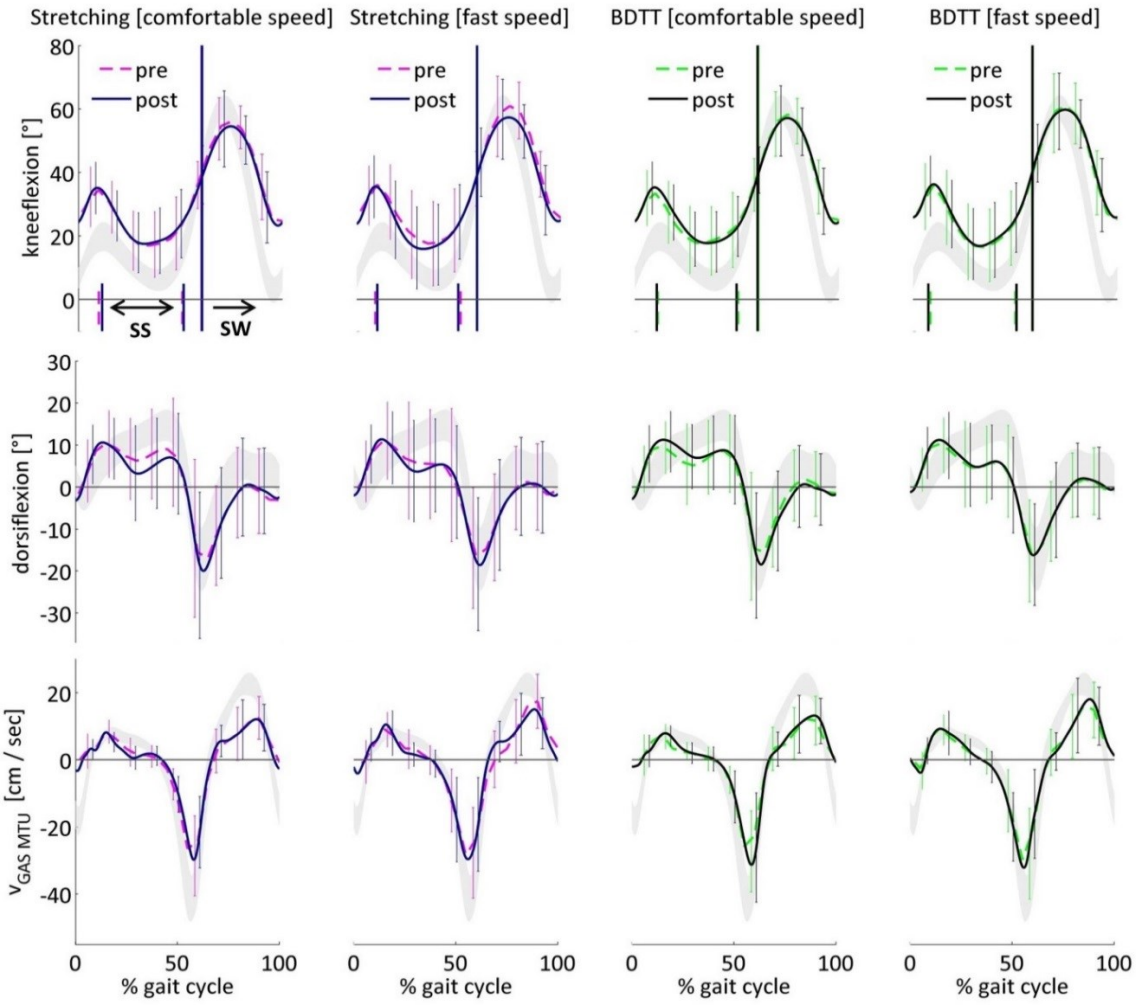

Fig. 5-4 Ensemble group average traces for sagittal knee and ankle kinematics and calculated Gastrocnemius muscle-tendon unit velocity (vGAS MTU) before and after stretching as well as before and after backward-downhill treadmill (BDTT) at comfortable and at as fast as possible speed. Error bars show 1 SD. Grey shaded areas show reference of typically developing mean and 1SD while walking at comfortable speed of 1.30 (SD 0.16) m/s from our lab. Vertical lines below x-axis in top row indicate single stance (SS) or separate stance from swing.

5.4.2. Functional mobility assessment

No significantly different treatment response occurred (all P≥0.138). As shown in Table 5-1, only after BDTT, Gross-Motor-Function Scores and Timed Up-and-Go time improvements reached significance (P=0.022-0.050). All changes after stretching were not significant (P≥0.335).

Table 5-1 Results of the 3D gait analysis and the functional ambulatory mobility tests.

	Stretching		**BDTT**		**ANCOVA Treatment differences**	
	Pre Mean(SD)	Post Mean(SD)	Pre Mean(SD)	Post Mean(SD)	*P*	η^2
Comfortable walking speed						
velocity [m/s]	1.10(0.17)	1.12 (0.20)	1.12(0.17)	1.15(0.17)	0.640	0.007
cadence [steps/min]	122(13)	121(15)	**123(14)**	**126(15)***	0.051	0.111
step length [cm]	53.9(6.9)	55.5(7.0)	54.8(5.0)	55.0(5.9)	0.450	0.011
mean DF $_{\text{single stance}}$ [°]	8.4(9.4)	6.6(8.8)	7.5(7.3)	8.7(7.2)	**0.041**†	**0.120**
mean KF $_{\text{single stance}}$ [°]	22.3(9.6)	22.3(9.7)	22.7(8.9)	23.1(7.8)	0.747	0.003
min HF $_{\text{single stance}}$ [°]	2.2 (8.1)	3.4 (5.8)	2.0(6.6)	3.1(5.2)	0.889	0.001
peak DF $_{\text{swing}}$ [°]	2.3(9.5)	2.5(10.5)	3.6(7.1)	2.9(8.7)	0.338	0.028
peak KF $_{\text{swing}}$ [°]	**59.7(7.0)**	**56.7(7.6)***	60.5(8.5)	60.7(7.2)	**0.037**†	**0.125**
peak HF $_{\text{swing}}$ [°]	46.5(8.8)	46.5(6.9)	45.8(7.6)	47.7 (7.5)	0.238	0.042
mean toe clearance [cm]	2.2(0.6)	2.4(0.8)	2.4 (0.8)	2.4 (0.7)	0.075	0.093
peak GAS vel. $_{\text{swing}}$ [cm/sec]	16.5(6.2)	16.7(6.7)	16.8(5.4)	18.1(6.8)	0.419	0.020
Peak PF moment [Nm/kg]	1.18(0.30)	1.20(0.27)	1.21(0.24)	1.22(0.26)	0.902	0.001
Peak PF Power [W/kg]	2.12(0.61)	2.29(0.73)	2.19(0.6)	2.35(0.8)	0.978	0.063
Fastest possible walking speed						
velocity [m/s]	1.45(0.20)	1.41(0.24)	**1.44(0.21)**	**1.51(0.17)***	**0.017**†	**0.160**
cadence [steps/min]	**150(12)**	**143(14)***	145(16)	151(18)	**0.004**††	**0.225**
step length [cm]	57.9(7.4)	59.4(8.6)	59.7(7.3)	60.3(6.4)	0.651	0.006
mean DF $_{\text{single stance}}$ [°]	6.2(10.8)	6.2(9.7)	6.4(7.8)	6.9(7.3)	0.674	0.005
mean KF $_{\text{single stance}}$ [°]	**25.4(11.6)**	**21.4(11.5)***	23.7(10.8)	23.6(9.6)	0.129	0.068
min HF $_{\text{single stance}}$ [°]	1.7(8.9)	2.7(6.9)	0.7(7.4)	3.4 (7.2)	0.360	0.025
peak DF $_{\text{swing}}$ [°]	3.5 (9.2)	3.4(10.5)	3.9(7.7)	4.2(8.0)	0.786	0.002
peak KF $_{\text{swing}}$ [°]	**64.9(9.5)**	**59.6(8.6)****	63.8(10.6)	64.3(8.3)	**0.003**††	**0.243**
peak HF $_{\text{swing}}$ [°]	49.3(8.5)	49.3(6.9)	48.8(7.6)	50.7(7.8)	0.200	0.049
mean toe clearance [cm]	2.8(0.7)	2.6(0.9)	2.8 (0.9)	2.9 (0.6)	0.191	0.051
peak GAS vel. $_{\text{swing}}$ [cm/sec]	22.4(7.2)	20.2(7.2)	20.7(7.6)	22.8(9.3)	**0.023**†	**0.148**
Peak PF moment [Nm/kg]	1.23(0.28)	1.23(0.25)	1.29(0.26)	1.26(0.29)	0.664	0.006
Peak PF Power [W/kg]	2.69(0.81)	2.76(0.87)	2.54(0.86)	2.84(0.92)	0.403	0.021
Functional ambulatory mobility tests						
GMFM D [Score 0-100]	88.2(8.9)	89.1(8.7)	**87.4(9.2)**	**90.0(7.1)***	0.218	0.088
GMFM E [Score 0-100]	90.7(8.5)	91.0(7.0)	**90.7(8.0)**	**92.6(6.2)***	0.138	0.125
Timed-Up-and-Go [sec]	7.9(1.9)	7.2(2.2)	**8.1(1.6)**	**6.8(1.3)***	0.497	0.028

BDTT: backward downhill treadmill training, DF: Dorsiflexion, PF: Plantarflexion, KF: Knee flexion, HF: Hipflexion. GAS vel.: calculated Gastrocnemius muscle-tendon unit velocity, *significant effects for the pre-post comparisons of each treatment *p<0.05 (** with <0.01), the ANCOVA p-value refers to the difference between treatments: the dependent variables were the post-values and the covariate were the pre-treatment values with treatment as between-subjects factor, † significant treatment differences †p<0.05 (††p<0.01), η2=partial effect size with benchmarks: η2 > 0.059 for medium and η2 > 0.138 for large effects.

5.4.3. Instrumented muscle-joint biomechanical assessment

Table 5-2 Results of the muscle-joint biomechanical assessment**.**

	Stretching		BDTT		ANCOVA Treatment difference	
	Pre Mean(SD)	Post Mean(SD)	Pre Mean(SD)	Post Mean(SD)	P	η^2
Active ankle-joint assessments						
Peak moment [Nm]	13.8(5.0)	14.0(6.0)	15.0(7.9)	16.6(8.2)	0.470	0.041
Passive ankle-joint assessments						
Peak moment[Nm]	5.3(1.2)	7.0(2.5)*	5.1(0.8)	6.3(1.7)	0.532	0.023
Peak DF [°]	-0.2(10.9)	1.7(8.1)	-0.3(9.3)	2.6(8.4)	0.710	0.008
DF at fixed moment [°][a]	-8.8(9.6)	-10.0(9.6)	-9.3(8.0)	-8.3(8.2)	0.344	0.053
Joint stiffness [Nm/°]	0.15(0.07)	0.14(0.04)	0.13(0.05)	0.14(0.04)	0.345	0.052
Muscle-tendon properties						
Medial Gastrocnemius morphometrics [at rest]						
Fascicle angle [°]	23.5(4.3)	24.5(4.0)	22.7(4.4)	23.5(3.6)	0.670	0.011
Thickness [cm]	1.5(0.4)	1.5(0.3)	1.5(0.4)	1.5(0.4)	0.788	0.004
Fascicle length [cm]	3.8(0.9)	3.7(0.9)*	3.9(0.9)	3.8(0.7)	0.893	0.001
Muscle length[cm]	19.9(2.7)	20.4(2.9)	20.0(2.6)	20.1(2.7)	0.543	0.022
Tendon length [cm]	15.1 (3.7)	15.1(4.4)	15.0(3.8)	15.1 (3.7)	0.725	0.008
*Total strain [*resting length to max. length]						
Fascicle [%]	21.2(7.1)	27.9(6.0)*	20.2(6.4)	26.8(6.6)*	0.792	0.004
Muscle belly[%]	3.8(1.8)	4.9(1.5)**	4.0(1.6)	5.4(1.7)**	0.460	0.033
Tendon [%][b]	6.9(1.4)	6.1(1.6)	6.2(3.4)	8.0(3.9)	0.168	0.109
Passive resistive stiffness [between 20-80% common passive joint moment]						
Muscle belly [Nm/cm][c]	5.9(2.7)	4.7 (1.2)	5.2 (2.1)	4.2 (1.9)	0.774	0.005
Tendon [Nm/cm]	3.8 (1.5)	4.3(1.1)	3.8 (1.6)	3.8 (1.1)	0.364	0.049

BDTT: backward downhill treadmill training, DF: Dorsiflexion, a fixed moment refers to 80% common passive resistive moment, b tendon strain was calculated by a using straight line approximation for tendon length, c force (moment) was not partitioned within the triceps-surae muscles group; *significant effects for the pre-post comparisons of each treatment *p<0.05 (** with <0.01), the ANCOVA p-value refers to the difference between treatments: the dependent variables were the post-values and the covariate were the pre-treatment values with treatment as between-subjects factor ,† significant treatment differences †p<0.05 (††p<0.01). η2=partial effect size with benchmarks: η2 > 0.059 for medium and η2 > 0.138 for large effects.

Concerning plantarflexor strength, one subject was excluded due to problems in data acquisition. Treatments did not have significantly different effects on strength (P=0.470) and neither stretching nor BDTT caused any significant change (P≥0.204). During the passive rotational tests, EMG interference was not significantly different between pre and post in BDTT or stretching (all muscles P≥0.109). Mean knee alignment was 2-3 (SD 6-7)° with no difference between treatments (P=0.544). Rotational ankle speed (°/sec) during the passive motion was 13.9 (SD 2.4) and 9.3 (SD 2.4) pre and post stretching and 16.1 (SD 8.8) and 9.8 (SD 3.7) pre and post BDTT. Both were significantly slower at post-assessments (P=0.005 and P=0.032) with no difference between treatments (P=0.915). Muscle morphometric traces are shown in Fig. 5-5. Test-statistics are shown in Table 5-2. Treatment effects on passive dorsiflexion were not significantly different concerning end range values or evaluations at matched torques (P=0.710 and P=0.344). The increases in peak dorsiflexion after stretching (+2.0 [SD 6.0]°, P=0.163) and after BDTT (+2.8 [SD 6.0]°, P=0.085), as well as the increases in peak resistive joint torque after stretching (+1.7 [SD 2.4] Nm, P=0.047) and after BDTT (+1.2 [SD 1.9] Nm, P=0.069) were not significantly different between treatments. Also no effects on ankle joint stiffness were noted (P=0.345).

With respect to morphometrics, none of the parameters indicated a significantly different treatment response (all P≥0.543). Subsequent pre-post comparisons separated for each treatment showed that muscle or tendon rest length was not significantly altered (all P≥0.356). Yet, total passive strain of the muscle belly and fascicles was similarly, significantly increased after both treatments (29-35%, all P=0.002-0.023). No significant changes in passive resistive stiffness of the muscle and tendon were observed (P≥0.082). In addition, after stretching and BDTT fascicle length at rest decreased by ~3%, reaching significance after stretching (P=0.046) but not after BDTT (P=0.438).

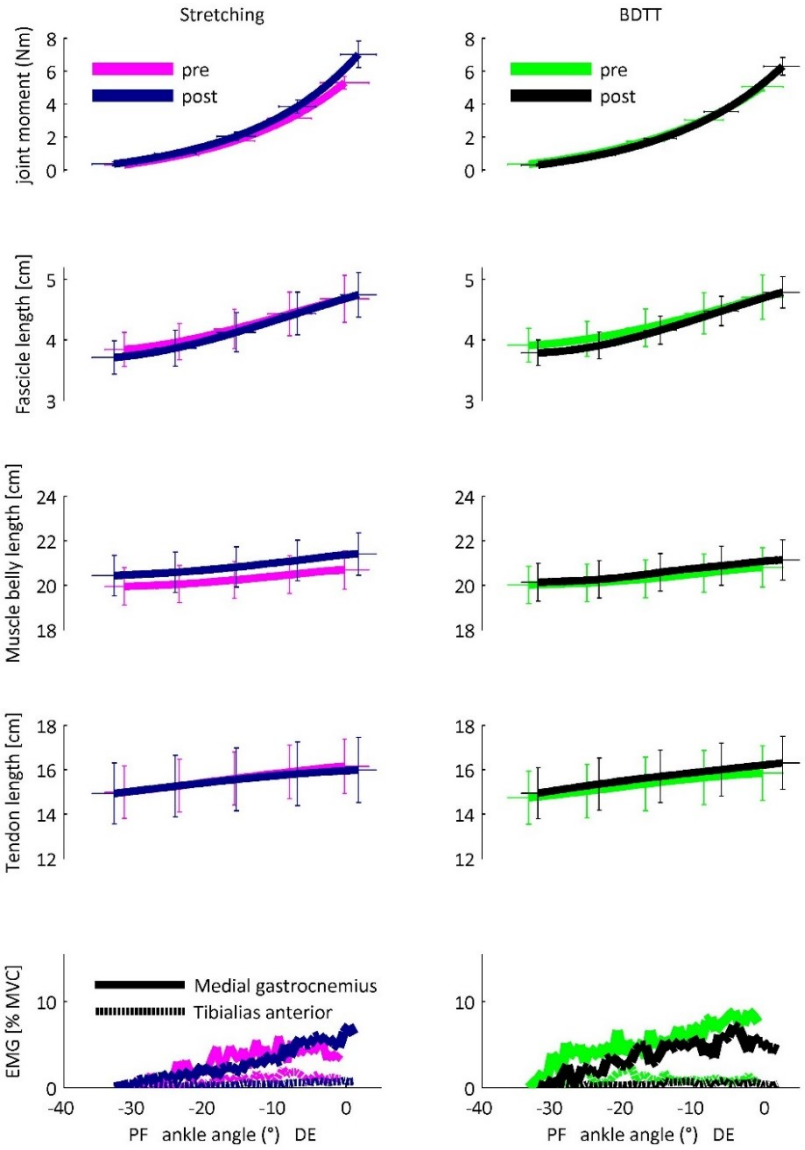

Fig. 5-5 Ensemble group average traces for instrumented muscle-joint biomechanical stretch assessment concerning the passive ankle joint moment, the medial Gastrocnemius muscle morphometrics and shank muscle activity during passive dorsiflexion stretches**.** Left side: Traces before and after stretching, Right side: Traces before and after backward-downhill treadmill (BDTT). Error bars show 1 SEM. For clarity, error-bars of EMG values and Soleus EMG traces have been omitted from the lowest graphs. Soleus muscle activity was similarly low as the Tibialis anterior activity.

5.5. Discussion

Focusing on stretching, passive dorsiflexion was not significantly increased and muscle stiffness was unaltered which contrasts findings in SCP-children that primarily relied on wheeled mobility (Theis et al., 2015). Yet, also in our sample larger peak resistive torques were tolerated, similar to studies in healthy adults (Blazevich et al., 2014). This may reflect altered tolerance to passive stretch but structural joint tightness could have impeded further joint flexibility. In comparison to Theis et al. (2015), each stretch was held shorter in our study (20 vs. 60 sec.) but the intervention volume was larger (+27%). However, we think that methodological aspects were of minor importance for the different outcome since our participants had a considerably higher ambulatory status (lower GMFCS-level). They may actually use their plantarflexors on short length during full weight-bearing gait which may dominate their muscles' response (Hösl et al., 2016). Therapeutic interventions in ambulatory SCP-children may therefore need to train aspects of gait to elicit positive changes during walking. On the other hand, normalizing muscle morphometrics and improving gait may not necessarily occur in concert since sustained stretch treatment with an ankle-foot orthotic improved walking velocity and foot lift despite inducing Gastrocnemius fascicle length decline (Hösl et al., 2015). As adverse outcome after stretching, knee flexion during swing declined. This may suggest worsened muscle function, since the Gastrocnemius usually initiates knee flexion into swing (Neptune et al., 2001). Yet, the reduced knee flexion did not negatively impact toe clearance and may disappear after 5 weeks since treatment baseline was similar in the group that started with stretching. Healthy adults also reduce knee flexion during swing phase as an acute response to intense static plantarflexor stretch which had been associated with transient plantarflexor weakness (Apti et al., 2016) but in our longitudinal study, SCP-patients showed no such drop in strength. Therefore the stiff-knee gait of the SCP-patients seems not to be induced by plantarflexor weakness. However, other neural aspects might play a role. Some stretch exercises might have tensioned the sciatic nerve (Coppieters et al., 2006), since the children were positioned with straight knees and flexed hips. This may cause impaired neural drive to hamstring muscles during gait and limit swing phase knee flexion. While detailed mechanism need to be investigated, our findings show that manual, static plantarflexor stretching may not be emphasized in SCP-patients with a high ambulatory status.

Although after BDTT deficits in passive dorsiflexion could not be substantially improved, gains in dorsiflexion during gait were significantly larger than after stretching. Moreover, participants were able to walk faster and increases in ambulatory mobility were found. In our study the higher walking velocity had been achieved by an increase in cadence. This faster reciprocal leg motion might reflect improved coordination. It had been previously also speculated that faster walking speed und improved postural balance after flat backward gait training (Hao and Chen, 2011; Kim et al., 2013) could be a sign of altered neuromuscular control and reorganization of muscle synergies. Higher cadences are

also a typical strategy in SCP (Abel and Damiano, 1996), which generally necessitate little change in ankle joint kinetics (Ardestani et al., 2016) and accordingly the increases in peak ankle joint power marginally failed to reach significance (+12%, P=0.054). We also found a treatment difference concerning faster Gastrocnemius MTU lengthening velocities in swing after BDTT. Previously, Bar-On et al. (2014b) showed that higher Gastrocnemius MTU lengthening velocities during gait are related to higher stretch reflex thresholds during passive examination, so, in comparison to stretching, dynamic stretch sensitivity might have been attenuated after BDTT.

Concerning the muscle-joint properties, no increase in muscle length or thickness was found, strength was not increased and also the passive resistive stiffness of the tendon was not altered. Muscle-tendon loadings during BDTT were probably too low to provide a homeostatic perturbation: Generally, in able-bodied persons, large tendon deformation during training is necessary for increasing tendon stiffness (Arampatzis et al., 2007; Bohm et al., 2014) but our previous ultrasonographic analysis of the spastic calf during backward downhill walking showed that the tendinous tissue of the Gastrocnemius is used on rather short length (Hösl et al., 2016). Apart from that, our participants with SCP did not report about muscle soreness. Reasons for the lack of muscle soreness could be that we gradually adjusted our training or that duration and intensity (slope and beltspeed) was less than in studies on healthy adults (Hoang et al., 2007; Hoffman et al., 2014). However, in comparison to adults, susceptibility to eccentric exercise-induced muscle damage is considerably reduced in healthy children and adolescents, too (Chen et al., 2014) and also alterations in spastic muscle composition could be detrimental.

From a methodological point of view, ~6-8% of passive tendon strain during assessments of muscle joint-properties was higher than previous values of ~2-3% in healthy adults (Csapo et al., 2013; Monte et al., 2006). Noteworthy, the tendon length defined here is approximated as a straight line and neglecting curvature leads to overestimation of strain values (Monte et al., 2006). Besides, this method integrates the Achilles tendon and the run-out from the deep MG aponeurosis. The absolute tendon strain values may therefore be interpreted with caution. To which extent these differences are affected by SCP pathology or age needs to be determined, too.

For both stretching and BDTT, Gastrocnemius muscle and fascicle strain was significantly increased which could be the combined results of somewhat larger dorsiflexion and larger tolerated stretch-moments. Conversely, even though it remains fairly speculative, we cannot totally refute conceptions about sarcomere remodeling. In the past, Gastrocnemius biopsis in SCP-patients showed that in-vivo sarcomeres are extremely long with pulled apart contractile proteins (Mathewson et al., 2014), probably due to difficulties in adding new sarcomeres. A change in the resting length of sarcomeres might have lowered the resting tension within myofibres and improved their potential to elongate during stretch. Consequently, the reduction in resting fascicle length, particularly after stretching, may

not necessarily reflect sarcomere loss. Investigating active fascicle force-length relationships (Barber et al., 2012) could give an indirect, non-invasive hint in future. Eventually, since the increase in muscle and fascicle strain with both interventions was similar but the effects during walking differed, it is debatable if the changes in passive muscle and fascicle strain are functionally decisive.

Apart from the current treatments, alternative modes of stretching or treadmill training showed more promising outcomes in ambulatory SCP-patients. First, a combination of cyclic, robotic plantarflexor stretching with dorsiflexor strengthening could improve ambulatory function without including specific walking exercises (Wu et al., 2011). Second, opposed to BDTT, forward uphill training also increased the toe-lift during swing phase of gait (Willerslev-Olsen et al., 2015). Both of these alternatives increased dorsiflexor strength which could be very influential. Uphill walking also necessitates more concentric Gastrocnemius fascicle excursion in SCP-patients (Hösl et al., 2016). This may better train push-off, since gains after strength training may be specific in terms of contraction mode (Roig et al., 2009). The motoric transfer of a braking action during BDTT to increased propulsion may be too challenging. To offer plantar- and dorsiflexor training with varying coordinative and contractile demands, forward and backward-training on slopes may be combined.

5.6. Limitations

First, this study was limited to a rather small convenience sample which was subjected to a cross-over study receiving both treatments and the interpretation may be impacted by the predominance of bilateral participants. More participants may be required to detect changes in muscle morphometrics and a larger scale RCT with parallel grouping may be preferable in future. Second, gains in passive dorsiflexion were fairly small and it remains unknown if both therapies can cope with any natural decline; ~2° in 3 months (Maas et al., 2014). Third, the testing is limited by the precision of the hand-held dynamometer. Fourth, the manually geared muscle-joint testing was executed slightly slower during post testings but a) no different EMG interference occurred and b) this change in angular velocity was not related to changes in stiffness on muscle, tendon or joint level (P=0.157-0.798). Nevertheless, motor-driven dynamometry would probably be more consistent. However, such tests were partly performed at similar velocities (10-20 °/sec) in SCP-patients (Theis et al., 2015,de Gooijer-van de Groep et al., 2013, Barber et al., 2011) while some manually-geared tests were also performed faster (30-60°/sec) (Peng et al., 2011, Alhusaini et al., 2010). The EMG-data showed low position dependent activation during the slow stretch maneuvers (Fig. 4), in agreement with previous investigations (Bar-On et al., 2014a). Still, the angular rotations were below reported stretch reflex thresholds of 21–71°/s for spastic plantarflexors (Willerslev-Olsen et al., 2013) and the EMG interference is consistent with proposed thresholds (<10% MVC) in SCP for dynamometric dorsiflexion

tests (Maas et al., 2014). Fifth, although the time each leg was focused during therapy was similar, the training volumes differed: The total end-range duration per leg during stretching was ~304 min (910 repetitions). On the treadmill, ~18.000 steps were performed. Since one eccentric contraction takes up ~20% of each step (Hösl et al., 2016), the eccentric contractions lasted ~60 min in total (~one step per sec.).

5.7. Conclusion

BDTT helps to achieve faster forward walking velocities compared to static stretching. Whether BDTT also altered neuromuscular control or reduced dynamic Gastrocnemius stretch sensitivity needs to be determined. No superiority of static stretching over BDTT was noted in any parameter. Passive ankle joint mobility was not increased after stretching or BDTT. Stretching particularly deteriorated knee flexion during swing and therefore we argue that, since manual static stretching is labor intensive and physically demanding to therapists, it may not be emphasized in high-functioning, independent ambulators with SCP. More intense BDTT, e.g. a steeper negative slope, or more frequent training might be necessary to alter muscle-tendon morphometrics and increase strength of spastic plantarflexors.

Conflict of interest

None of the authors has any commercial or other interests that create conflict of interest for the work presented

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6. Main findings and conclusions

The overall objective of this thesis was to investigate several non-invasive treatment strategies for calf muscle pathology of children with Cerebral Palsy (CP) and equinus deformity. More specifically, the aim was to gain knowledge about the responsiveness of their muscle-tendon structures by using brightness mode ultrasonography on the medial gastrocnemius. In addition, this thesis sought to promote the understanding of gastrocnemius structure-function relationship in children with CP.

The **first study** focused on stretch-immobilization against equinus via ankle-foot bracing. A pre-post setting was applied. Additionally, the values of children with CP were compared to a group of untreated healthy controls. Prior to the treatment, the current findings supported previous results on gastrocnemius muscle-tendon pathology in children with CP. Children with CP displayed reduced muscle belly thickness, muscle belly length, fascicle length and increased tendon length of the gastrocnemius muscle with respect to the control participants. Furthermore, their fascicles were shown to be less extensible upon stretch. The treatment, which on average lasted for 4 months, showed that the gastrocnemius muscle belly atrophied. This was caused by a reduction in muscle belly thickness and fascicle length. Thus, muscle architecture seemed to further deteriorate. This was probably caused by disuse or decreased muscle excursion related with the orthotics. However, the extent of ankle joint contracture was alleviated and passive dorsiflexion increased. Still, passive dorsiflexion primarily increased when clinically assessed with flexed and not with extended knees. Very likely, morphological structures distal to the gastrocnemius muscle belly were targeted during bracing. In addition, the distal tendon extensibility seemed to increase after bracing. Yet, this might have been prone to methodological flaws, since we did not account for slack length of the tendon during testing. Assuming that, similar to findings in animal studies (Blanchard et al., 1985; Tardieu et al., 1977), the tendon adaptation could have been stimulated first and the tendon could have initially also gotten more compliant during stretch-immobilization, the fascicles of the gastrocnemius might have been unstrained during brace wear. This in turn could have triggered a loss of sarcomeres in series and would fit to the obsverved reductions in fascicle length.

On the other hand, the children's walking patterns improved. They selected a faster natural walking velocity, landed with a flatter foot to floor angle and increased dorsiflexion during the swing phase of gait. Nevertheless, decreased ankle plantarflexion moments during the first and second half of the stance phase were found. Since a large portion of these ankle moments is typically generated passively in CP children (Crosbie et al., 2012; Dallmeijer et al., 2011; Eek et al., 2011), the passive stretch resistance of the plantarflexor muscles probably reduced. Having said that, reduced ankle moments during push-off potentially also reflected weaker plantarflexor muscles. Both findings might be attributed to the decreased gastrocnemius muscle bulk after the treatment. Although the active

plantarflexor strength was not instrumentally assessed, the architectural deteriorations of the muscle suggest that the gastrocnemius' capacity for force production reduced. But, worth noting is that the the gastrocnemius muscle belly atrophy might have also provided some benefits. It could have helped the dorsiflexing muscles to exert foot lift during walking, since the thinner gastrocnemius might have produced less antagonistic resistance during the swing phase. Arguably, these functional gains may somewhat outweigh the atrophic effects. To potentially also induce gastrocnemius muscle belly growth, orthotic braces may need to extend the knee to exert stretch on the whole gastrocnemius muscle-tendon unit or, next to bracing, complementary training could be necessary.

In the **second study**, the goal was to evaluate alternative training stimuli for the gastrocnemius of children with CP. In this cross-sectional investigation, the contractile activity of the medial gastrocnemius was measured while walking on sloped surfaces, namely during forward-uphill and backward-downhill gait, as well as during flat-forward walking. During flat-forward gait, the study revealed that, despite having shorter fascicles length at rest, children with CP also worked at shorter relative fascicle length than controls. This is an important finding. It suggests that muscles of children with CP could voluntary work on short fascicle length in order to compensate for their increased sarcomere length (Mathewson et al., 2014). This may enable the use of sarcomeres in a configuration where they are able to produce enough active forces and also avoid that contractile filaments are stretched beyond overlap (see section 1.5.1.4). In addition, no difference in eccentric fascicle excursion during the stance phase between CP patients and control subjects existed. In contrast to a frequent assumption (e.g. Pitcher et al., 2015), increased eccentric loadings may thus not be responsible for the genesis of contracture in children with CP. Still, those patients that were able to exert less isometric plantarflexor force during strength tests, experienced more eccentric fascicle excursion during gait. Therefore the gastrocnemius fascicles of children with CP may indeed face difficulties resisting tensile forces during the stance phase.

When walking backward-downhill, the gastrocnemius functioned as a brake and displayed increased eccentric excursion during the landing phase. During the uphill-walking condition concentric fascicle action was increased throughout the push-off phase. It was concluded that both training modes could therefore offer benefits for children with CP. Yet, taking the positive outcomes of eccentric training on animal muscles (Butterfield et al., 2005; Lynn and Morgan, 1994) and on healthy human muscles (Duclay et al., 2009) into account, it was subsequently hypothesized that backward-downhill gait could stimulate sarcomere-genesis in series and induce strength gains in children with CP. Both factors should help to alleviate the impact of equinus pathology.

Consequently, during the **third study,** backward-downhill treadmill walking was evaluated. We compared its effectiveness against manual static calf stretching. Both treatments were administered over periods of 9 weeks in a cross-over study. Manual static calf stretching was applied as 'control

treatment'. Stretching was considered to be representative for the standard physiotherapeutic management. After backward-downhill treadmill training the children achieved faster overground walking velocities and improved their functional ambulatory mobility, as revealed by Timed up and go tests, 10m walk tests and GMFM D and E assessments. Still, no significant gains in passive or active ankle dorsiflexion during gait were found. Since stretching tended to cause a decline in dorsiflexion during the single stance phase of gait, backward-downhill treadmill training was nevertheless statistically superior.

Contrary to our expectations, no signs of muscle growth (increases in fascicle length or muscle belly thickness) were found after backward-downhill training. Accordingly, no improvement in plantarflexor strength could be documented. Apart from that, the passive resistive stiffness of the tendon was also not altered. This is in contrast to Foure et al.'s (2013) findings on eccentric plantarflexor training in healthy adult controls. Notably, the second study of this thesis revealed that while walking backward-downhill, the series elastic element (tendon+aponeurosis) of the gastrocnemius probably worked on rather short length. This might not have been sufficient to induce major tendon adaptations with the conducted training. In able-bodied persons, large deformation of tendons seems to be necessary for increasing their stiffness (Arampatzis et al., 2007a; Bohm et al., 2014). Hence, muscle-tendon loadings in the current study were probably too low to induce an adaptive response within the muscle-tendon complex of children with CP.

Concerning the static stretching treatment**,** no superiority over backward-downhill walking was noted in any parameter of gait or with respect to the values on muscle morphometrics and joint contracture. Stretching even seemed to be counterproductive during walking because children displayed reduced knee flexion during the swing phase of gait. Some stretch exercises might have tensioned the sciatic nerve (Coppieters et al., 2006), since the children were positioned with straight knees and flexed hips during some exercises. This might have caused impaired neural drive to hamstring muscles during gait and thereby limited the swing phase knee flexion.

Similar to the effects of backward-downhill walking, passive dorsiflexion was not increased after stretching and muscle belly stiffness was also not significantly altered. This contradicts with another study on static stretching in CP children (Theis et al., 2015). The study of Theis et al. (2015), however, primarily included children that relied on wheeled mobility. The very mobile children who were included in the current study might have actually often used their gastrocnemii on short muscle-tendon and fascicle length during gait. This loading regime probably dominated their muscular response and prevented increases in fascicle length. Thereby any major increase in passive ankle joint extensibility was probably prevented. As expected, manual stretching was also not sufficient to induce muscle growth or change tendon properties.

Eventually, both treatments did not significantly change passive end-range dorsiflexion, as well as the resistive stiffness values on muscle-tendon and joint level. Nevertheless, an increase in passive muscle and fascicle strain after stretching and treadmill training was found. These larger passive strains could have been the combination of somewhat larger dorsiflexion and larger tolerated stretch-moments during the tests. However, only the gains in passively tolerated moments after stretching surpassed statistical significance. In addition, even though we did not quantify the untreated progression of muscle pathology in CP children, it was concluded that manual, static plantarflexor stretching may not be emphasized in patients with a relatively high ambulatory status. Therapeutic interventions may most likely need to directly train aspects of gait to elicit positive changes during walking in patient populations with CP (Boyd and Graham, 1999; Moreau et al., 2016; Romeiser Logan, 2013). When opting for backward-downhill gait as a treatment, more intense training, e.g. a steeper decline, or more frequent training sessions might be necessary.

7. Implications for orthopedics and therapists

First, ankle foot orthotics used as splints remain a valuable treatment option for children with CP. They were shown to be capable of improving gait. As had been already acknowledged by others (National Collaborating Centre for Women's and Children's Health (UK), 2012), muscle wasting and potential weakness resulting from immobilization with orthotics may need to be weighed against these benefits. Quite frequently, the major rationale for the use of orthotics in CP is to prevent progressive joint contracture. Considering results from Sweden, 62% of ankle-foot orthotic users among CP children also manage to maintain their level of passive dorsiflexion (Wingstrand et al., 2014). All treatments presented in this thesis (braces, manual stretching, as well as backward-downhill treadmill training) were able to statistically maintain passive dorsiflexion with extended knees. Thus, they also prevented disease progression. More specifically, with ankle foot bracing, 76% of the children increased their maximal passive dorsiflexion which seems to be a rather promising result. Notably, Wingstrand et al. (2014) reported that as little as 9% of CP children generally display major gains in passive dorsiflexion with orthotics. However, there is a clear need for alternative treatments. From age 5-6 years onwards, the compliance with orthotics among children with CP seems to progressively decline (Wingstrand et al., 2014).

Second, concerning manual static calf stretching, we could not provide any major evidence to support its widespread use. Our results even suggest that pathology partially progresses. Previous

recommendations from reviews on stretching in CP concluded to prescribe alternatives, e.g. orthotics (Pin et al., 2006), to allow children to stretch and move (Wiart et al., 2008) or to rather cancel stretching during therapy (Novak et al., 2013). The results of the current thesis may to some extent agree with all three of those statements. Manual stretching in ambulatory and very mobile children with CP should thus probably be replaced with more active training modalities. Resistance exercises and group workouts in combination with interactive gaming could likely be future avenues to be explored.

Third, although backward-downhill gait was not able to improve plantarflexor strength or induce muscle growth, it was beneficial for gait and ambulatory mobility. It thus deserves its spot in pediatric physical therapy. Treadmills are already commonly applied in CP (Willoughby et al., 2009). They are often used to train gait quality by executing multiple movement repetitions with or without therapeutic guidance. Thereby, they aim to change motor behavior. While sophisticated treadmills with virtual realities (Sloot et al., 2015a; van der Krogt et al., 2014), partial body-weight support (Dodd and Foley, 2007) or robotic assistance (Meyer-Heim et al., 2009) are applied in CP patients, the latter options may yet be most suitable for more severely affected children. They also require bulky or expensive equipment. Modifications of the slope and walking direction can be easily implemented with low cost treadmills. This can be locally used in physiotherapeutic practices or home-settings, especially for children with a relatively high ambulatory status. Backward gait in particular seems to put high demands on coordinative skills (Hoogkamer et al., 2014). As a consequence, flat-backward (Abdel-Aziem and El-Basatiny, 2017; Kim et al., 2013) or backward-uphill walking (Kim et al., 2016) strategies have been already explored in CP research as well. All these approaches may help to train motor control. In addition, eccentric training modalities also warrant further attention. Robotic exercise, e.g. by means of isokinetic devices (Moreau et al., 2013), could be used for the treatment of calf muscles in CP, too. In hindsight, by training uphill walking we might have been able to reach higher contractions intensitiessince uphill walking necessitated more neural activity from the gastrocnemius than backward-downhill gait (see 4.4.5). Whether this would have also increased dorsiflexion during gait remains arguable. Willerslev-Olsen et al. (2014b) observed an increased toe to floor distance after uphill treadmill training in CP patients, but did not report changes in maximum dorsiflexion during swing phase of gait. Therefore, lifting the foot was probably achieved by hip flexion which is a major modulation of gait when walking uphill. The potential effects of uphill walking may thus also encompass habitual adaptations of gait which needs to be assessed.

Finally, by overseeing the current results and the evidence from the scientific literature, further considerations for pediatric orthopedics may emerge: As with orthotics, other 'conventional' treatments seem to increase passive dorsiflexion at the expense of reducing plantarflexor muscle thickness, e.g. BoNT injections (Park et al., 2014) or surgeries (Shortland et al., 2004). Both aspects may be causally related which is a quite controversial aspect: In healthy subjects, thinner muscles were

associated with more joint range of motion in the past (Chleboun et al., 1997; Kawakami et al., 2003; Magnusson et al., 1997). Speculatively, augmented contractile material in parallel within calf muscles could be responsible for the natural decline in dorsiflexion with growths among typically developing subjects (Weide et al., 2015). Hence, gains in joint range of motion should not be prioritized per-se during treatment of children with CP. Remarkably, restrictions in dorsiflexion may even be energetically beneficial in neurologically healthy adults since there is an inverse relationship between locomotor economy and ankle joint flexibility in walking or running (Hunter et al., 2008, Craib et al., 1996; Hunter et al., 2011). If little active strength is available, limited dorsiflexion may also help to augment ankle moments during walking (Mueller et al., 1995). Concerning passive dorsiflexion excursion, values greater than 10° were even associated with increased oxygen cost during locomotion (Gleim et al., 1990). On the other hand, equinus pathology in neurologically intact persons is negative because it overloads the foot and equinus probably emerges due to sedentary life styles or limited exposure to range of motion (Amis, 2014).

Interestingly, in unilaterally affected children with CP, increased passive ankle joint stiffness seems to be positively associated with faster walking velocities (Crosbie et al., 2012). The stiff hemiparetic leg may be used as a passive spring (Fonseca et al., 2004). This would be in-line with a compensatory role of joint contracture during gait. Nevertheless, less available passive dorsiflexion was associated with slower velocities in unilaterally affected children (Crosbie et al., 2012). In bilaterally affected children, more ankle joint range of motion during gait seems to decrease energy expenditure (Pouliot-Laforte et al., 2014). It may be therefore speculated that gains in ankle joint range of motion in children with CP will functionally be most beneficial if joint stiffness can be preserved. Active stiffening of contractile tissue due to accurately timed muscle activity may either be energetically less efficient or could be too complex from a coordinative perspective. In the future, discriminating non-pathological from pathological joint stiffness seems to be an important task for research. Also the exact nature and consequence of limited joint range of motion in CP needs to be disentangled further.

Finally, although the current finding of elongated gastrocnemius tendons in CP children relative to controls confirms previous studies (Wren et al., 2010), the terminology ‘achillo-tendon shortening’ is frequently used in the context of equinus in CP. The author strongly recommends avoiding this. It may lead less experienced surgeons to target tendons in CP children too often. In addition, the judgement of such tendon alterations appears controversial. In healthy adults, longer Achilles tendons seemed to be efficient during locomotion (Craib et al., 1996; Hunter et al., 2011). Kovanen et al. (1984) reasoned that the longer the tendon relative to the whole muscle, the higher the proportion of elastic energy to be stored and released. It should hence be discussed whether longer tendons are pathological or represent an adaptive strategy of the musculoskeletal system in CP. It is noteworthy that the gastrocnemius’ tendon not only seems to be rather long, but also more compliant than its muscle belly

in CP children (Kruse et al., 2016b; Theis et al., 2016). This is a very atypical finding. Mechanical properties of tendons in CP therefore clearly warrant further attention.

8. General limitations

It needs to be taken into account that all experiments presented in this thesis were conducted on children classified in GMFCS Level I and II. They were able to walk without assistive devices. As can be suspected for manual static stretching, children that primarily rely on wheeled mobility might react in a different way (Theis et al., 2015). For the first and third study, both children with uni- and bilateral pathology were included. Yet, structure-function relationships may be different in both groups. It had been shown that more plantarflexor strength in the hemiparetic leg of unilaterally affected children seems not to be related to faster walking velocities (Crosbie et al., 2012) which generally contrasts findings in bilaterally affected children (Dallmeijer et al., 2011; Eek et al., 2011). Thus, more homogenous groups might have been preferable. Increasing the sample sizes would have also been a theoretical option, but this would have likely required a multi-center approach.

Apart from that, the ankle-foot orthotics used in the first study reached below the knee and thus reasonably targeted the soleus. Due to the ultrasound technique in use, we were not able to precisely visualize the deeper lying soleus simultaneously with the gastrocnemius muscle. Previous research on below knee casting in CP children by Brouwer et al. (1998) revealed a rightward shift of the active ankle angle-force curve when measuring the plantarflexor strength of the subjects with flexed knees pre and post treatment. In principle, this could have been induced by serial sarcomere adaptations of the soleus muscle. Although ultrasound for fascicle tracking works best with superficial muscles (e.g. the gastrocnemius or the vastii), the soleus needs to be investigated in more detail in CP.

Concerning our third study, it may be argued that the frequency of the training (three sessions per week) was too little. Yet, three supervised sessions was the maximum that was feasible for organizational purposes. This was also in the range of other, more effective stretching or strength training interventions for children with CP (see section 1.6.1.5. and 1.6.1.6.). Moreover, it appears to be in accordance with general recommendations for strength training in children and adolescents (McCambridge and Stricker, 2008). Since children with CP require care from multiple disciplines, e.g. from occupational, speech or psycho-social therapists, further training sessions were difficult to integrate into everyday life. This emphasizes the importance of home-based interventions for children with CP in the future. Moreover, we might have not integrated sufficient time for acclimatization to the backward walking protocol. As Verschuren et al. (2016) stressed, interventions in children with CP frequently need more time for adaption to learn proper execution of the exercises. The effective time of training is thus reduced. Consequently, the treatment periods could have theoretically lasted longer.

9. Methodological considerations

This thesis might be restricted by the accuracy of some measurement approaches. Concerning 2D ultrasonographic imaging of the gastrocnemius muscle, acceptable reproducibility has been established in typically developing children (Legerlotz et al., 2010), as well as in children with CP (Mohagheghi et al., 2007). Mohagheghi et al. (2007) reported that the difference between repeated measures during passive assessments was ≤8.1% for fascicle length and ≤8.0% for muscle belly thickness. The findings of the current thesis concerning the effects of ankle-foot bracing are outside these limits. However, care should be taken not to over-interpret the 3% reduction in fascicle length after manual stretching. Notwithstanding these accuracy limits, studies on healthy young adults on average also showed declines in resting gastrocnemius fascicle length of -5.1% (Nakamura et al., 2012) or -4.6% (Blazevich et al., 2014) after static plantarflexor stretching. Yet, in contrast to the current results, these findings did not reach significance.

Due to the altered sarcomere length in children with CP, inference from fascicle length to the underlying cellular structures is complicated. By using brightness mode ultrasound, actually the white connective tissue between and alongside fascicles had been measured. Taken the increased passive gastrocnemius fascicle strains after stretching or backward downhill walking into account, alterations in stretch sensation could have likely played an important role. However, if this had been the sole reason for increased fascicle strains, it would have been fairly likely that children who underwent prior stretching treatments would have tolerated greater strains since the assessment set-up and the training stimulus was rather similar for stretching. Alternatively, it cannot be completely ruled out that the connective tissue properties changed. Such changes are yet difficult to capture non-invasively. Moreover, in contrast to the minor decline in fascicle length after manual stretching during the third study, the more pronounced reduction in fascicle length after bracing during the first study was on average accompanied by decreased passive strain values. Only the latter findings would be in-line with actual serial sarcomere-loss within the fascicles.

Concerning the hand-held dynamometry used in the second and third study, the examiner held the device stationary, while the participant exerted force against it. Therefore the examiner may unconsciously provide a bias (Hebert et al., 2015). Also some other successful training interventions reported gains of ~28-30% in maximal plantarflexor strength in children with CP (Stackhouse et al., 2007; Scholtes et al., 2012; Jung et al., 2013). This is close to the accuracy limits of hand-held dynamometry in children with CP (Taylor et al., 2004; van Vulpen et al., 2013) and smaller changes might be missed. However, since plantarflexor moments neither changed during strength tests nor during gait in the third study, the author is confident that neither stretching nor backward-downhill walking induced meaningful effects on strength.

Furthermore, the passive ankle moment-angle assessments were also manually geared and can hence be prone to inconsistencies. Still, intra-rater ICCs and the typical error (SEM) for 13 subjects with CP revealed acceptable reliability. ICC values ranged between 0.86-0.99 for peak passive moments (SEM: 0.77 Nm), peak dorsiflexion (SEM 1.1°), dorsiflexion at fixed moments (1.3°) or joint stiffness (0.03 Nm/°). Finally, fairly low muscle activity during manual examination was noted. The ankle motion was continuously guided during the tests. To achieve smaller EMG interference, stationary moment-angle relationships would be an alternative (Benard et al., 2010).

10. Future lines of research

Several parameters of orthotics should be explored in more detail. First, it needs to be determined if the use of orthotics during ambulatory and resting periods, or during day and night time has similar effects on muscle morphometrics. Results in young CP children (≤ 4 years) showed that day-night and sole daytime use of ankle foot orthotics seems to have similar effects on passive dorsiflexion (Zhao et al., 2013). However, it is common practice to additionally subscribe ankle foot orthotics during the night. Mol et al. (2012) reasoned that this was more beneficial, since sleeping CP children are quite relaxed and night use may help to prolong the stretch stimulus. The children would probably elsewise sleep in pronounced equinus posture. Interestingly, there also seems to be no extra sleep disturbance caused by night orthotics in CP children (Mol et al., 2012). Second, to the best my knowledge, the impact of the wearing time of orthotics on muscle architecture has not been quantified. In the first study of this thesis, the recommendation was to wear the brace throughout the night. If additional day use was scheduled, only 1-2 hours should have been spent without the brace. In the past, it had been reported that at least 6 hours are necessary to prevent progressive contracture in CP (Tardieu et al., 1988). Still, a study from Sweden reported that only 53% of children with CP regularly wear ankle-foot orthotics for more than 7 hours a day (Wingstrand et al., 2014). Consequently, our current protocol may be considered as a rather extensive approach. In the future, heat sensitive sensors may be implemented and actual wearing times can be documented. This can be used as co-variates when assessing treatment effects (Maas et al., 2014). Third, the effect of knee-ankle-foot orthotics and ankle-foot orthotics may be compared. Notably though, compliance with knee-ankle-foot orthotics was reported to be very limited due to severe discomfort (Maas et al., 2014). Recommending knee-ankle-foot orthotics to target the gastrocnemius (Sees and Miller, 2013) may thus be a theoretical rather than a practical option. Fourth, the orthotics may be instrumentally combined with active movement training. This could be done by incorporating an external device that was able to give feedback to the patients (Zhao et al., 2011). Fifth, concerning the ankle-foot orthotic which was used in this thesis, the incorporated hinge blocked plantarflexion motion completely. This might have led to

learned non-use of the plantarflexors during gait. Therefore, the effectiveness of more dynamic hinge constructions with varying mechanical resistance may be explored.

Apart from that, the use of ultrasound during gait may be capable may be capable of providing valuable information on other muscle pathologies in CP, e.g. for the tibialis anterior in children with foot drop or for the rectus femoris and vastii in children with crouch or stiff knee gait. Wireless ultrasound techniques will perhaps also enable visualization of muscles during uncompromised overground gait.

Also tendons and their properties warrant further attention in CP. As previously described, elongated tendons are a quite typical finding in CP children and not limited to the calf muscles. Remarkably though, the Achilles tendon is typically lengthened during surgery (Dietz et al., 2006) while the patellar-tendon is shortened (Böhm et al., 2017) to improve walking. It appears reasonable that also patella-tendon properties should be explored in more detail in CP. Previous investigations in typically developing children (O'Brien et al., 2010) or in juvenile athletes (Mersmann et al., 2015) focused on the patella-tendon properties by combining ultrasound and dynamometry. This could be also done in CP children. Similar to cellular muscle tissue characteristics, also properties of tendon tissue are likely altered. It had been speculated that increased muscle tone creates persisting tendon strains below injury threshold and leads to a tendinopathic like state in CP children (Gagliano et al., 2013). In their review on tendinopathies in otherwise healthy controls, O'Neill et al. (2015) speculated that muscle weakness and poor neuromuscular co-ordination are related to tendinopathic states. Both of these risk factors are major constrains in CP children. Biopsis of tendon tissue during surgery could be informative.

Eventually, according to a dynamical system driven theory, the abnormal movement patterns in CP may be somewhat optimal for a biomechanically and neurologically limited system (Holt et al., 1996). These movement patterns will persist as long as the stability of the system can be sufficiently 'perturbed'. The author of this thesis believes that the 'perturbations' induced by altered muscle-tendon properties secondary to conservative interventions may often reach a magnitude that is too low to induce an actual change of the movement or gait pattern in CP children. Future research could focus on the interplay between muscle-tendon properties and neural coordination. It should explore concepts such as muscle synergies (Steele et al., 2015; Tang et al., 2015) in more detail. Hao and Chen (2011) and Kim et al. (2013), for example, speculated that the benefits of backward gait training are transmitted via altered neuromuscular control and reorganization of muscle synergies.

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Statutory Declaration
Eidesstattliche Erklärung

I declare that I have authored this thesis independently, that I have not used other than the declared sources / resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

Ich erkläre, dass ich die vorliegende Dissertation selbständig und nur unter Verwendung der angegebenen Hilfsmittel angefertigt habe. Alle Zitate sowie sinngemäße wörtliche Wiedergaben, die anderen Werken entnommen wurden, sind unter Angabe der Quelle kenntlich gemacht. Die Abbildungen, Diagramme und Tabellen sind von mir erstellt, sofern diese nicht als Entlehnung gekennzeichnet sind. Weder diese noch eine andere Arbeit wurde von mir an einer anderen Universität oder Hochschule zum Zwecke der Einleitung eines Promotionsverfahrens vorgelegt.

Prien am Chiemsee, 31.05.2017 _____

Matthias Hösl