CHAPTER 1

GENERAL INTRODUCTION
INTRODUCTION

Children with cerebral palsy (CP) often develop an impaired gait, which is mainly characterized by reduced ankle dorsiflexion range of motion and limited push-off power. When left untreated, gait impairments will likely progress with age. CP is the most common cause of childhood movement impairments, affecting two cases in every 1000 children born (Yeaggin-Allsopp et al., 2008; Wichers et al., 2001; Himmelmann & Uvebrant, 2018). CP is mainly characterized by movement and posture disorders related to non-progressive disturbances in the developing fetal or infant brain (Rosenbaum et al., 2007). The gross motor functioning levels in children with CP vary greatly and are classified using the Gross Motor Classifications System (GMFCS). Children with GMFCS levels I-II are considered mildly impaired and can walk without walking aids. Children with GMFCS level III require walking aids. Children with GMFCS levels IV-V are unable to walk independently with or without walking aids and are wheelchair bound. Spastic Cerebral Palsy (SCP) is the most common subtype of the motor disorders in CP (around 80% of children with CP) (Goldsmith et al., 2016; Himmelmann & Uvebrant, 2018). Symptoms of children with SCP are characterized by a disinhibition of the neuromuscular reflex arc through the descending neural pathways. Such disinhibition of this reflex results in excessive muscle activation in response to stretch, in particular to fast elongations (Sheean, 2002). Besides spasticity, involuntary muscle background activations and soft-tissue changes (e.g. muscle adaptations) result in functional limitations such as an reduced ankle dorsiflexion range of motion (van den Noort et al., 2017). Therefore we aim to extend our knowledge regarding mechanisms contributing to ankle joint hyper-resistance in children with SCP.

Hyper-resistance of muscles may in part explain joint hyper-resistance, limiting the active and passive range of joint motions. Understanding underlying mechanisms of increased joint and muscle hyper-resistance, including soft-tissue adaptations are important because joint hyper-resistance increases with age when neglected (Kerr Graham & Selber, 2003; Davids et al., 2014). In children with SCP, increases in ankle joint hyper-resistance results in ‘equines gait’ characterised by increased plantar flexion angles during the stance phase in gait (Goldstein & Harper, 2007). In typical gait, triceps surae muscles (TS) are predominantly responsible for: 1) generating sufficient plantar flexion moment over an adequate ankle range of motion to control the forward progression of the centre of mass during mid-stance), 2) providing lifting power to decelerate the falling centre of mass (in late stance), 3) generating propulsive power driving the leg into the swing phase (i.e. at pre-swing) (Zelik & Adamczyk, 2016), and (4) allow dorsal flexion of the foot during the swing phase by allowing extension of the TS muscles. In children with SCP, ankle joint hyper-resistance results in an ‘equines gait’ characterized by a predominantly plantar-
flexed orientation of the foot during stance, causing: 1) a limited base of support, 2) insufficient propulsion, and 3) hindering initial contact with heel strike. In this thesis we focus on measurements and determinants of triceps surae muscle (TS) hyper-resistance in ambulant children and adolescents with SCP (i.e. GMFCS-I-III).

Interventions to reduce TS hyper-resistance

In ambulant children with SCP, clinicians frequently aim to improve and prevent progression of plantar flexion of the foot during stance, by interventions directed to reduce TS hyper-resistance. This is important in the growing child where the TS muscles need to adapt to match the growth of the lower leg. Depending on the suspected mechanisms contributing to TS hyper-resistance, different interventions may be applied to reduce TS hyper-resistance (Koman et al., 2004). Typically interventions start at a young age, aiming to increase or maintain joint range of motion. Such interventions often include weekly physiotherapy sessions stretching the muscles (from age ≈ 1 year), wearing ankle foot orthoses (AFO’s) (from age ≈ 2 year). When effects of such interventions are insufficient because of spasticity, treatment might be augmented by medical interventions that aim to manage neural disturbances via efferent denervation (e.g. Bontulinum NeuroToxin-A injections combined with progressive casting) and/or via afferent denervation, (e.g. Selective Dorsal Rhizotomy). When such treatments remain insufficient, surgical lengthening of the TS is considered to increase the TS muscle-tendon complex length to reduce TS hyper-resistance (Perry et al., 1974). Despite early treatment initiation and a decreased necessity (or use) of correcting orthopaedic surgery, ankle dorsiflexion range of motion (ROM) in children with GMFCS II-III remains reduced by about 30 degrees (Nordmark et al., 2009; Tardieu et al., 1982). Although most interventions show short-term improvements in ankle dorsiflexion ROM (Nieuwenhuys et al., 2016), recurrences on the long-term are frequently reported (Fry et al., 2007; Tedroff et al.; Moore et al., 2008). Improving patient-specific clinical decision making and evaluation of treatment outcome requires comprehensive insight into mechanisms underlying ankle joint hyper-resistance and TS hyper-resistance in children with SCP. The aim of this thesis is to extend knowledge regarding mechanisms contributing to TS hyper-resistance in children with SCP and their effects on ankle joint hyper-resistance.

Determinants of length-force characteristics of the muscle-tendon complex

To extend knowledge of mechanisms contributing to TS hyper-resistance we need to evaluate how components constituting the TS muscle-tendon complex affect the passive and active length-force characteristics. The triceps surae muscle group (TS) consists of tri (three) muscles, the m. gastrocnemius medialis and lateralis and the m. soleus. In this thesis our main focus is on the m. gastrocnemius medialis (GM)
because of its functional significance in locomotion and because it is presumably morphologically altered in children with SCP (van den Noort et al., 2017). The GM originates from the medial femur condyle and inserts on the calcaneus, it is a bi-articular muscle affecting both the knee and ankle joints.

The muscle-tendon complex of the GM consists of a muscle belly arranged in series with a tendon between the origin and insertion. In response to neural excitation, the muscle belly contracts and pulls the origin and insertion towards each other. The force generated by the muscle-tendon complex generates a flexion moment at the knee joint and a plantarflexion moment at the ankle. The muscle-tendon complex is capable of resisting elongation both actively and passively, as such it can resist extension moments at the knee joint and dorsal flexion moments at the ankle joint. The extent to which a muscle-tendon complex is capable of generating the active and passive force are defined by the active and passive length-force characteristics of each muscle. Variations in structural and physiological properties of components constituting the muscle-tendon influence the length-force characteristics of a muscle.

The muscle belly of the GM consists of striated muscle fibres arranged in a certain orientation (pennation angle) with respect to the line of pull of the muscle belly. Muscle fibres are composed of myofibrils, which are constructed by the smallest contractile unit, the sarcomere. Sarcomeres comprise contractile proteins actin and myosin, arranged in parallel with the giant protein titin. The active force, generated by a single sarcomere depends on the amount of overlap and interaction between the actin and myosin proteins (i.e. sarcomere length) (Huxley & Hanson, 1954). Sarcomere maximum force is generated at its optimum length, which is the length at which actin and myosin have optimal overlap. The amount of passively generated force by the sarcomeres is mainly related to the length of titin (Wang et al., 1991).

Multiple sarcomeres arranged in series make up a long myofibril. Multiple myofibrils arranged in parallel are enclosed by the sarcolemma (i.e. the cell membrane) and together constitute a muscle fibre. Optimum muscle fibre force is proportional with the number of sarcomeres (i.e. myofibrils) arranged in parallel within the muscle fibre. Optimum muscle fibre length and length range of active fore exertion are proportional with the number of sarcomeres arranged in series. With more sarcomeres arranged in series (i.e. long fibres, low titin length changes) passive resistance to elongation is low (compliant). With more sarcomeres arranged in parallel (i.e. thick fibres with more titin proteins are arranged in parallel) passive resistance to extension is higher. This can be explained with the analogy of stretching a rubber band, imagine the effort of stretching one rubber band. Next, imagine stretching two similar rubber band arranged in parallel. Stretching the two parallel arranged bands the same distance doubles the effort. By increasing the amount of parallel arranged material, the resistance to extension increases.
Muscle fibres of the GM are oriented at an angle with respect to the line of pull (pennation angle), such orientations influences the length-force characteristics of the muscle-tendon complex. In a muscle in which the fibres are oriented parallel with respect to the line of pull (parallel-fibred muscles), length changes and force generated by the summed muscle fibres contribute fully to the length range of active force exertion and the force generating capacity of the muscle belly. However, muscle fibres in most muscles-tendon complexes (e.g. the GM) are oriented at an angle with respect to the line of pull (pennate-fibred muscles). Because of the pennation angle, optimum muscle force is smaller than the optimum force of the summed fibres (i.e. summed sarcomeres arranged in parallel). Similarly, when the pennation angle does not change during contraction, larger pennation angles decrease the length range of active force exertion. Such reduced length of active force exertion are compensated by rotations of muscle fibres during lengthening and shortening of the muscle belly, increasing the length range. A pennate-fibred muscle built up by the same amount of contractile material compared to a parallel-fibred muscle can have more parallel arranged sarcomeres, thus have a higher force generating capacity. In addition, increases in parallel arranged sarcomeres in a pennate muscle contributes to increases in muscle-tendon length.

The tendon consists of parallel arranged collagen fibres that are constructed of fibrils made up of multiple tropocollagen molecules. Besides providing a force pathway between the origin and insertion (myotendinous pathway), it also increases optimum length and extensibility compared to a muscle-tendon complex without tendinous components. Because of the elastic properties of the tendon, the length range of active and passive force exertion increases with longer tendons (if material properties and thickness have not changed). In summary, dependent on tendon slack length, thickness and material properties, the tendon affects both the active and passive length-force characteristics of the muscle-tendon complex. In case of a pennate muscle, the aponeurosis contributes to the serial elastic component as the aponeurosis and tendon together are the tendinous structures.

Besides the above-mentioned structures contributing to the GM length-force characteristics, other structures such as the connective tissues between muscle and tendon fibres may provide alternative force pathways affecting the GM length-force characteristics of the muscle-tendon between their origin and insertion. Muscle fibres are surrounded by non-contractile connective tissues: 1) endomysium surrounds individual muscle fibres, 2) perimysium surrounds bundles of muscle fibres (i.e. muscle fascicle), and 3) epimysium surrounding the muscle belly. The quantity and quality of the connective tissues influence the active and passive length-force characteristics of the muscle-tendon complex in vivo (Alnaqeeb et al., 1984).
In summary, various components constituting the GM contribute to the active and passive length-force characteristics of the GM. Secondary to neural disturbances of the GM in children with SCP, aberrant adaptations of components constituting the GM are believed to influence the GM length-force characteristics and as such TS hyper-resistance (van den Noort et al., 2017). To gain further insight into aberrant GM adaptations in children with SCP we first need to understand how the GM adapts in typically developing (TD) children.

**Gastrocnemius medialis muscle growth in TD children and adolescents**

Skeletal muscles are highly adaptable organs, able to adapt in size and force generating capacity to meet changes in functional demands. With growth, skeletal muscles need to increase in length to adapt to longer bones (Haines, 1932), and increase the force generating capacity to accommodate increases in functional demands related to increases in body size. Most of our knowledge of human anatomy is based on cadaveric studies. Thorough understanding of how the aforementioned tissues change and influence the length-force characteristics of muscle-tendon complexes are still mostly lacking in humans. In TD children aged 5-12 years, it has been shown that GM length increases by uniform scaling of both fascicle length and increases of the physiological cross-sectional area (Bénard et al., 2011). However, the mechanisms regulating growth during adolescence may differ from those regulating growth during childhood, as increases in sex hormone play an important role in the process of organ maturation (Round, 1999).

**Gastrocnemius medialis muscle growth in SCP children**

In SCP children, besides neural disturbances, hyper-resistance may also be related to aberrant morphological adaptations of the GM compared to that in TD children (van den Noort et al., 2017). GM muscle morphology in children with SCP differs from that of TD children (Moreau et al., 2010; Noble et al., 2014). Muscle bellies of children with SCP are typically smaller and shorter compared to those in TD children, implying a potential growth defect (Noble et al., 2014; Barber et al., 2012; Tardieu et al., 1979). In children with SCP normalised GM volume has been shown to be 22% smaller than that in TD children (Barber et al., 2011b, 2016). Deficits in muscle volume may be related to shorter muscle fascicle lengths (Mohagheghi et al., 2008; Barber et al., 2011A), a smaller physiological cross-sectional area ($A_{fasc}$) (Malaiya et al., 2007; Lieber et al., 2003; Barber et al., 2011A), or both. Since muscle volume strongly correlates to muscle power (Moreau et al., 2010), muscle volume deficits in children with SCP will probably affect its power generating capacity, particularly during the push-off phase. In addition, variations in GM geometry affect both active and passive length-force characteristics.
Shorter muscle fascicles, as seen in the gastrocnemii of children with SCP, likely result in a shift of optimum length towards shorter muscle-tendon complex lengths and in increased passive resistance to extension. Ambiguity in the literature exists whether fascicle lengths ($\ell_{\text{fasc}}$) are different in children with SCP compared to TD (Malaiya et al., 2007). A smaller $A_{\text{fasc}}$ in SCP children results in lower maximal muscle force because of less parallel arranged sarcomeres. In addition, a smaller $A_{\text{fasc}}$ also likely results in a shift of optimum length towards shorter muscle-tendon complex lengths and reduces passive resistance to extension. For the pennate GM, $\ell_{\text{fasc}}$ and $A_{\text{fasc}}$ largely determine the active and passive slack length and the length range of active force exertion. Shorter $\ell_{\text{fasc}}$ or a smaller $A_{\text{fasc}}$ influence muscle-tendon complex hyper-resistance in children with SCP.

In contrast to shorter $\ell_{\text{fasc}}$ and lower values of $A_{\text{fasc}}$, tendon lengths ($l_{t}$) are often found to be longer in SCP children compared to those in TD children (e.g. Gao et al., 2011). Increases in $l_{t}$ are expected to result in a shift of the length-force characteristics of the muscle-tendon complex towards longer lengths, and a decrease in the resistance to extension (Kalkman et al., 2018). To understand how adaptations of the GM influence TS hyper-resistance in children with SCP, we need a method to assess GM morphology and TS hyper-resistance.

**Measurements of triceps surae hyper-resistance**

In clinical practice and in biomechanics in general (Honert & Zelik, 2016; Bobbert et al., 1986), assessment of TS hyper-resistance are commonly inferred from the assessment of foot sole range of motion (Gracies et al., 2010) and interpreted as ankle joint range of motion. Note that such an interpretation, mostly implicitly, assumes a rigid foot (Tardieu et al., 1976). Over the last 40 years, several studies have shown, in violation of the indicated assumption, that the foot deforms upon application of external forces (Iwanuma et al., 2011; Tardieu et al., 1976; Wrbaškić & Dowling, 2007; Tardieu et al., 1977; Carlson et al., 2000; Huijing et al., 2013; Bruening et al., 2012). In SCP children, such foot flexibility was found to be more prominent compared to that in TD children (Huijing et al., 2013; Tardieu et al., 1977) (hence the characteristic mid-foot break in gait). In a recent study by Huijing et al. (2013), X-ray images of the ankle joint were made. Fig. 1 shows that in a child with SCP, of 15 degrees of total dorsal flexion rotation of the foot, only 7 degrees of rotation correspond to true ankle dorsal flexion. Such differences suggest that limitations in ankle dorsiflexion range are underestimated and that the TS muscles remain relatively short, particularly during gait (Pothrat et al., 2015; Zandbergen et al., 2018). Foot deformations need to be taken into account to improve assessments of TS extensibility during physical examination.
In order to accurately estimate the contribution of TS hyper-resistance to dorsal foot, (“ankle”) hyper-resistance insight into foot flexibility is required. The contribution of triceps surae hyper-resistance to foot (“ankle”) hyper-resistance likely reduces with increases in foot flexibility. In children with SCP, increased foot flexibility is expected to contribute considerably to foot hyper-resistance compared to TD children.

Figure 1. Analysis of X-rays for a SCP subject after talus alignment (adapted from Huijing et al. 2013)
A) The foot in a position corresponding to externally applied 0 Nm moment. B) The foot in a position corresponding to externally applied 4Nm dorsal flexion. The talar bone was aligned with that in panel A. Such talar bone alignment allows distinction of foot deformation contribution to foot plate movements. This figure shows that in response to externally applied 4 Nm dorsal flexion, 7 degrees of total 15 degrees of foot dorsal flexion corresponded to actual talo-crural joint rotations. This indicates that the remaining 8 degrees of foot dorsal flexion corresponds to internal foot deformations.

In-vivo measurements of GM geometry using ultrasound
At the beginning of the 21st century, with the ultrasound (US) imaging techniques becoming more accessible, investigating the muscle-tendon complex in-vivo became available. Ultrasound imaging allows for non-invasive quantification of various components constituting the GM muscle-tendon complex. However, ultrasound does not allow quantification of sarcomere numbers or their lengths.
Ultrasound images are generated by specular and diffuse reflections of ultrasound waves at interfaces between tissues with different acoustic impedances. A large difference in acoustic impedance yields a large fraction of the ultrasound wave to be reflected. The location of a reflector (interface between tissues) is determined based on the time of flight and the estimated velocity of sound through the tissues. Most ultrasound sound waves received by the transducer arise from scattering of the ultrasound waves by diffuse reflection from small structures. Scattering results in varying intensities of the ultrasound waves received by the transducer due to constructive and destructive interactions of the ultrasound waves. This results in ultrasound images that are speckled or grainy in appearance. The interfaces between muscle fibres and connective tissues act as specular reflectors, reflecting the ultrasound waves from the ultrasound transducer back to the transducer. Such reflection allows for identification of the muscle belly and estimation of the orientation of muscle fibre bundles (fascicles). Mainly using US imaging, numerous studies investigated in-vivo GM geometry in humans. Most US assessments are performed using conventional two-dimensional (2D) B-mode US images, with the examiner choosing a, presumably, suitable orientation and location of the ultrasound transducer. Such 2D method restricts morphological measurements to one image plane, while the parameter of interest may not be observable within this plane. However, true morphological analysis requires a three-dimensional (3D) approach, providing out-of-plane measurements using 3D reference points. More recent US examination combined 2D B-mode US images with a motion capture system to generate 3DUS images (Prager et al., 1998). Recent applications of 3DUS imaging have enhanced insight regarding typical childhood growth of the GM. These 3DUS imaging approaches by our group have been proven time-consuming and technically limited, as only small segments of large anatomical structures (i.e. muscles) could be reconstructed, taking a long time to process. Since the width of the GM muscle belly exceeds the width of the ultrasound probe, images obtained by a single sweep do not cover the whole muscle. As such it is impossible to assess the GM muscle volume and GM physiological cross-sectional area. In order to also assess muscle volume and physiological cross-sectional area, technical advancements are required to combine US images obtained from multiple sweeps.
GENERAL AIM AND OUTLINE OF THIS THESIS

The general aim of this thesis is to extend knowledge regarding mechanisms contributing to TS hyper-resistance in children with SCP.

We first aim to assess how changes in GM geometry relate to changes in ankle dorsal flexion range of motion during adolescence. Chapter 2 presents the results of a cross-sectional study of how muscle geometry varies over an age range corresponding to the adolescent growth spurt period in typical developing adolescent males.

Chapter 3 describes technological advancements of the 3DUS ultrasound approach capable of assessing morphological characteristics of entire body segments including whole muscles. The validity of this approach was tested on cadaver muscles.

TS hyper resistance is typically assessed by quantification of dorsal foot sole range of motion and commonly interpreted as being directly related to TS extensibility. To discriminate between foot sole rotation related to deformation within the foot and rotations related to actual changes in TS length during physical examination, more insight in this mechanism is needed. In Chapter 4, we described a method to assess foot flexibility and we present results on how foot flexibility affects typical assessment of TS extensibility based on foot sole rotations in both TD and SCP children.

In Chapter 5, we compare GM geometry and GM extensibility in children with SCP to TD children. We aim to evaluate how alterations in GM geometry relate to GM extensibility.

In Chapter 6, we present a case study of a 6-year-old girl undergoing a standard intervention of combined serial casting, Botulinum NeuroToxin-A and physiotherapy to improve gait. The aim of the case study was to evaluate the effect of this combined intervention on gait, spasticity, foot flexibility and GM muscle morphology.

Finally, in Chapter 7 the main findings of this thesis are summarised and discussed. This chapter also addresses clinical implications and suggestions for further research.
REFERENCES


