CHAPTER 7

GENERAL SUMMARY
AND DISCUSSION
GENERAL SUMMARY

The overall aim of this thesis was to elucidate on mechanisms contributing to hyper-resistance of the triceps surae muscle (TS) in children with spastic cerebral palsy (SCP). During gait, TS hyper-resistance in children with SCP and the associated ankle dorsal flexion hyper-resistance frequently results in abnormal plantar flexed orientations of the foot. Clinical interventions aim to improve gait and prevent progression of abnormal plantar flexion angles by reducing TS hyper-resistance.

To extend knowledge regarding the underlying mechanisms of TS hyper-resistance, we investigated various determinants of TS hyper-resistance in children and adolescents with SCP and in typically developing (TD) children. In this context, we particularly focussed on the morphology of the gastrocnemius medialis muscle (GM), which is part of the TS. The results of this thesis showed that relations between GM morphology and TS hyper-resistance differ between TD and SCP children. Previously, it has been shown in TD children, that structural components constituting the GM muscle-tendon complex (i.e. muscle fibres, aponeuroses and tendons) grow uniformly (Bénard et al., 2011). This indicates that with childhood growth, structural components constituting the GM muscle-tendon complex are scaled, preserving the internal ratios. In Chapter 2, we provided new insights in GM growth in TD adolescent males. We found that longitudinal growth of the GM is mediated by increases in physiological cross-sectional area ($A_{fasc}$) and not by increases in fascicle length ($l_{fasc}$) or tendon length ($l_t$). We hypothesised, that increases in $A_{fasc}$ may have contributed to a decreased ankle dorsal flexion range of motion in the older TD adolescent males. In a similar way, altered GM growth in children and adolescents with SCP may also be related to a reduced ankle dorsal flexion range of motion.

In addition to an altered GM growth, ankle dorsal flexion range of motion in children with SCP may also be influenced by foot deformations. Previous research showed that foot deformations in children with SCP may cause erroneous assessments of TS extensibility when based on foot sole rotations (Huijing et al., 2013). Passive ankle dorsal flexion range of motion (ROM) and TS hyper-resistance are often clinically inferred from assessments focussed on foot sole ROM. Foot sole flexion angles are measured as the angle between the foot sole and the shank (Fig. 1). Clinicians often use the examination of “foot” ROM to infer TS muscle extensibility (Gracies et al., 2010), however, caution is required. In Chapter 4, we showed that during physical examination, foot deformations in both TD and SCP children contribute to foot sole ROM by on average 30%. Based on these findings, we advise clinicians and researcher to take foot deformations into account when evaluating TS hyper-resistance during physical examination in children with SCP, but also in TD children.
To evaluate differences in whole GM morphology in both SCP and TD children, we improved and extended an existing 3-dimensional ultrasound (3DUS) technique (Fry et al., 2004; Bénard et al., 2011) to enable fast reconstructions of 3DUS images of entire muscles by using multiple ultrasound sweeps (Chapter 3). The improved technology now allows for in-depth assessments of muscle volume, physiological cross-sectional area, fascicle length, and tendon length of the m. gastrocnemius medialis (GM) and the m. vastus lateralis muscle (van der Zwaard et al., 2018). In Chapter 5, we showed using the improved 3DUS technique, that GM geometry of TD and SCP children mainly differed in the muscle belly and tendon growth rates. GM muscle volume in children with SCP was smaller compared to that in TD children, particularly in children with a larger body mass (i.e. at an older age). In contrast to TD children, GM muscle volume of children with SCP was only correlated to \( A_{\text{fasc}} \) and not to \( L_{\text{fasc}} \) (Chapter 5, Fig. 4). This finding suggests that muscle growth in children with SCP is not related to increases in fascicle, but to radial increases instead. In contrast to the slower growth rates in muscle volume in children with SCP, the longitudinal increases of tendinous structures exceeded those in TD children. Interestingly, the longitudinal increase in tendinous structures in children with SCP even exceeded the increase in their lower leg length. In children with SCP, but not...
in TD children, extension of the muscle-tendon complex between two standardised externally applied loading condition (GM extensibility) was negatively correlated to both $A_{\text{fasc}}$ and $l_1$. These findings imply that the muscle-tendon complexes of SCP children with a lower $A_{\text{fasc}}$ and a shorter $l_1$ are more compliant towards extension compared to SCP children with higher values of $A_{\text{fasc}}$ and $l_1$. As growth of $A_{\text{fasc}}$ and $l_1$ both contribute to longitudinal GM muscle-tendon complex growth, it is concluded that growth of these components negatively affects GM extensibility in children with SCP. Based on the observation that muscle growth in children with SCP is characterised by hampered longitudinal growth of muscle fascicles, interventions targeting longitudinal fascicle growth and preventing longitudinal tendon growth are likely favourable to improve muscle-tendon complex extensibility.

In Chapter 6, we presented a case study in which we showed short-term improvements in gait of a child with spastic paresis after Botulinum Neurotoxin-A, serial casting and physiotherapy treatment. Short-term gait improvements were mainly explained by a reduction of spasticity and increased foot flexibility rather than the anticipated longitudinal growth related changes of the GM muscle-tendon complex. The outcome of this case study questions the rationale of administering BoNT-A in triceps surae muscles and subsequent serial casting to induce longitudinal fascicle growth.

To conclude, it has been shown that substantial morphological variations characterises children with SCP. The results of this thesis suggest that a few morphological features should be taken into account to improve clinical interventions and to further research that aims to reduce TS hyper-resistance in children with SCP. Assessments described in this thesis are ready to be used in a clinical setting to support clinical decision making, and to evaluate interventions that aim to reduce TS hyper-resistance in children with spastic cerebral paresis.

**Functional limitations related to GM morphology in children with SCP**

The geometry of the GM muscle-tendon complex (morphological characteristics) determines both the active and passive length-force characteristics of the muscle (Woittiez et al., 1983). Several GM morphological characteristics related to TS hyper-resistance have been identified in children with SCP. In Chapter 5, we showed that different conclusions were obtained from group comparisons between children with SCP and TD children as opposed to conclusions derived from individual data of only children with SCP. The muscle-tendon complex consists of the muscle belly and the tendon, each with different characteristics contributing to the length range of active and passive force exertion. In Chapter 5, we showed that muscle volume in children with SCP is smaller compared to in TD children. A smaller GM volume may be due to a low number of sarcomeres arranged in-series, but in case of a pennate muscle also
to a low number of sarcomeres arranged in-parallel. The total number of sarcomeres arranged in-parallel within the GM is reflected by the muscle's physiological cross-sectional area ($A_{fasc}$). On average, there was no significant difference in $A_{fasc}$ between SCP and TD children, because of large inter-individual variations. However, most values of $A_{fasc}$ of children with SCP were outside the 95% confidence interval of the TD children (Chapter 5, Figs. 5 and 6). This suggests that $A_{fasc}$ in children with SCP is lower at similar age and body mass values compared to that of TD children, which is line with results of previous studies (D’Souza et al., 2019; Malaiya et al., 2007). Although we did not examine the force generating capacity of GM, a smaller $A_{fasc}$ indicates a lower force generating capacity (Morse et al., 2008). Because of the GM’s pennate architecture, a smaller $A_{fasc}$ also implies a shorter muscle belly, and therefore a shift of optimum force towards shorter muscle-tendon complex lengths (cf. Swatland, 1980; Bénard et al., 2011; Heslinga & Huijing, 1992). At a similar muscle volume, $A_{fasc}$ is larger in SCP children compared to TD children (who have a larger $l_{fasc}$), particularly with large muscle volumes (Chapter 5, Fig. 4B). In addition, smaller values of $A_{fasc}$ imply a lower number of parallel-arranged sarcomeres and titin proteins, and hence a lower passive resistance to extension (i.e. more compliant muscle-tendon complex). In children with SCP, we found that $A_{fasc}$ was negatively correlated with muscle-tendon complex extensibility (Chapter 5), indicating that SCP children with a smaller $A_{fasc}$ had a lower resistance to acute elongations of the muscle. We expected to find similar effects in TD children, however, in these children $A_{fasc}$ correlated positively with muscle-tendon complex extensibility. This finding may be explained by simultaneous increases in $l_{fasc}$ and $A_{fasc}$ in TD children (Fig. 2), in which positive effects of having longer fascicles on the extensibility may have outweighed the negative effects of a larger $A_{fasc}$.

Regarding the muscle fascicle length ($l_{fasc}$), we showed that $l_{fasc}$ (i.e. bundle of muscle fibres) was shorter in children with SCP compared to TD children. Shorter fascicle lengths in children with SCP are possibly related to a lower number of sarcomeres in-series, which implies a shift of the passive and active length-force curve of the muscle-tendon complex towards shorter lengths (Williams & Goldspink, 1978). Such a shift implicates an increase in both passive and active forces at shorter lengths. Optimum muscle-tendon complex length moves to shorter muscle-tendon complex lengths, or in terms of ankle joint angles, optimum GM length shifts to more plantar flexion angles. Passive resistance to dorsal flexion is increased for a given dorsal flexion angle, resulting in an increased stiffness at lower dorsal flexion ankle joint angles. In addition, shorter $l_{fasc}$ will potentially promote higher elongation rates in the muscle spindles, and as such enhance the velocity dependent stretch reflex in children with SCP (cf. Gracies, 2005; Tardieu et al., 1982; Van Dyke et al., 2014). Taken together, aberrant short GM fascicles in children with SCP affect both the active and passive ankle dorsal flexion ROM and may also increase spastic reflexes.
In contrast to our expectations, we did not find any difference in muscle-tendon complex length between SCP and TD children. However, at standardised net ankle moments, tendon structures were longer in children with SCP compared to TD children (Gao et al., 2011; Wren et al., 2010; Barber et al., 2012). Generally, a longer tendon slack length shifts the optimum length of the muscle-tendon complex to a longer length and increases the length range of active force exertion, when compared to a muscle-tendon complex with a shorter tendon at slack length. However, because there was no difference in GM muscle-tendon complex length between SCP and TD children, a longer tendon may compensate for the short fascicles in children with SCP. If the tendon properties in SCP and TD children would be similar, the larger tendon length in SCP implies a reduced tendon stiffness (i.e. increased compliance). However, our results showed the opposite effect for SCP children. The length of tendinous structures (aponeurosis + tendon length) was negatively correlated with muscle-tendon complex extensibility, indicating that children with longer tendinous structures have a reduced muscle-tendon complex extensibility. As we did not expect the tendinous structures to lengthen acutely, and - if any - a very slightly, it may explain the reduced extensibility. In addition, reduced extensibility may also be explained by shorter and presumably less extensible muscles fascicles accompanying the longer tendinous structures (Fig. 2A).

In summary, morphology of the GM in children with SCP is characterised by a smaller muscle belly volume, shorter fascicles with a smaller $A_{\text{fasc}}$ and longer tendinous structures compared to those found in TD children. Particularly in children with SCP with longer tendinous structure ($l_{\text{art}}$), $l_{\text{fasc}}$ is found to be shorter compared TD (Fig. 2A). While $A_{\text{fasc}}$ is on average lower in children with SCP, values of $A_{\text{fasc}}$ are higher in SCP children with short $l_{\text{fasc}}$ compared to that in TD children (Fig. 2C). Shorter fascicles and lower values of $A_{\text{fasc}}$ imply a shift of the active length-force curve towards shorter length. A lower value of $A_{\text{fasc}}$ results in lower muscle force generating capacity. Shorter $l_{\text{fasc}}$ and lower values for $A_{\text{fasc}}$ have opposing effects on the passive extensibility of the muscle belly. However, in our cross-sectional group of children with SCP (GMFCS I-III), $A_{\text{fasc}}$ was negatively associated with $l_{\text{fasc}}$ (Fig. 2C). This implies that muscles of children with SCP are comprised of relatively large $A_{\text{fasc}}$ and short $l_{\text{fasc}}$, likely in part explaining TS hyper-resistance and associated ankle dorsal flexion hyper-resistance.
Figure 2. Regression analysis of individual geometrical characteristics of m. gastrocnemius medialis related to tendinous structure length and fascicle length at 0 Nm ankle condition. A) Plot relating individual $l_{fasc}$ (fascicle length) to $l_{a+t}$ (tendinous structure (aponeurosis and tendon) length). B) Plot relating individual $A_{fasc}$ to $l_{a+t}$. C) Plot relation individual $A_{fasc}$ to $l_{fasc}$. Indicating, that while in TD longer fascicle are related to a larger physiological cross-sectional area, in SCP longer fascicles are related to a smaller physiological cross-sectional area. The shaded area represents the 95% confidence interval for the TD group. No regression lines are drawn and no coefficient of correlation is indicated for data not showing a significant coefficient of correlation.

Possible explanations for aberrant GM morphology in children with SCP

During growth, the TS muscle-tendon complex needs to grow longitudinally to preserve the range of motion of the ankle joints. In the preceding chapters, we have shown that the ability or inability of the foot, tendon and/or muscle belly to adapt, likely influences ankle hyper-resistance and triceps-surae extensibility in children with SCP. This inability of the fascicles to increase appeared as the most apparent growth impairment of children with SCP. Several mechanisms may play a role in the hampered longitudinal fascicle growth. Either the mechanical stimulus for growth is attenuated, the growth potential of the muscle is reduced, or both are impaired in children with SCP compared to that in TD. Based on our data and findings of others we will discuss how both scenarios may explain aberrant GM adaptations in children with SCP.

Is the mechanical stimulus for longitudinal fascicle growth attenuated in children with SCP?

Although the mechanisms underlying regulations of the number of sarcomeres are far from understood in healthy muscles (Huijing & Jaspers, 2005), it is generally acknowledged that addition of sarcomeres in-series requires a mechanical stimulus (Huijing & Jaspers, 2005). In children with SCP, spasticity and a sedentary lifestyle with increases in both $A_{fasc}$ and lengths of tendinous structures may precede or resolve the mechanical stimulus required for longitudinal muscle fascicle growth.
Spasticity results in involuntary spastic reflexes in response to muscle fibre lengthening. Movements that extend these sensitive muscles are likely avoided or result in involuntary contractions. The muscle-tendon complex, but more specifically the muscle fibres are kept short, resulting in a reduced strain on the muscle fibres. This theory is arguable, as others have found increased sarcomere lengths in SCP compared to TD children, showing that muscle fibres in SCP children are strained to a larger extent compared to TD children (Mathewson et al., 2014, 2015). However, these comparisons were made in a condition that requires higher externally applied moments in SCP children compared to that in TD children (i.e. 0 degrees foot sole angle, i.e. perpendicular to the shank), likely related to TS hyper-resistance in children with SCP (c.f. Bénard et al., 2010). In addition, children with SCP tend to have a more sedentary lifestyle (Longmuir & Bar-Or, 2000), which may result in less mechanical loading and less contractile activity compared to active TD children, reducing the stimulus to add sarcomeres in-series. In addition, longitudinal fascicle growth seems to require contractile activity (cf. Dyke et al., 2012; Jaspers et al., 2004), hampered longitudinal muscle fascicle growth in children with SCP may as such also be related to both altered contractile and mechanical stimuli.

The human TS consists of three pennate muscles, in which not only fascicle lengths and tendon length, but also the physiological cross-sectional area contributes to the optimum length of the muscle-tendon complexes. In Chapter 2, we showed that in adolescent males, increases in $A_{\text{fasc}}$ of the GM mediates increases in muscle-tendon complex length because of the pennate architecture of this muscle. We hypothesised that during adolescence in both SCP and TD adolescents increases in $A_{\text{fasc}}$ will compensate for the lack of longitudinal fascicle growth. Shortening immobilization studies have shown that beside shortening adaptations of muscle fibre and tendinous structures, atrophy of the pennate fibre also results in shorter muscle belly lengths (Heslinga et al., 1995). In our group of children with SCP, $A_{\text{fasc}}$ was smaller than in TD children, particularly for children with a smaller body mass (see Chapter 5). However, the increases in $A_{\text{fasc}}$ with age were similar between groups, which indicates that children with SCP demonstrate normal increases of $A_{\text{fasc}}$ (normal trophy or hypertrophy) during childhood (Fig. 2B). Note that these increases in $A_{\text{fasc}}$ in children with SCP also contribute to increases in muscle belly length compensating for the lack of $L_{\text{fasc}}$ growth.

Children with SCP tend to have a more sedentary lifestyle compared to TD children (Nooijen et al., 2014), which may consequently result in a reduced mechanical stimulus for muscle growth. However, such an explanation may not be true for every individual SCP subject, as some children with SCP had a very large $A_{\text{fasc}}$ (larger than some TD children), which indicates a strong radial trophic potential despite of a reduced longitudinal increase in $L_{\text{fasc}}$ (Fig. 2C). Possibly, this larger $A_{\text{fasc}}$ is a result of a higher number of fibres at birth in these SCP children compared to other SCP...
children, due to a different timing of the brain lesion (Gough & Shortland, 2012). Taken together, overall muscle bellies of children with SCP remain short and small compared to those in TD children. Further research is needed to explain why muscle bellies of children with SCP are characterised by large variations in $A_{\text{fasc}}$ and only limited variations in $l_{\text{fasc}}$. More research is required to further our knowledge of conditions, mechanisms and interactions between growth mechanisms resulting in longitudinal fascicle growth.

The results presented in Chapter 5 suggest that the longitudinal growth of tendinous structures was not impaired in children with SCP, but instead was enhanced compared to that of TD children. The suggested enhanced tendinous growth instead of longitudinal muscle fascicle growth is in line with the effects of lengthening immobilization experiments in young animals (Tardieu et al., 1977; Williams & Goldspink, 1973). Results of these immobilization studies in young animals show that changes in muscle-tendon complex length are mainly a result of an increase in tendon length. Lengthening immobilization of innervated muscle and denervated soleus muscles of young and adult rabbits, has shown that tendon growth in the innervated muscle of young animals was enhanced (Blanchard et al., 1985). In adult animals, however, increases in muscle-tendon complex were mediated only by changes in sarcomere number, with no changes in tendon length. The results of this thesis together with the data on rodents suggest that the tendon length in children and young animals increases in length to reduce straining (Wren, 2003). In an innervated muscle, muscle contractions may enhance the straining of the tendon, and increase the stimulus for longitudinal tendon growth (Wren et al., 2010). In addition, enhanced tendon adaptations may protect sarcomeres from peak stresses during growth (cf. (Maas & Finni, 2018)). Taken together, in children with SCP spasticity induced tendon straining may enhance tendon growth, which attenuates the stimulus for longitudinal fascicle growth.

Foot flexibility may also contribute to the attenuation of the stimulus for longitudinal fascicle growth. In both children with SCP and TD children, foot flexibility reduces the lengthening of the muscle-tendon complex relative to the degree of dorsal flexion ROM of the foot. During gait, foot flexibility in TD children at initial contact in gait is resolved during mid-stance by muscles activation (e.g. m. tibialis posterior and m. peroneus brevis) rigidifying the hind foot to provide a ‘rigid’ lever arm for efficient push-off (cf. Semple et al., 2009). In children with SCP, possibly due to impaired selectivity, weakness, joint laxity, and/or bony deformation, foot flexibility results in functional limitations (Theologis, 2013). Based on the results of Chapter 4 we cannot conclude that the effects of foot flexibility are increased in children with SCP compared to that of TD children. In both children with SCP and TD children, foot deformations significantly decreased TS extensibility upon dorsiflexion of the foot sole. Therefore, the TS muscle-tendon complexes of children with relatively more
foot flexibility are likely subjected to less straining of the muscle-tendon complex compared to children with less foot flexibility and as such contribute to a reduced stimulus for longitudinal fascicle growth.

All together, the results presented in this thesis indicate that the mechanical stimulus for longitudinal fascicle growth may be reduced in SCP due to spasticity, hypertrophy, tendon growth, or effects of foot deformation. Based on our results we can only speculate on which of these factors should be addressed to enhance the stimulus for longitudinal fascicle growth in children with SCP. Further research is required to investigate how management of spasticity, hypertrophy, enhanced tendon growth and foot deformation improves longitudinal fascicle growth in children with SCP.

**Is the growth potential of skeletal muscle intrinsically impaired in children with SCP?**

Increasing evidence suggests that the growth potential may be intrinsically impaired in children with SCP (see below). In general, the muscle belly grows (trophies) when the rate of synthesis of contractile proteins exceeds the rate of protein degradation. Mechanotransduction is the phenomena in which cells or nuclei sense mechanical stimuli affecting the rate of protein synthesis (Goldspink, 1999; Huijing & Jaspers, 2005). Muscles are composed of multinucleated muscle cells (i.e. muscle fibre) and extracellular matrixes (ECM). The growth potential of a muscle in children with SCP can be intrinsically impaired by several factors involved in muscle protein synthesis and degradation:

1) **Number of myonuclei**

   During muscle growth, the number of myonuclei within muscle fibres increase proportionally with the increase in muscle fibre size (White et al., 2010; Meer et al., 2011). Satellite cell (SC) mediated myonuclear growth, increases the myonuclear content and the amount of DNA available for transcription. The myonuclear density within a muscle fibre remains constant with proportional increases in muscle fibre size and the number of myonuclei, allowing sufficient template for transcription from DNA into mRNA to accommodate the radial and longitudinal addition of sarcomeres. Compared to TD children, a lower number of satellite cells per muscle fibre (on average 40% of that in TD children) has been reported in hamstrings muscle biopsies of children with SCP (GMFCS I-V) (Smith et al., 2013; Dayanidhi et al., 2015). A reduced population of satellite cells results in a reduced potential to increase the myonuclear content. Surprisingly, length immobilisation in a genetically modified mouse with a lower satellite cell (SC) concentration showed normal addition of sarcomeres in-series, but only radial growth was affected (Kinney et al., 2017). Whether the low number of SCs and a reduced inability of these SCs to proliferate...
is an intrinsic property of the neurological disorder (SCP) is unclear and remains to be determined. Note that the lower number of satellite cells in muscles of children with SCP may likewise be the result of a lower mechanical stimulation of the muscle fibres (as discussed above) (Boers et al., 2018).

2) **Regulation of the rate of transcription & translation**
The rate at which available DNA is transcribed and translated into proteins is determined via several steps: 1) The first step is gene transcription during which DNA is copied into strands of RNA which implicates. RNA polymerase makes copies (mRNA) of specific segments of the DNA required for protein synthesis. 2) Next, ribosomes translate the mRNA strand into a peptide. The rate of mRNA translation depends on the amount of mRNA, ribosomes and amino acids and the initiation and elongation factors that regulate the rate at which ribosomes move along the mRNA strand (van Wessel et al., 2010). Recently, it has been shown that in children with SCP aged 15 years, transcriptional activity of ribosomal DNA genes was reduced compared to that in TD children (Von Walden et al., 2018). This implies that the lower availability of ribosomal RNA reduces the potential to translate mRNA into proteins. In addition, this study also showed that expression levels of transcription factors involved in ribosomal biogenesis were reduced in children with SCP. Formerly, it has been shown that muscle hypertrophy is preceded by ribosomal biogenesis (Von Walden et al., 2018; Nader, 2014). Reduced ribosomal biogenesis, leads to a reduced number of ribosomes, which may reduce the rate of protein synthesis in children with SCP.

3) **Factors involved in protein synthesis and protein degradation.**
The rate of proliferation and differentiation of satellite cells and the transcription and translation of muscle proteins is orchestrated by growth factors and cytokines. Growth factors (e.g. IGF-1 and MSTN) stimulate or inhibit signalling pathways resulting in changes in the rate of protein synthesis or degradation. IGF-1 plays an important role in all phases of satellite cell myogenesis (Chakravarthy et al., 2000; Allen & Boxhorn, 1989), stimulating the rate of protein synthesis and inhibiting the rate of protein degradation via the Akt/mTOR in animal experiments. In children with SCP, serum levels of IGF-1 have been shown to be lower than in TD children of the same age (Ali et al., 2007). The major source for IGF-1 in the circulation is the liver, in particular during growth, and therefore the low IGF-1 serum levels in children with SCP could be a sign that growth is reduced in these children. In contrast to lower serum levels of IGF-1, higher expression levels of IGF-1 mRNA transcripts have been reported in the hamstring muscle of children with SCP (Smith et al., 2012). At present, one can only speculate what the net effect of IGF-1 on muscle growth is. More research is required to figure out what the local effects of increased IGF-1
expression are on myogenesis and sarcomerogenesis. In contrast to growth factors that stimulate muscle growth, other factors (e.g. myostatin (MSTN) and Interleukin-6 (IL-6)) inhibit protein synthesis and stimulate protein degradation, resulting in reduced muscle growth or even atrophy. Muscles deficient for MSTN expression tend to be larger, suggesting that MSTN inhibits protein synthesis and stimulates protein degradation (Hoogaars & Jaspers, 2018). MSTN mRNA expression levels in the m. biceps brachii of SCP children have been shown to be elevated by 2.3-fold compared to TD children (Von Walden et al., 2018). Elevated expression levels of MSTN that negatively regulates muscle mass potentially explains reduced growth in children with SCP. IL-6 is a cytokine that is secreted in response to increased mechanical loading (Juffer et al., 2014). Increased local concentrations of IL-6 slows down protein synthesis and increases protein breakdown, resulting in reduced myogenesis or atrophy (Haddad et al., 2005). In addition to affecting the rate of protein synthesis, IL-6 activates satellite cell proliferation and differentiation, increasing the myonuclear density and thereby the potential for increases in muscle mass (see for review Bakker & Jaspers, 2015). Cytokine gene expression levels for IL-6 were found elevated in children with SCP as mRNA levels of IL-6 were 2.1-fold higher in skeletal muscles of children with SCP compared to those in TD (Von Walden et al., 2018). Further investigations are warranted to study whether factors regulating growth are impaired in SCP children because of an attenuated mechanical stimulus or due to intrinsically impaired growth mechanisms.

Are mechanisms related to longitudinal tendinous growth enhanced in children with SCP?

In Chapter 5, we found that tendon growth is increased in children with SCP compared to that in TD. Similar to others (Gagliano, 2013), we speculated that spasticity may have contributed to enhanced longitudinal tendon growth. Several factors are involved in tendon growth (see for review (Magnusson & Kjaer, 2019). Either the mechanical stimulus, or the growth potential for longitudinal tendon growth is enhanced in children with SCP. In contrast to adults, in children, the tendon cores are still metabolically active until adulthood is reached (Heinemeier et al., 2013), potentially explaining why longitudinal adaptations of tendon are possible in children and not so much in adults (Wren, 2003; Blanchard et al., 1985). Tendon cores in adults are mostly metabolically inactive, whereas adaptations in adult tendon predominantly occur in the outmost layers of the tendon (i.e. increasing the cross-sectional area) (Gumucio et al., 2014). Tendinous structures predominantly consist of extracellular matrixes (ECM), composed of collagen fibrils arranged in-parallel (especially collagen type 1 (Col1a)) capable of withstanding strong tensile forces. In addition to ECM, tendinous structures comprises tenocytes, proteoglycans and water. Several factors are involved in growth and maintenance of
the ECM. Once such factor is the Transforming Growth Factor β1 (TGF-β1), TGF-β1 signaling increases in response to resistance training, resulting in increases in Col1a synthesis (Gumucio et al., 2015; Heinemeier et al., 2003). In children with SCP, both systemic TGF-β1 concentrations and local expression of TGF-β1 (tendon biopsies) are higher compared to TD children (Pingel et al., 2019; Von Walden et al., 2018). In addition, mRNA expression of Col1a was shown to be elevated in samples of hamstrings tendons of quadriplegic SCP children compared to that of TD (Gagliano, 2013). Taken together, in addition to spasticity-induced increases in the mechanical stimulus for growth, factors regulating tendon growth were found to be elevated in children with SCP.

**Future research**

Based on the results of this thesis, we have extended our knowledge regarding the mechanisms contributing to TS hyper-resistance. Given the lack of effective treatments to alter muscle properties in children with SCP, more research in this field is urgently required. Reference databases of morphological characteristics of TD children and children with SCP with growth and in response to interventions are required to allow for context-specific interpretation of individual data. The described comprehensive assessment provides relevant information to identify aberrant morphological characteristics. Besides identification of morphological characteristics in children with SCP and optimizing their interventions, more research is needed to further unravel factors responsible for the development of these aberrant morphological characteristics. More specifically, the following research questions require further investigations.

1) **What mechanisms are responsible for hampered longitudinal fascicle growth?** Animal models for spasticity and/or biopsies of very young children with and without SCP are required to obtain more insight in whether satellite cell dysfunction is an intrinsic feature in children with SCP or whether it is an effect of the reduced muscle activity and mechanical loading.

2) **What mechanisms are responsible for enhanced tendon growth?** Research is warranted to investigate whether TGF-β1 serum levels are increased in children with SCP as a result of neural disturbances (Dobolyi et al., 2012), because of increases in spasticity-induced mechanical loading, or both. And can we use TGF-B1 inhibitors to reduce synthesis of collagen in muscles and tendons of children with SCP? (Potter et al., 2016; Booth et al., 2001)
3) Can training interventions reduce the imbalance between muscle and tendon growth in children with SCP? Recent studies have shown that resistance exercises improve function in children and adults with SCP (Kirk et al., 2016; Willerslev-Olsen et al., 2014). In addition, a systematic review has shown that in children and adolescents with SCP of strength training induces muscle hypertrophy (Gillett et al., 2016). These results indicate that it may be favourable for SCP children to participate in exercise training programs that stimulate muscle hypertrophy and possibly also the addition of sarcomeres in-series.

Clinical implications

Comprehensive assessment of morphological characteristics of the GM provides relevant information to identify and monitor aberrant morphological characteristics in children with SCP. In addition, results presented in this thesis suggest that interventions that aim to reduce TS hyper-resistance should focus on increasing muscle volume by stimulating longitudinal fascicle growth, while preventing enhanced tendon growth. Although the exact form and composition of such interventions is currently unknown, clinical assessments of morphological characteristics allow clinicians to optimise existing interventions to achieve their intended goals.
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