Knee joint mechanics and semitendinosus muscle morphology in spastic paresis

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The work presented in this thesis was conducted at the Department of Human Movement Sciences, Faculty of Behavioural and Movement Sciences, Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, The Netherlands, in cooperation with the Department of Rehabilitation Medicine and Department of Orthopaedic Surgery, VU University Medical Center, The Netherlands and Pediatric Orthopaedic Department and Laboratory for Movement Analysis, University Children’s Hospital Basle, Basle, Switzerland.

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Knee joint mechanics and semitendinosus muscle morphology in spastic paresis

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CHAPTER 1

General introduction
General introduction

Spastic paresis (SP) due to cerebral palsy and hereditary spastic paraplegia often leads to limitations in daily activities such as walking (Gage et al., 2009, Fink, 2013). Secondary to the neurological disorder part of children with SP develop knee extension limitations, which can contribute to a further decrease in walking ability (Johnson et al., 1997, Gage and Novacheck, 2001). It is generally assumed that shortening and/or stiffening of the hamstring muscle-tendon units (MTU) also referred to as “hamstring contracture” can contribute to knee extension deficits (Novacheck, 2009). Therefore, surgical lengthening of medial hamstring muscles including the semitendinosus (ST) muscle is frequently applied (Novacheck, 2009). However, surgery is only partly successful in restoring gait (Adolfsen et al., 2007, Dreher et al., 2012b, Dhawlikar et al., 1992, Zwick et al., 2002). To improve the outcome of surgical interventions in children with SP requires a detailed understanding of knee joint mechanics, underlying mechanical and morphological muscle characteristics and their relation to gait as well as knowledge of muscle adaptation after surgical lengthening.

Spastic paresis in cerebral palsy and hereditary spastic paraplegia

Cerebral palsy

Cerebral palsy occurs in 2 per 1000 live birth in Western European countries and is, thereby, the most common physically disabling condition in childhood (SCPE, 2002). Cerebral palsy is an umbrella term for a group of permanent neurological disorders characterized by impaired development of movement and posture causing limitations in activity such as walking (Rosenbaum et al., 2007). The cause of cerebral palsy is a prenatal, perinatal or early postnatal brain injury or lesion of the foetal or infant brain (Rosenbaum et al., 2007, Graham et al., 2016). Typical examples are a white matter damage due to preterm birth, brain malformation or birth asphyxia (Graham et al., 2016). The location of brain lesions varies with gestational age (Graham et al., 2016). Intra-amniotic infections, perinatal infections, placenta abnormalities and foetal growth retardation are associated with the development of cerebral palsy (Graham et al., 2016). Although the primary cause of cerebral palsy is neurological and non-progressive, over time children may develop secondary musculoskeletal impairments (Rosenbaum et al., 2007). Three different subtypes of motor disorders are distinguished in cerebral palsy: spastic, dyskinetic and ataxic cerebral palsy, of which spastic cerebral palsy is the most common one (Graham et al., 2016, SCPE, 2002, Winter et al., 2002). The effects of cerebral palsy are also described by the anatomic distribution of the motor disorder (i.e. bilateral or unilateral) (Rosenbaum et al., 2007). In the studies of this thesis, children with spastic cerebral palsy have been included who were bilaterally affected, but the upper limbs were not or much less involved compared to the lower limbs. The pathology of this group of children is frequently referred to as having spastic diplegia (Graham et al., 2016).
Hereditary spastic paraplegia

The prevalence of hereditary spastic paraplegia ranges from about 1-10 in 100,000 born children (Fink, 1993). Most patients with a hereditary spastic paraplegia have gait impairments due to muscle weakness and spasticity for which a genetic mutation is the major cause (Fink, 2013). There are currently more than 50 genetic types of hereditary spastic paraplegia known (SPG numbered from 1-56), with a wide variation in severity of spasticity and weakness (Fink, 2013). The early childhood onset of hereditary spastic paraplegia (such as SPG3A and SPG4) has been mainly reported as relatively non-progressive and clinically very similar to spastic diplegic cerebral palsy (Fink, 2013, Kai et al., 2014). In the study of chapter 4 and 5 of the current thesis children with early childhood onset of hereditary spastic paraplegia and children with spastic cerebral palsy were included. In this thesis both groups together are classified as SP.

Figure 1.1 Normal gait pattern (A) compared to flexed knee gait pattern in spastic paresis (B) in mid-stance (MSt), in terminal stance (TSt) and terminal swing (TSw) for the right leg. Note excessive hip and knee flexion during stance and lack of knee extension at TSw in spastic paresis.
Walking limitations and neuromuscular impairments in spastic paresis

A common gait deviation in children with SP is flexed knee gait (Wren et al., 2005, Wolf et al., 2011). This gait deviation is characterized by increased hip and knee flexion during the stance phase of the gait cycle with or without heel rise and is often associated with a reduced knee extension in terminal swing (Figure 1.1) (Rodda and Graham, 2001, Becher, 2002, Cooney et al., 2006). Treatment of this gait pattern is important as it is known that walking ability in these children declines with age (Johnson et al., 1997, Gage and Novacheck, 2001).

There are several primary or secondary impairments related to SP, which are assumed to underlie the pathologic gait pattern in SP, e.g. spasticity, muscle weakness, poor muscle selectivity, muscle fatigue, pain as well as bone deformations, joint restrictions, muscle morphology alterations and muscle tissue alterations (Gage et al., 2009, Fink, 2013). These impairments can be described in relation to walking limitations within the International Classification of Functioning, Disability and Health for Children and Youth (ICF-CY) – which is a holistic framework that describes the consequences of a disease in the domains of body function and structure, activities and participation (WHO, 2008) (Figure 1.2).

**Figure 1.2** The International Classification of Functioning, Disability and Health for Children and Youth (ICF-CY) applied to walking limitations and some assumed underlying impairments in body function and structures in spastic paresis (SP) resulting in a pathologic gait. The focus of the current thesis will be on the impairments in body structure (printed in bold letters).
Impairments in body function (e.g. spasticity, muscle weakness and poor selectivity) that contribute to a flexed knee gait pattern, are mainly of neural origin (caused by SP) and are referred to as impaired muscle activation in this thesis, while impairments in body structure (e.g. bone deformations, joint restrictions and muscle morphology alterations) - generally develop secondary to the dysfunction of the nervous system – are referred to as passive structures in this thesis. Both impaired muscle activation as well as affected passive structures contribute to a pathologic gait in SP (Gage et al., 2009, Fink, 2013) (Figure 1.2). The focus of this thesis will be on impairments in passive structures, more specifically on knee joint restrictions and hamstrings muscle morphology alterations as well as their relation to a flexed knee pattern during gait. Impairments in passive structures, which have been developed during growth, are generally presumed to contribute to deterioration of walking performance. Orthopaedic surgery including surgical lengthening of medial hamstring muscles and bony procedures to improve knee extension is frequently applied in severe cases (Stout et al., 2009). The specific procedures, treatment outcomes, and side effects of surgery are described below.

**Knee joint mechanics**

In clinical practice, resistance to knee extension is measured by the popliteal angle (Figure 1.3). For such assessment one leg is extended while the hip of the examined leg is maintained at 90° and the lower leg is moved manually towards knee extension at a slow speed till increased resistance is perceived (Reimers, 1974).

Upon knee extension several structures around the knee and in the thigh are stretched and exert an increasing reaction force, resulting in a net knee moment resisting knee extension. The structures that contribute to the net knee flexion moment are the knee flexion muscles (i.e. biceps femoris brevis and longus, semimembranosus, semitendinosus, gracilis and gastrocnemius), knee joint capsule, ligaments, nerves and blood vessels as well as their supporting connective tissues. It is generally assumed that the resistance against knee extension in SP is predominantly caused by the hamstring muscles (i.e. biceps femoris brevis and longus, semimembranosus and semitendinosus) (Reimers, 1974). To what extent other structures contribute is unknown. The popliteal angle is a measure of the maximum knee angle that can be attained at a hip angle of 90°. If net knee moments are measured as function of knee angle over the full range of knee extension motion, this may provide estimate of (1) the stiffness of structures that span the knee joint and (2) the slack length of these structures. Measurements of knee moment-angle relations together with assessment of muscle morphological variables may provide insight in how morphological alterations contribute to knee extension limitations.

To the best of our knowledge, the first joint moment-angle measurements for children with SP have been reported for the ankle joint by the group of Tardieu (Tardieu et al., 1982a, Tardieu et al., 1982b). These studies argued the importance of measurements of the joint
moment-angle curve to determine alterations in mechanical muscle properties in SP and the effects of treatment (Tardieu et al., 1982a, Tardieu et al., 1982b). Since then, several studies have shown that for SP and typically developing (TD) children the ankle moment-angle curves are substantially different (Barber et al., 2011a, Tardieu et al., 1982a, Alhusaini et al., 2010, Bénard et al., 2010). In SP children, moment-angle curves in the ankle diverge, i.e. less dorsal flexion with increasing moments compared to TD children (Barber et al., 2011a, Tardieu et al., 1982a, Alhusaini et al., 2010, Bénard et al., 2010). In addition, some studies have shown a shift of joint angles corresponding to 0 Nm ankle moment towards plantar flexion (Tardieu et al., 1982a, Barber et al., 2011a). The results of these studies into limitations of ankle dorsal flexion suggest an increased stiffness as well as a shorter slack length of the ankle plantar flexors in children with SP.

Figure 1.3 Popliteal angle measurements. The measured leg is hold in a hip angle of 90°, while the contralateral leg is extended. The measured leg (in the figure the left leg) is slowly moved towards knee extension till increased resistance is perceived. The knee angle is measured defining the neutral position (full extension) as 0° with increasing knee angle towards knee flexion. Measured angle is indicated by the dotted line in the figure.

To what extent the moment-angle curves of the knee joint are altered in children with SP is unknown. Theoretically, there are different options how the net knee flexion moment-angle curve differs between children with SP and TD children (Figure 1.4). An increased
stiffness of tissues around the knee would lead to an increased slope of knee moment-angle curve, while a shorter slack length of tissues would shift the curve towards more knee flexion. A combined effect of shorter and stiffer tissues around the knee is also conceivable (Figure 1.4). Note that of the abovementioned differences between TD and children with SP all will lead to a larger popliteal angle (i.e. a more flexed knee angle).

Figure 1.4 Possible difference in knee moment-angle curve between typically developing children and children with spastic paresis. The blue curve represents the curve of typically developing children, the green curve shows an increased slope of knee moment-angle curve, while the red curve shows a shift in knee moment-angle curve towards more knee flexion. The orange curve shows the combined effect of an increased slope and shift towards more knee flexion. All options would lead to a more flexed knee angle at equal resistance, as estimated by popliteal angle.

**Gross muscle morphology in spastic paresis**

The muscle belly together with the tendon forms the muscle-tendon unit (MTU). Via tendons the muscle is anchored to the bone. A skeletal muscle is mainly composed of muscle fibers which consists myofibrils arranged in parallel. Myofibrils consist of many in series arranged sarcomeres (Figure 1.5).
Figure 1.5 Architecture of skeletal muscle. A skeletal muscle consists of muscle fibres that consist of sarcomeres arranged in series and in parallel. Muscle fibres are enclosed by endomysium. A bundle of muscle fibres (referred to as a muscle fascicle) is surrounded by perimysium. The fascicles form the muscle belly, which is supplied by blood vessels and innervated by nerves. The muscle is attached by tendons to the bone. (Image ©copyright Wikimedia commons)

The passive length-force characteristics of a MTU are determined by the in series compliance of both tendon and muscle belly. The stiffness of the muscle belly is determined by the number of sarcomeres in series within muscle fibres, by the number of sarcomeres arranged in parallel, as well as by intrinsic mechanical properties of sarcomeres and intramuscular connective tissue.

A decrease in number of sarcomeres in series shifts the passive length-force curve to a shorter length, leading to a shorter slack length of the muscle fibre and an increased slope of the passive length-force curve (Figure 1.6A). A decreased in physiological cross-sectional area (i.e. less sarcomeres arranged in parallel) – on the other hand – leads to a decreased slope of passive length-force curve (Figure 1.6B). A longer tendon results in a longer slack length of the MTU and shifts the length-force curve to longer length and decreases its slope (Figure 1.6C).
Figure 1.6 Effects of changes in muscle morphology (left) on the passive length-force curve of muscle belly/muscle-tendon unit (right). Initial curve in blue and effects of muscle morphology on the curve are printed in red. A: a decrease in number of sarcomeres in series results in a shorter slack length of the muscle fibres and shifts the passive length-force curve towards a lower length and also leads to an increased slope of the curve; B: a decrease in sarcomeres in parallel leads to a decreased slope of passive length-force curve. C: a longer tendon results in a longer slack length of the muscle-tendon unit and shifts the length-force curve to higher length and decreases its slope.

Compared to TD children, children with SP generally have a lower muscle volume, a smaller physiological cross-sectional area, a shorter muscle belly length, shorter fascicle length and a longer tendon length (Barrett and Lichtwark, 2010). However, there also seem to exist substantial differences between muscles concerning morphological alterations (Handsfield et al., 2016, Barrett and Lichtwark, 2010). These differences between muscles might be caused by differences in anatomy (e.g. bi-articular versus mono-articular), morphology (parallel versus pennate fibre architecture), action at the joint (e.g. flexors versus...
extensor muscles), and adaptation of specific muscles to SP. In addition, conditions (i.e. joint angles and joint moments) in which muscle morphology was assessed may contribute to differences in measured morphological variables. Furthermore, the heterogeneity of the population of children with SP (e.g. age, previous treatment, level of selectivity of muscle activation, walking ability) may affect results regarding the comparison of muscle morphology between SP and TD children. Therefore results obtained for one muscle cannot be generalized to other muscles and one should account for the heterogeneity within the SP population.

Regarding data on muscle morphology of ST in children with SP only a few studies were encountered in the literature cf. (Lampe et al., 2006, Noble et al., 2014, Handsfield et al., 2016). A 35% lower muscle volume of ST and a 20% shorter muscle belly in children with SP compared to TD have been reported (Noble et al., 2014, Handsfield et al., 2016), while tendon length, fascicle length and physiological cross-sectional area have not been assessed yet. Insight in these morphological characteristics of spastic ST may improve our understanding on how ST muscle affects knee joint mechanics in children with SP with a flexed knee gait.

Orthopaedic surgical procedures to improve knee angles towards extension

Surgical procedures to improve knee extension can be divided in (1) soft tissue procedures aiming to restore MTU length (including hamstrings lengthening) and (2) bony procedures to correct fixed joint limitations and restore lever arm dysfunction (Stout et al., 2009). These surgical procedures are commonly applied in a single event multi-level surgery (SEMLS) (McGinley et al., 2012, Narayanan, 2012, Lamberts et al., 2016). SEMLS is indicated when fixed joint limitations occur and conservative treatments including physical therapy, orchotics and injections with Botulinum Toxin A did not succeed in preserving and/or restoring sufficient knee range of motion (Damiano et al., 2009, Molenaers et al., 2006, Hagglund et al., 2005).

Hamstrings lengthening

In the past, it was common to release the hamstring muscles including the ST proximally at their origin at the ischial tuberosity (Sharps et al., 1984). Later proximal lengthening was replaced by distal lengthening with more possibilities to fine-tune surgery in relation to knee and hip function during gait (Baumann et al., 1980). Nowadays, distal medial hamstrings lengthening with or without lateral hamstrings lengthening is mostly performed (Dreher et al., 2012b, Novacheck, 2009).

Medial hamstrings lengthening is preferred above combined lateral and medial hamstrings lengthening, because medial hamstrings are assumed to contribute to flexion of the knee in midstance and endorotation-adduction movement of the hips in terminal swing and, thereby, limiting movement during gait. Leaving the lateral hamstrings intact may reduce
the risk of hyperextension of the knee after surgery (Kay et al., 2002). In medial hamstrings lengthening the distal ST and gracilis tendons are lengthened by Z-plasty (Figure 1.7A&B) and the semimembranosus muscle by aponeurotomy (Figure 1.7C) (Bleck, 1987).

**Figure 1.7 Medial hamstrings lengthening.** A: Z-plasty of tendon of semitendinosus muscle (i.e. the distal free tendon is obliquely divided on a distance of 2-4 cm; the free ends are fixed to each other); B: Z-plasty of tendon of gracilis muscle; C: aponeurotomy of the semimembranosus muscle (i.e. the distal semimembranosus aponeuroses is incised with several v-incisions).

**Bony procedures**

Medial hamstrings lengthening is frequently combined with bony procedures to decrease knee flexion deformity and, thereby, improve gait. Either a wedge of the femur is removed on the anterior side to proportionally lengthen the posterior side of the femur (supracondylar extension osteotomy) (Stout et al., 2008) (Figure 1.8A) or 8-shaped plates are placed on the anterior growth plate of the distal femur (hemiepiphysiodesis) to promote ‘guided growth’ (i.e. growth on the anterior side stops, while it continues on the posterior side) (Figure 1.8B). Hemiepiphysiodesis is only possible before skeletal maturity is reached (Klatt and Stevens, 2008, Macwilliams et al., 2011, Al-Aubaidi et al., 2012).
General introduction

Figure 1.8 Bony procedures to increase knee angle towards knee extension. A: a wedge of the femur is removed to proportionally lengthen the posterior side of the femur (supracondylar extension osteotomy); B: 8-shaped plates are placed on the anterior growth plate of the distal femur and fixated with screws (hemiepiphysiodesis) to promote ‘guided growth’ (i.e. growth on the anterior side stops, while it continues on the posterior side). Thereby, the posterior side is lengthened in relation to the anterior side.

Functional outcome of surgeries

Over the last decades, effects of SEMLS including medial hamstrings lengthening and bony procedures around the knee on gait characteristics have been frequently investigated (for review see Lamberts et al., 2016). Generally, gait kinematics improve after SEMLS (Lamberts et al., 2016). However, medial hamstring lengthening has been frequently related to side effects of SEMLS as increased anterior pelvic tilt, and lumbar lordosis as well as hyperextension of the knee during gait (Adolfsen et al., 2007, Dreher et al., 2012b, Dhawlikar et al., 1992, Zwick et al., 2002). Also, the persistence of flexed knee gait or recurrence after initial success have been described (Dhawlikar et al., 1992, Dreher et al., 2012b, Chang et al., 2004). Side effects of medial hamstrings lengthening are thought to be a consequence of over-lengthening and weakening of hamstring muscles (Dreher et al., 2012b, Dhawlikar et al., 1992, Zwick et al., 2002). However, little is known about changes of the hamstring
muscles after surgery and how these changes are related to functional outcome and side effects of surgery.

Aims and outline of thesis

The general aim of this thesis was to investigate effects of medial hamstring lengthening in children with spastic paresis on knee joint mechanics, morphological characteristics of the semitendinosus (ST) muscle and gait and, thereby, identify factors that may contribute to a favourable or unfavourable outcome.

Specific aims were:

1. to develop a method to assess knee joint mechanics and ST morphology,
2. to determine how knee joint mechanics and ST morphology differ between children with spastic paresis and typically developing children,
3. to assess the longitudinal effects of medial hamstrings lengthening on knee joint mechanics and ST morphology, and
4. to evaluate how changes in knee joint mechanics and ST morphology are related to changes in gait kinematics after medial hamstrings lengthening

For this purpose several studies were performed:

Chapter 2 provides a detailed description of the method to assess knee joint mechanics by instrumented hand-held dynamometry. It reports on its reliability in SP and TD children and shows differences in knee moment-angle characteristics between children with SP and TD children.

Chapter 3 describes a newly developed method to assess the morphology of ST muscle with three-dimensional ultrasound (3D US). To evaluate the validity of this 3D US protocol, morphological variables of ST muscle obtained by 3D US are compared with cadaveric dissection. In addition, a detailed description of ST muscle is provided.

In Chapter 4 and Chapter 5 the methodologies described in chapter 2 and chapter 3 are applied to study knee joint mechanics and morphological muscle characteristics in children with SP selected for medial hamstrings lengthening.

Chapter 4 describes knee moment-angle characteristics and ST morphology prior to surgery and compares these to those of TD children. In addition, the relationship between knee moment-angle characteristics and ST morphology is presented.

Chapter 5 investigates the short-term (up to 20 weeks after surgery) and medium-term (up to 20 months) effects of medial hamstrings lengthening on knee moment-angle characteristics and ST morphology, as well as gait kinematics.

In Chapter 6 the main findings of this thesis are summarized and discussed. This chapter provides an evaluation of the methods used and reflects on the outcome of the measurements before and after surgery. Furthermore, clinical implications of this study are discussed and recommendations for future research are made.
CHAPTER 2

Assessment of net knee moment-angle characteristics by instrumented hand-held dynamometry in children with spastic cerebral palsy and typically developing children

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ABSTRACT

The limited range of motion during walking in children with spastic cerebral palsy (SCP) may be the result of altered mechanical characteristics of muscles and connective tissues around the knee joint. Measurement of static net knee moment-angle relation will provide insights into these alterations, for which instrumented hand-held dynamometry may be applied.

The aims of this study were: (1) to test the measurement error of the net knee moment-angle characteristics, (2) to determine the correlation between knee extension angle measurement at a standardized knee moment and popliteal angle from common physical examination and (3) to compare net knee moment–angle characteristics in SCP versus typically developing children.

With the child lying in sideward position, the knee was extended by moving the lower leg by a hand-held force transducer on a low friction cart. Force data were collected for a range of knee angles. Data were excluded when activity (EMG) levels of knee extensor and flexor muscles exceeded the EMG level during rest by more than two standard deviation. The net knee flexion moments were calculated from recorded force data and measured moment arm. Reliability for knee angles corresponding with 0.5, 1, 2, 3, and 4 Nm knee net flexion moment was assessed by standard error of measurements (SEM) and smallest detectable difference (SDD).

For between day comparison, SEMs were about 5 degrees and SDDs were below 14 degrees for knee angles at 1-4 Nm net knee flexion moments. In SCP children, the knee angle measured at 4 Nm knee net flexion moment was not related to the popliteal angle (r=0.52). The slope at 4 Nm of the knee moment-angle curve in SCP children was significantly higher than that in typically developing children.

We conclude that the presented knee hand-held dynamometry allows assessment of net knee flexion moment-knee angle characteristics in typically developing and SCP children and can be used to identify clinically relevant changes as a result of treatment. Overall stiffness of structures that contribute to the net knee flexion moment at the knee (i.e. muscles, tendons, ligaments) is elevated in SCP children.
Introduction

Cerebral palsy is a neurological disorder which involves impaired muscle function that restricts the ability to move and to maintain posture, causing limitations in daily activities (Rosenbaum et al., 2007). Cerebral palsy is the most common physically disabling condition in childhood (SCPE, 2000). In children with cerebral palsy, the prevalence of the spastic motor disorder is high (Himmelmann and Uvebrant, 2014). The level of daily limitations varies substantially, ranging from children who only show limitations in sports activities to children who have no independent mobility (Palisano et al., 1997). In children with spastic cerebral palsy (SCP), crouch gait is a common gait deviation, which is characterized by excessive hip and knee flexion during stance (Rodda and Graham, 2001, Becher, 2002, Gage et al., 2009). This gait pattern is often associated with a lack of knee extension in terminal swing, which restrains step length (Cooney et al., 2006). Weakness of the soleus muscle has been described as a major risk factor for the development of crouch gait (Gage et al., 2009). In addition, children may develop hamstring muscle contractures (i.e. a high resistance to knee extension when the hip is kept in a flexed position) with or without a knee-joint extension limitation (Gage et al., 2009). The mechanisms underlying the ethiology of hamstrings contracture and knee extension limitations and how mechanical tissue properties besides alterations in muscle excitation contribute to crouch gait in SCP children is largely unknown.

Part of the children with SCP walking in crouch gait needs surgical lengthening of the hamstring muscle-tendon unit (MTU) (Molenaers et al., 2006). In most cases, such an intervention is effective in improving gait (Dhawlikar et al., 1992, Dreher et al., 2012b). However, recurrence and reoperation rates are substantial (Dhawlikar et al., 1992). Also, there are reports of overcorrection of the hamstring muscles leading to a hyperextension of the knee and an increase in lumbar lordosis and anterior pelvic tilt (Dreher et al., 2012b, Dhawlikar et al., 1992).

In clinical practice, resistance to knee extension due to muscle, ligament and joint stiffness is estimated by the popliteal angle (Reimers, 1974) and is used as indication for surgical lengthening. The popliteal angle is measured with the ipsilateral hip hold flexed at 90° while the knee is passively extended to the knee angle at which increased resistance is perceived. The contralateral hip is kept fully extended at 0° (Reimers, 1974). Assessment of the popliteal angle, despite being widely used, has some methodological limitations: (1) The maximal attainable knee joint angle is subjectively determined, (2) correct position of hip and pelvis is difficult to maintain manually during popliteal angle measurements and (3) the variability of popliteal angle measurements is affected by voluntary and/or involuntary muscle activation (Kilgour et al., 2003, Ten Berge et al., 2007, Herrington, 2013, McNee et al., 2007).

In previous research it has been emphasized that the current indication of surgical lengthening of hamstring muscle based on popliteal angle may be insufficient and that there
is a need of estimation of muscle length to indicate surgical lengthening (Delp et al., 1996, Arnold et al., 2006, Thompson et al., 2001). Muscle length is currently estimated using musculoskeletal modelling, without individual morphological data (Delp et al., 1996, Arnold et al., 2006, Thompson et al., 2001).

An approach that allows measurement of morphological determinants of the length-force characteristics directly in relation to altered mechanical behaviour of the joint may increase our knowledge of mechanisms underlying limitations in range of motion (ROM) in SCP. Morphological determinants of the length-force characteristics involve muscle belly length, fascicle length, physiological cross-sectional area and pennation angle; all of which can be measured by ultrasound imaging (Kellis et al., 2009). For the ankle joint such an approach has been developed (Barber et al., 2011b, Barber et al., 2011a, Huijing et al., 2013). However, such an approach has not previously been described for the knee joint.

A first step towards a similar approach for the knee is to determine the mechanical properties of tissues around the knee by measuring net knee moment as a function of the knee angle over the full ROM (Tardieu et al., 1982a). Net knee flexion moment-angle curves were measured in healthy adults with instrumented hand-held dynamometry at standardized low muscle activation (Silder et al., 2007, van der Krogt et al., 2008), but to our knowledge neither in SCP nor TD children. In addition, available methods for assessment of net knee moment-angle characteristics have not been evaluated for their precision i.e. measurement error (Silder et al., 2007, van der Krogt et al., 2008).

Therefore, the aims of this study were: (1) to present a hand-held dynamometry approach and to determine its measurement error for assessment of net knee moment-angle characteristics in SCP and TD children in repeated measurements, (2) to determine the correlation with popliteal angle and (3) to compare net knee moment-angle characteristics between SCP and TD children.

**Methods**

**Study population**

The study protocol was approved by the Medical Ethics Committee of the VU University Medical Center (VUmc), Amsterdam (The Netherlands). All children and their parents gave their written informed consent.

Children with SCP were recruited from a group who were under medical treatment in the VUmc from April to October 2012. Patients included had: (1) a clinical diagnosis of SCP (SCPE, 2000, Rosenbaum et al., 2007), (2) a Gross Motor Function Classification System (GMFCS) Expanded and Revised Class I-III (Palisano et al., 2008) and (3) were 8-16 years old. Patients that were currently involved in treatment that could affect the structural properties of the hamstring muscles were excluded. Specifically, this includes (1) medication that influences neuromuscular properties, treatment with Botulinum toxin A or
24-hour casting within three months before measurements or (2) selective dorsal rhizotomy or surgery within one year prior to measurements. The control group consisted of age
matched TD children.

Experimental protocol

Subjects were lying on their left side on a comfortable examination table. The right leg was measured with the right hip positioned in 70° flexion. The left hip was put in a comfortable slightly flexed position (20°-40°). To prevent pelvic tilt and hip movement during measurements, pelvis and upper leg were tightly secured to the setup - the pelvis with an adjustable frame and foam blocks on both sides of the trunk and the upper leg with a bandage. The right lower leg was resting on a low friction cart (appropriately fastened) with the ankle in plantar flexion (about -20°) to minimize effects of gastrocnemius on knee moment (Figure 2.1). This setup was designed such that in future experiments simultaneous ultrasound imaging of medial hamstrings would be possible.

Figure 2.1 Top view of hand-held dynamometry measurement setup. Children were positioned on their left side on a treatment table, with the hip of the measured (right) leg at 70° flexion (A). Pelvis and upper leg were tightly secured – the upper leg with a bandage (B) and the pelvis with an adjustable frame (C1) and foam blocks on both sides of the trunk (C2). The lower leg was positioned on a low-friction movable plate (D). The lower leg was manually moved with a hand-held force transducer (E) through its range of motion with stops for 10 seconds every 5°.
Changes in knee angle were measured using a Twin Axis Goniometer SG150 (Biometrics Ltd, UK) of which one part was placed on the lateral side of the upper leg along the line between greater trochanter and the lateral condyle of the femur. The other part was placed on the lower leg along the line between the fibula and the lateral malleolus (Piriayaprasarth et al., 2008). The goniometer was attached to the skin when the knee was in 90° flexion. Technical clusters of three markers (Optotrak, NDI, Canada) were attached to verify whether movement of upper leg or pelvis occurred. Use of anatomical landmarks (trochanter major, lateral epicondyle, caput fibulae and lateral malleolus), defined tibia and fibula lengths as well absolute knee angles. These latter were used for calibration of the goniometer.

Force applied at the lower leg was measured using a custom-made hand-held device instrumented with a bi-directional force transducer with an accuracy of 0.5 N (HBM Darmstadt, Germany). The measurement device was attached to the lower leg with a neoprene strap so that forces could be measured during both pushing as well as pulling the lower leg. The moment lever arm was measured on the line between lateral epicondyle and lateral malleolus, as the distance from the point of application of the force measurement device to the lateral epicondyle, taken as an estimate of the location of the knee axis.

Activity levels of m. biceps femoris, m. gastrocnemius medialis, m. rectus femoris and m. vastus lateralis were assessed using surface electromyography (EMG). Skin preparation and placement of EMG electrodes were performed according to SENIAM guidelines (Hermens et al., 1999). Force and knee angle were sampled at 100 Hz by a GSV-3USBx2-amplifier (ME-measuring systems GmbH, Germany) and Mobi system (TMSI, The Netherlands). EMG activity was sampled at 1000 Hz by a Porti or Mobi system (TMSI, The Netherlands). All signals were stored on a PC for off-line analysis.

To comfort the child, and distract him/her from the measurement a movie was played. Prior to the assessment of net knee moment-angle characteristics, the subject was asked to fully relax the leg for ten seconds, which was used to assess rest EMG (EMG-rest). As preparation for the measurement, three flexion-extension cycles from knee flexion of about 110° to knee extension of maximal 20° were performed. To avoid a knee range of motion for which the joint axes of rotation translate, knee extension between 0 and 20° was not included (Iwaki et al., 2000, Hill et al., 2000). The leg was moved only within the range that was possible without obvious EMG bursts determined by visual inspection and/or discomfort experienced by the child. After these cycles, the lower leg was pulled into a flexion position of about 110° and then slowly released till the cart stopped. From that position, the knee was extended in steps of ~5°. At each knee angle, the position was maintained for 10 seconds to allow effects of stress-relaxation. Therefore, only the last three seconds of each step were used for data analysis. After the maximal attainable extension angle was measured, the leg was slowly released and pulled towards flexion again.
This procedure was repeated five times (for a typical example of three measurement cycles and selected data points, see Additional file 1, Figure A1.1).

The standard method as described by Reimers was used to measure popliteal angle (Reimers, 1974).

**Data analysis**

Joint angle and force data were low-pass filtered at 1 Hz. For each knee angle, force and joint angle data were time averaged over the last three seconds of every ten second measurement interval. The net knee moment was calculated by multiplying the force measured at the force transducer by moment arm.

Since artefacts appeared to be present, below 100 Hz, all EMG data were off-line high-pass filtered at 100 Hz (Potvin and Brown, 2004, Staudenmann et al., 2010) to monitor muscle activity. To obtain an envelope the filtered EMG was rectified and low-pass filtered at 5 Hz. Mean and standard deviation (SD) of the envelope data from the resting EMG was retrieved. A threshold level was set at mean $+ 2$ SD. When mean EMG during the last three seconds of every ten second measurement interval (i.e. the interval were joint and force data was retrieved) exceeded this threshold for one of the four muscles, data corresponding to these knee joint angles were excluded from further analysis. A repetition was included for further analysis if it consisted of at least four data points, of which at least one data point lower than 0.5 Nm and at least one data point higher than 3 Nm.

Using all combinations of angles and net knee moment of a repetition, a curve was fitted by a third order polynomial function, which was retrieved based on a stepwise regression analysis using different functions.

$$y = ax^3 + bx^2 + cx + d \text{ (Equation 2.1)}$$

Where $y$ represents net knee moment and $x$ knee angle, $a$, $b$, $c$ and $d$ are constants determined by the fitting procedure. For comparison of repetitions, data of each repetition was fitted. As the fits of the data of the repetitions were very similar (see Additional file 2), we pooled the data for the between and within-day comparison. This increased the number as well as the range of data points that can be used for fitting the data. From the fitted curves, knee angles at 0.5, 1, 2, 3 and 4 Nm and the slope at 4 Nm were derived. Higher moments were not included because these were not measured in all subjects. Knee angles at different knee moments were used for statistical analysis.

**Study design**

We assessed (1) within-session reliability and measurement error from five repetitions on one day (for details see Additional file 2). Based on these data, we included three repetitions in the measurement protocol; (2) measurement error of between-day measurements from sessions on two different days; and (3) measurement error of within-day from two sessions.
on the same day. Between the two sessions, all devices were removed and the child walked around for 5 minutes.

The within-day measurement error was assessed, in addition to the between-day error, to distinguish between (1) variation as a result of possible differences in tissue properties between days and (2) variation as a result of differences in positioning of the child and alignment of the goniometer.

**Statistics**

Within-session reliability was analysed with intraclass correlation coefficients (ICC) for single measurement using variance components of subject and repetition determined by a Restricted Maximum Likelihood Estimation (RMLE) (Vet et al., 2011) (for details see Additional file 2).

The measurement errors of between-day and within-day measurements were calculated by the standard error of measurements (SEM). The SEM was determined by the standard deviation of the difference (SD\(_{\text{difference}}\)) between days and SD\(_{\text{difference}}\) between sessions, respectively (Vet et al., 2011).

\[
\text{SEM} = \frac{\text{SD}_{\text{difference}}}{\sqrt{2}} \quad \text{(Equation 2.2)}
\]

To obtain an indication of measureable change beyond measurement error in knee angle of individual patients over time, the smallest detectable difference (SDD) was calculated. The SDD has been defined as change outside the 95 % limits of agreement (Vet et al., 2011).

\[
\text{SDD} = \pm \text{SEM} \times \sqrt{2} \times 1.96 \quad \text{(Equation 2.3)}
\]

This equation is only valid in the absence of systematic difference between sessions and days. This absence was tested by Paired Sample T-Test for knee angles at 0.5, 1, 2, 3 and 4 Nm.

Differences between SCP and TD in anthropometric parameters, age, maximum knee angle, maximum moment and the slope at 4 Nm were tested using Independent T-Tests. Differences in net knee moment-angle characteristics between SCP and TD were tested with repeated measurement ANOVA (factors: group x moment). Correlation between knee angle at 4 Nm and the popliteal angle was calculated by the Pearson correlation coefficient (Pearson’s \(r\)). The level of significance was 0.05 for all statistical tests. Values are presented as means ± standard deviations (SD).

**Results**

Each measurement session took about 45 minutes. Maximum displacement of the pelvis (12±6 mm) and upper leg (14±4 mm) did not differ between SCP and TD children.
Displacement of the pelvis and upper leg between days and within days yielded very similar results. The largest displacement of pelvis and upper leg was always reached when the knee was maximally extended.

**Within-session reliability**

Repeating net knee moment-angle measurements five times within one session (i.e. subject stays within the setup) resulted in similar net knee moment-angle characteristics (for typical examples, see Figure 2.2).

At all net knee flexion moments tested, a single repetition yielded ICCs higher than 0.65 and SDDs ranging from 3.1° to 11.4°. Differences in ICCs between SCP and TD were similar. At all net knee flexion moments tested, averaging knee angles over three repetitions resulted in ICCs higher than 0.85 (for detailed information see Additional file 2).

**Measurement error between days**

Four subjects (3 SCP, 1 TD) could not be included on the second day due to technical or planning problems. Five SCP children and three TD children had EMG-activity higher than the EMG threshold during at least two repetitions on one of the two measurement days and were excluded from analysis of between day reliability. Therefore, between-day reliability was assessed in seven TD children and three SCP children (GMFCS I, II & III). Two to nineteen days were in between the two measurement days.

For TD and SCP children, at all moments absolute differences of mean values of the first and the second day were below 8° (Table 2.1). Overall values of the first and second day did not significantly differ from each other.

<table>
<thead>
<tr>
<th>Knee angle at</th>
<th>TD (between-day) n=7</th>
<th>SCP (between-day) n=3</th>
<th>TD (within-day) n=7</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 Nm</td>
<td>7.8° ±6.2°</td>
<td>5.5° ±1.6°</td>
<td>6.5° ±4.9°</td>
</tr>
<tr>
<td>1 Nm</td>
<td>5.8° ±4.2°</td>
<td>3.8° ±3.0°</td>
<td>6.7° ±2.7°</td>
</tr>
<tr>
<td>2 Nm</td>
<td>5.0° ±2.7°</td>
<td>4.5° ±5.5°</td>
<td>6.6° ±2.0°</td>
</tr>
<tr>
<td>3 Nm</td>
<td>5.5° ±2.5°</td>
<td>4.4° ±6.8°</td>
<td>6.1° ±2.5°</td>
</tr>
<tr>
<td>4 Nm</td>
<td>6.0° ±2.7°</td>
<td>4.4° ±7.2°</td>
<td>5.3° ±2.4°</td>
</tr>
<tr>
<td>maximum Nm</td>
<td>5.2° ±3.4°</td>
<td>6.8° ±10.1°</td>
<td>5.0° ±3.0°</td>
</tr>
</tbody>
</table>

TD= typically developing children, SCP=spastic cerebral palsy. Units are degrees

The SEMs were about 5° at knee angle corresponding to 0.5-4 Nm, which yielded SDD values from 17° (at 0.5 Nm) to 12.7° (at 4 Nm). SEM and SDD for knee angles at maximum measured knee moments were similar to those a 4 Nm (Table 2.2).
Figure 2.2 Example of five subsequently performed repetitions of knee moment-angle measurements. Typical examples of a child of the TD group (A) and the SCP group (B). Grey dots: measured data. Black lines: 3rd order polynomial fit. White symbols: calculated knee angles at 0.5, 1, 2, 3 and 4 Nm.
Table 2.2 Within- and between day measurement errors: Standard error of measurement (SEM) and smallest detectable difference (SDD).

<table>
<thead>
<tr>
<th>Group</th>
<th>Knee angle at</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 Nm</td>
<td>1 Nm</td>
<td>2 Nm</td>
<td>3 Nm</td>
<td>4 Nm</td>
<td>maximum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>SDD</td>
<td>SEM</td>
<td>SDD</td>
<td>SEM</td>
<td>SDD</td>
<td>SEM</td>
<td>SDD</td>
<td>SEM</td>
</tr>
<tr>
<td>TD (between-day)</td>
<td>6.1°</td>
<td>17.0°</td>
<td>5.0°</td>
<td>13.8°</td>
<td>4.2°</td>
<td>11.7°</td>
<td>4.3°</td>
<td>11.9°</td>
</tr>
<tr>
<td>TD (within-day)</td>
<td>6.1°</td>
<td>16.8°</td>
<td>5.4°</td>
<td>14.9°</td>
<td>5.2°</td>
<td>14.5°</td>
<td>5.0°</td>
<td>13.3°</td>
</tr>
</tbody>
</table>

TD = typically developing children, Units of SEMs SDDs are in degree.

SEMs and SDDs, were calculated for TD only, as sample size of SCP children was considered too small. For SCP children, differences in knee angles between days were within the range of those shown for TD children. Therefore, similar SEMs and SDDs for SCP children are expected (Figure 2.3, Table 2.1).

Figure 2.3 Between-day reliability of hand-held dynamometry approach to measure knee moment-angle characteristics. X-axis: knee angle at 0.5, 1, 2, 3 and 4 Nm measured at day one. Y-axis: knee angles measured at day two. Dotted arrow: between days error. TD (n=7) and SCP (n=3).

Measurement error within a day

Absolute mean values of the first and the second session measured on the same day, differed 7° or less at all assessed net knee moments tested (Table 2.1), but differences were not systematic. SEMs and SDDs were similar to between-day measurements (Table 2.2).
These results show that measurement error of the net knee moment-angle characteristics within a day was similar as that of between-day measurements (see for example the comparison of data for two sessions and two days at 4 Nm, Figure 2.4).

**Figure 2.4 Bland and Altman plot for knee angles at 4 Nm.** Net knee moment at day one and day two (between-day) (●) and session one and session two (within-day) (○). X-axis: mean values of day one and day two measurements as well as mean values of session one and session two. Y axis: difference between the knee angles (day one minus day two as well as session one minus session two). Dotted line: smallest detectable difference (SDD) of the between day reliability. Dashed line: SDD of the within-day reliability.

**Correlation to popliteal angle**

For SCP children, the knee angle measured at 4 Nm net knee flexion moment was not related to the popliteal angle (Figure 2.5; r=0.52; p=0.12).

**Difference in net knee moment-angle characteristics between SCP and TD**

Eleven SPC and eleven TD were included, but one SCP child and two TD children could not perform three repetitions without significant EMG activity on at least one of the days and were excluded from comparison between the SCP and TD group. The groups did not differ in age, gender and anthropometrics (Table 2.3; p<0.05).
Assessment of net knee moment-angle characteristics

Figure 2.5 Correlation between popliteal angle and hand-held dynamometry in SCP. X-axis: popliteal angle. Y-axis: knee angle at 4 Nm measured with hand-held dynamometry. (n=10)

Table 2.3 Anthropometric and subject data ± standard deviation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Gender (female/ male)</th>
<th>Body length (cm)</th>
<th>Body mass (kg)</th>
<th>Femur length (cm)</th>
<th>Fibula length (cm)</th>
<th>GMFCS (I-III)</th>
<th>Popliteal angle (degree)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TD</td>
<td>11.6 ±1.7</td>
<td>5/4</td>
<td>150.2 ±13.5</td>
<td>41.4 ±10.6</td>
<td>34.9 ±4.3</td>
<td>32.4 ±3.7</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>SCP</td>
<td>12.7 ±1.7</td>
<td>6/4</td>
<td>155.9 ±12.3</td>
<td>46.7 ±11.2</td>
<td>36.8 ±3.7</td>
<td>33.1 ±3.4</td>
<td>I (4), II (3), III (3)</td>
<td>56 ±16.5</td>
</tr>
</tbody>
</table>

TD= typically developing children, SCP=spastic cerebral palsy, GMFCS=Gross Motor Function Classification System.

In SCP children, knee angles measured from 0.5-4 Nm net knee flexion moments ranged from $72.8° ± 7.9°$ (at 0.5 Nm) to $49.7° ± 12.1°$ (at 4 Nm) and in TD from $69.2° ± 5.9°$ (at 0.5 Nm) to $40.0° ± 9.4°$ (at 4 Nm) (Table 2.4). Repeated measures ANOVA did not reveal an effect of groups (p=0.100). However, a significant interaction effect was shown between group and net knee flexion moment (p=0.010), which indicates that net knee moment-angle curves of SCP and TD diverged with higher net knee flexion moments (Figure 2.6). The slope at 4 Nm was significantly higher in SCP children (p=0.017), indicating a higher increase in net knee moment as a result of knee extension. The maximum measured angle in TD children was lower (i.e. knee was more extended) than in SCP children (p=0.043), while the maximum measured moment did not differ (p=0.318) (Table 2.4).
Table 2.4 Knee angles ±standard deviation and mean differences.

<table>
<thead>
<tr>
<th></th>
<th>CP (n=10)</th>
<th>TD (n=9)</th>
<th>Mean difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee angle at 0.5 Nm</td>
<td>72.8° ±7.9°</td>
<td>69.2° ±5.9°</td>
<td>3.6°</td>
</tr>
<tr>
<td>1 Nm</td>
<td>66.8° ±8.6°</td>
<td>61.4° ±5.7°</td>
<td>5.4°</td>
</tr>
<tr>
<td>2 Nm</td>
<td>58.9° ±9.8°</td>
<td>52.0° ±7.1°</td>
<td>6.8°</td>
</tr>
<tr>
<td>3 Nm</td>
<td>53.7° ±11.0°</td>
<td>45.4° ±8.3°</td>
<td>8.3°</td>
</tr>
<tr>
<td>4 Nm</td>
<td>59.7° ±12.1°</td>
<td>40.6° ±9.4°</td>
<td>9.8°</td>
</tr>
<tr>
<td>Maximum knee angle</td>
<td>42.8° ±12.1°</td>
<td>32.8° ±6.6°</td>
<td>9.9°*</td>
</tr>
<tr>
<td>Maximum knee moment</td>
<td>7.4 Nm ±3.1 Nm</td>
<td>6.2 Nm ±1.6 Nm</td>
<td>1.2 Nm</td>
</tr>
<tr>
<td>Slope at 4 Nm</td>
<td>0.3 ±0.1</td>
<td>0.2±0.1</td>
<td>0.1 *</td>
</tr>
</tbody>
</table>

TD = typically developing children, SCP=spastic cerebral palsy; *significantly different p<0.05

Figure 2.6 Differences of knee moment-angle characteristics of SCP and TD children. Black line: knee-moment-angle characteristics of SCP children (n=10). Grey line: knee-moment-angle characteristics of TD children (n=9). Values are means±SD.
Discussion

We investigated measurement error of a hand-held dynamometry approach to measure net knee moment-angle characteristics in children (TD and SCP). The results are: (1) with a SEM of about 5°, this approach allows assessment of changes in individuals for knee extension angles of at least 14° between repeated measurements at positive net knee flexion moments (>0.5 Nm), (2) in SCP children the knee angle measured at 4 Nm knee moment is not related to the popliteal angle and (3) SCP children show a steeper slope of the net knee moment-angle curve at 4 Nm measured by hand-held dynamometry than TD children.

Agreement of between day measurements of instrumented hand-held dynamometry

The evaluation of the measurement error depends on the goal of the (clinical) intervention (e.g. expected effects of treatment). Based on popliteal angle measurements, surgical lengthening of hamstring MTU is expected to result in a change of the 4 Nm knee flexion angle of more than 20° (Dhawlikar et al., 1992). Since the SDD was 13° at 4 Nm knee flexion moment, the presented dynamometer approach allows assessment of clinically meaningful changes.

Our results show that between days within one subject differences, greater than 14° in knee angles at positive net knee flexion moments (>0.5 Nm), can be assessed (i.e. differences greater than 14° are beyond the measurement error). SDD was higher for the 0.5 Nm knee angle (17°). It should be noted that when measuring a group of subjects repeatedly, SDDs are reduced by a factor square root of sample size (Vet et al., 2011). For example, for a group of 10 subjects, the smallest difference that can be detected by instrumented hand-held dynamometry is approximately 4° for net knee flexion moments between 1 and 4 Nm. Therefore, a difference of less than 5° between days can be determined in a relatively small group.

To the best of our knowledge, this is the first study that reports measurement error of net knee moment-angle characteristics while controlling for low EMG levels. The measurement of popliteal angle is to a certain extent comparable to the knee angle at 4 Nm as measured in the present study. The SDDs for between day popliteal angle measurements of (~18°) are somewhat higher [11] than those of the present study (~13°).

Our results indicate that the presented instrumented handheld dynamometer approach allows measurement of clinical relevant changes in net knee flexion moment-angle characteristics at knee angles corresponding with knee moments higher than 1 Nm. Differences in knee angles that can be determined with instrumented hand-held dynamometry are 5° smaller than those determined with the commonly used popliteal angle (18°).
Sources of error of instrumented hand-held dynamometry

Measurement errors between days may be the result of day-to-day variation in levels of muscle activity, displacement of pelvis and upper leg, mechanical muscle properties, position of the body in the setup (i.e. trunk and pelvis position; and hip and ankle angle), as well as alignment of the goniometer.

We used an EMG threshold to verify whether muscle activity of knee flexors and extensors was below the EMG threshold (see methods). This exclusion criterion minimized the effects of EMG-activity on the moment-angle curve, but small effects of EMG activity below the threshold could occur.

Minor displacements of pelvis and upper leg were found, but these were constant during different repetitions, sessions and days. Therefore, it is not likely that these have affected the measurement error between days.

The ankle was placed in a plantar flexed position (about -20° plantar flexion for both groups) to minimize effects of gastrocnemius muscle force on knee moment. In children with SCP, zero ankle moment assessed with the knee fully extended, has been reported at ≈-10° plantar flexion (Hug et al., 2013, Bénard et al., 2010). This implies a negligible mechanical effect of the gastrocnemius muscle at the knee for the ankle angle at which the subjects of the present study were tested.

The higher measurement error (i.e. higher SEM and SDD) at 0.5 Nm may be explained partly by friction between the cart to which the foot was attached and by the surface it was displaced on. On the toe region of the moment-angle curve, a relatively large range of knee angles at which the knee moment is near zero can be expected (Heerkens et al., 1985). In those conditions, a small difference in net moment due to some friction will have a relatively large effect on the assessment of the corresponding knee angle.

The unexpected similar measurement error for the within- and between-day measurements (see Table 2.2) indicates that measurement error between days cannot be ascribed to differences in mechanical properties of tissues, but was rather due to variation in the performance of the measurement itself. Despite the fact that we aimed to place each child in the same position for different measurement sessions (on the same day and on different days) and to align the goniometer with respect to anatomical landmarks on the skeleton (see methods), this procedure seemed to contribute largely to the measurement error between days, as well between sessions.

Assessment of mechanical properties of hamstring muscles in SCP

Assessment of the popliteal angle in clinical practice aims to determine knee extension limitations due to resistance of the hamstrings muscles and joint structures as well as to estimate their contribution to crouch gait. The popliteal angle in SCP children may be increased due to an increased stiffness of the structures that span the knee joint and/or a reduction in slack length of these structures resulting in a lower knee angle at 0.5 Nm
net knee moment. Therefore, interventions to decrease knee extension limitations in SCP children may be effective via distinctive mechanisms. These interventions may result either in a decrease of the slope of the net knee moment-angle curve (due to a decreased MTU stiffness) and/or a shift of the whole moment-angle curve to more extended knee angles (e.g. due to an increased MTU slack length). Such changes in anatomical structures may be distinguished by assessment of knee moment-angle characteristics, but likely not by a single point on the curve such as the popliteal angle.

To investigate to what extent differences in knee moment-angle curves are caused by differences in morphological determinants of muscle length-force characteristics (i.e. muscle belly length, fascicle length, physiological cross-sectional and pennation angle), ultrasound imaging of knee flexor and extensor muscles is deemed necessary.

The current approach (especially when combined with ultrasound measurements in future studies) provides an opportunity to assess differences in hamstring muscles properties between SCP and TD, as well as to evaluate the effects of different treatment modalities aimed at muscle lengthening (e.g. orthotics, serial casting, surgical lengthening of hamstring MTU). In addition, with our methods insight can be gained on how spasticity reduction (interventions like botulinum toxin injections and selective dorsal rhizotomy) affects mechanical and morphological properties of hamstring muscles.

The knee angle measured at 4 Nm net knee flexion moment was not related to the popliteal angle. The lack correlation may be explained by differences between popliteal angle measurements and hand-held dynamometry measurements. During popliteal angle measurements, muscle activity may affect the measured knee angle, as well as variations in hip and pelvis positions and magnitude of applied knee moment. The lower measurement error of hand-held dynamometry compared to the popliteal angle and the possibility to assess knee angles at a range of net knee moments, yield the possibility to improve the estimation of effects of hamstring muscle differences on ROM limitations and their impact on gait deviations in SCP children.

The similar knee angles at low net knee flexion moments together with a steeper slope of the curve towards higher knee moments in SCP children implies that SCP children had a reduced knee ROM within the same range of knee moments. The steeper slope of the net knee moment-knee angle curve in SCP children may be explained by increased stiffness of the MTU of the hamstring muscles, possibly due to a decreased length of muscle fascicles (Barber et al., 2011a). Furthermore, altered properties of non-muscular structures (i.e. nerves, blood vessels and their connective tissues) (Kuilart, 2005) as well as enhanced knee capsular stiffness may contribute to the steeper slope of the net knee moment-angle curve in SCP children. Altered tissue composition in muscles and tendons of SCP children may effect MTU stiffness (e.g. due to increased collagen content) (Booth et al., 2001, Friden and Lieber, 2003, Smith et al., 2011, de Bruin et al., 2014). Muscle biopsies could be used to deepen our understanding into causes of MTU stiffness in SCP.
Chapter 2

The steeper slope of the knee moment-angle curve in SCP children may contribute to the limited ROM during gait (i.e. excursion of hamstring MTU), particularly to the restricted knee extension in terminal swing (i.e. at maximum hamstring MTU length) (Cooney et al., 2006).

We assessed maximum knee moments ranging from around 4-12 Nm. In the swing phase during gait, knee flexion moments up to 0.5 Nm/kg have been reported in SCP and TD (i.e. 20 Nm for a child of 40 kg) (Adolfsen et al., 2007, Cupp et al., 1999). During gait, higher moments are found as result of activation of muscles. Measurement of knee moment-angle characteristics with the current approach in SCP children may allow differentiation of the contribution to knee moments by passive mechanical tissue properties (i.e. structural alterations as shortening or stiffening of the MTU) from that neural activation (i.e. increased reflex stiffness).

In order to improve decision making for treatment of hamstring muscles, future studies are needed to combine instrumented hand-held dynamometry with ultrasound measurements and to relate these outcomes of mechanical and morphological muscle properties to joint kinematics in crouch gait. Longitudinal studies are deemed necessary to evaluate if changes in mechanical properties of muscles, tendons and ligaments will lead to a decrease in functional impairments of walking in SCP children.

Limitations

Some limitations of the approach and the study need to be taken into account and may provide information to improve measurements of knee moment-angle characteristics in the future:

The current measurement protocol is quite time consuming for a standard clinical assessment (approximately 45 min) and needs specialized equipment (i.e. a custom made setup to position the child and a custom-made hand-held device for force measurement were used). However, for research purposes and for the indication of interventions with a high impact on the child (e.g. surgery) such an approach is considered justifiable.

In a considerable number of subjects (SCP and TD), EMG levels were higher than the set threshold (see methods). We imposed a very strict EMG threshold to minimize anticipated effects of muscle activity on the knee moment. EMG activity seemed to increase mainly due to resistance to stretch at increased knee extension angle and that children tried to assist the extension movement. It may be possible to decrease the number of exclusions due to EMG activity with feedback to the subject on muscle activity during measurement (Harlaar et al., 2000), as well as by increasing the number of repetitions.

In our experimental design, a limited number of sources of error could be distinguished from each other. The measurement error is a sum of different errors that can be the result of (a) differences in tissue mechanical properties, (b) differences in body position in the setup (i.e. differences in pelvis position and joint angles at hip and ankle), (c) different placement
of the goniometer on the skin, (d) instrumentation errors or (e) errors made by the examiner. To obtain insight in the contribution of these sources of error to the overall measurement error for the introduced approach would require an extended study design.

**Conclusions**

Instrumented knee hand-held dynamometry as presented in this study allows assessment of clinically relevant changes in net knee moment-angle characteristics in TD and SCP children. Valuable information about stiffness of the hamstring muscles and other structures spanning the knee joint can be obtained. This information can be useful to quantify functional changes in SCP after clinical interventions and to study mechanisms underlying the outcomes of these interventions.

**Acknowledgements**

The authors wish to thank Danny Koops, Léon Schutte and Guus Baan for designing and engineering the setup. Andrea Spierenburg and Rozemarijn Dekker are acknowledged for their assistance with measurements and Friso Elzinga for designing Figure 2.1. We are very grateful for the advice of Dick Stegeman on processing the EMG data.

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Appendix

Additional file 1

Selection of data points from raw measurement data:

Figure A1.1 Typical example of recorded angle and moment for three cycles of flexion and extension movement. As preparation for the measurement, three flexion-extension cycles from knee flexion of about 110° to knee extension of maximal 20° were performed (1). The leg was moved only within the range that was possible without obvious EMG bursts determined by visual inspection and/or discomfort experienced by the child. After these cycles, the lower leg was pulled into a flexion position of about 110° (2) and then slowly released till the cart stopped (3). From that position, the knee was extended in steps of ~5°. At each knee angle, the position was maintained for 10 seconds to allow effects of stress-relaxation (e.g. 4). Therefore, only the last three seconds of each step were used for data analysis (red bar). After the maximal attainable extension angle (5) was measured, the leg was slowly released (6) and pulled in small steps towards flexion again (7). In this example three repetitions are shown.
Additional file 2

Within session reliability of measurements of knee moment-angle characteristics

We assessed within-session reliability from five repetitions. Within a session the subject stayed within the setup. 11 children with SCP and 11 typically develop children were included. Six TD and six SCP were excluded for analyses due to beyond threshold EMG activity at least during one repetition.

Statistics

Variance components of the factors subject ($\sigma^2_p$), repetition ($\sigma^2_r$) and the residual error ($\sigma^2_e$) were determined using Restricted Maximum Likelihood Estimation (RMLE) (Vet et al., 2011). Subject variance represents the variance between subjects (i.e. variance of interest) and the repetition variance component represents the error variance, caused by repetition. The residual error is the sum of the interaction subject x repetition and a random error (e.g. caused by examiner or instrumentation).

Reliability was assessed by the intraclass correlation coefficients (ICC) for single measurements and the smallest detectable difference (SDD). Since we were interested in absolute agreement of measurements we used to calculate the ICC from the variance components (Vet et al., 2011, Roebroeck et al., 1993):

$$ ICC = \frac{\text{variance of interest}}{\text{variance of interest} + \text{error variances}} $$  \hspace{1cm} (Equation A2.1)

ICC values range between 0 and 1. An ICC towards 1 indicates that error variance is negligible compared to inter subject variance and that subjects can be well distinguished from each other (Vet et al., 2011). To obtain an indication of measureable true change in knee angle of individual patients over time, we calculated from the error variance the standard error of measurements (SEM) and smallest detectable difference (SDD) (Roebroeck et al., 1993). By definition, 95% of the differences between measurements lies within the limits of agreement. The SDD has been defined as difference outside the limits of agreement (Vet et al., 2011):

$$ SEM = \sqrt{\sigma^2_r + \sigma^2_e} \hspace{1cm} (Equation \hspace{0.1cm} A2.2.) $$

$$ SDD = \pm SEM \times \sqrt{2} \times 1.96 \hspace{1cm} (Equation \hspace{0.1cm} A2.3) $$

To determine the increase in reliability with number of repetitions, we divided the variance components of repetition and error variance by number of repetitions (Vet et al., 2011).
Table A2.1 Estimates of variance components ($\sigma^2$) of subject, repetition and the residual error variance for the knee angle at different knee moments.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>GMFCS</th>
<th>Sources of Variance</th>
<th>Degree of Freedom</th>
<th>$\sigma^2$ Knee angle at 0.5 Nm</th>
<th>1 Nm</th>
<th>2 Nm</th>
<th>3 Nm</th>
<th>4 Nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>TD</td>
<td>10.9±1.3 years</td>
<td></td>
<td>Subject</td>
<td>4</td>
<td>98.9</td>
<td>84.3</td>
<td>97.4</td>
<td>109.3</td>
<td>119.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Repetition</td>
<td>4</td>
<td>5.6</td>
<td>3.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Residual</td>
<td>20</td>
<td>8.4</td>
<td>4.2</td>
<td>1.9</td>
<td>2.1</td>
<td>2.0</td>
</tr>
<tr>
<td>SCP</td>
<td>13.9±0.6 years</td>
<td>GMFCS: I-II</td>
<td>Subject</td>
<td>4</td>
<td>32.2</td>
<td>42.0</td>
<td>56.0</td>
<td>68.1</td>
<td>79.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Repetition</td>
<td>4</td>
<td>3.2</td>
<td>0.1</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Residual</td>
<td>20</td>
<td>13.6</td>
<td>11.4</td>
<td>3.2</td>
<td>1.6</td>
<td>1.1</td>
</tr>
</tbody>
</table>

$TD = \text{typically developing children}, SCP = \text{spastic cerebral palsy}, GMFCS = \text{Gross Motor Function Classification System}$. Units for $\sigma^2$ knee angle at 0-4 Nm are degree$^2$.

Table A2.2 Intraclass correlation coefficient (ICC) and smallest detectable difference (SDD) for 1-5 repetitions.

| Group     | rep | Knee angle at 0.5 Nm | ICC | SDD | ICC | SDD | ICC | SDD | ICC | SDD | ICC | SDD | ICC | SDD |
|-----------|-----|----------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| TD n=5    | 1   |                      | 0.88| 10.4| 0.92| 7.4 | 0.98| 3.8 | 0.98| 4.0 | 0.98| 4.0 |     |     |
|           | 2   |                      | 0.93| 7.3 | 0.96| 5.3 | 0.99| 2.7 | 0.99| 2.8 | 0.99| 2.8 |     |     |
|           | 3*  |                      | 0.95| 6.0 | 0.97| 4.3 | 0.99| 2.2 | 0.99| 2.3 | 0.99| 2.3 |     |     |
|           | 4   |                      | 0.97| 5.2 | 0.98| 3.7 | 1.00| 1.9 | 1.00| 2.0 | 1.00| 2.0 |     |     |
|           | 5   |                      | 0.97| 4.6 | 0.98| 3.3 | 1.00| 1.7 | 1.00| 1.8 | 1.00| 1.8 |     |     |
| SCP n=5   | 1   |                      | 0.65| 11.4| 0.79| 9.4 | 0.94| 5.2 | 0.97| 3.8 | 0.98| 3.1 |     |     |
|           | 2   |                      | 0.79| 8.1 | 0.88| 6.7 | 0.97| 3.7 | 0.99| 2.7 | 0.99| 2.2 |     |     |
|           | 3*  |                      | 0.85| 6.6 | 0.92| 5.4 | 0.98| 3.0 | 0.99| 1.8 | 0.99| 1.8 |     |     |
|           | 4   |                      | 0.88| 5.7 | 0.94| 4.7 | 0.98| 2.6 | 0.99| 1.9 | 1.00| 1.6 |     |     |
|           | 5   |                      | 0.90| 5.1 | 0.95| 4.2 | 0.98| 2.3 | 0.99| 1.7 | 1.00| 1.4 |     |     |

$TD = \text{typically developing children}, SCP = \text{spastic cerebral palsy}$. ICC is dimensionless (0-1). Units of SDDs are in degree. *Averaging over three repetitions is sufficient to assess the net knee moment-angle curve, with more repetitions SDDs only slightly improve.
Assessment of net knee moment-angle characteristics

Results

Repeating net knee moment-angle measurements five times within one session (i.e. subject stays within the setup) resulted in similar net knee moment-angle characteristics (for typical examples, see Figure 2.1). The estimates of the variance components revealed that most of the variance in knee angle at particular net knee flexion moments was explained by subject (i.e. variance of interest), with maximum values for subject variance at 4 Nm. Residual error variance was the main source of error. Values of residual error variance were similar for moments ranging from 2-4 Nm and substantially higher at 0.5 and 1 Nm. Estimates of variance components are reported in Table A2.1.

At all net knee flexion moments tested, a single repetition yielded ICCs higher than 0.65 and SDDs ranging from 3.1° to 11.4°. Differences in ICCs between SCP and TD were similar (Table A2.2).

Conclusion

At all net knee flexion moments tested, averaging knee angles over three repetitions resulted in ICCs higher than 0.85. These data indicate that three repetitions are sufficient to assess measurement error of net knee moment-angle characteristics between days and sessions.
Freehand three-dimensional ultrasound to assess semitendinosus muscle morphology

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Jaap Harlaar
Jules G. Becher
Annemieke I. Buizer
Richard T. Jaspers

Chapter 3

ABSTRACT

In several neurologic disorders and muscle injuries, morphological changes of the musculus semitendinosus (ST) are presumed to contribute to movement limitations around the knee. Freehand three-dimensional (3D) ultrasound (US), using position tracking of two-dimensional US images to reconstruct a 3D voxel array, can be used to assess muscle morphology in vivo.

The aims of this study were (1) to introduce a newly developed 3D US protocol for ST and (2) provide a first comparison of morphological characteristics determined by 3D US with those measured on dissected cadaveric muscles.

Morphological characteristics of ST (e.g. muscle belly length, tendon length, fascicle length and whole muscle volume and volumes of both compartments) were assessed in six cadavers using a 3D US protocol. Subsequently, ST muscles were removed from the body to measure the same morphological characteristics.

Mean differences between morphological characteristics measured by 3D US and after dissection were smaller than 10%. ICCs were higher than 0.75 for all variables except for the lengths of proximal fascicles (ICC=0.58). Measurement of volume of proximal compartment by 3D US were not feasible, due to low US image quality proximally.

We conclude that the presented 3D US protocol allows for reasonably accurate measurements of key morphological characteristics of ST muscle.
Assessing semitendinosus muscle morphology

Introduction

The semitendinosus muscle (ST) is one of the medial hamstring muscles, spanning the hip as well as the knee joint. Morphological changes of ST are observed in several central neurologic disorders (e.g. cerebral palsy, stroke, multiple sclerosis, traumatic brain injury) (Gage et al., 2009, Martin et al., 2006, Keenan et al., 1988), following sport injuries (Silder et al., 2008) as well as after surgery (e.g. anterior cruciate ligament reconstruction with the ST tendon) (Nishino et al., 2006) and are presumed to contribute to movement limitations around the knee. As the morphology of a muscle is a major determinant of its mechanical properties (Gans and Bock, 1965, Gans and Gaunt, 1991, Huijing, 1996, Brand et al., 1986, Woittiez et al., 1983, Winters et al., 2011), knowledge of ST morphology may improve our understanding of ST function. Freehand three-dimensional ultrasound (3D US) has been shown to allow for accurate and standardized assessment of: (1) length of morphological variables (Barber et al., 2009, Fry et al., 2004, Weide et al., 2015), and (2) muscle volume (Weller et al., 2007, Barber et al., 2009, MacGillivray et al., 2009). Currently for ST no specific 3D US protocol is available.

The ST is divided by a tendinous inscription (also referred to as raphe) into a proximal and a distal compartment (van der Made et al., 2013, Woodley and Mercer, 2005, Kellis et al., 2012, Lee et al., 1988, Markee et al., 1955). Fascicles in both ST compartments are arranged in series and exert force at a small angle with the aponeuroses (Ward et al., 2009, Kellis et al., 2009). To assess morphological characteristics of ST by 3D US, detailed knowledge about ST morphology needs to be taken into account.

The aims of this study were to (1) introduce a newly developed 3D US protocol for ST and (2) provide a first comparison of morphological characteristics determined by 3D US data with those measured on dissected cadaveric muscles.

Methods

Data were collected using six human cadavers (one female, five males, age at death 81.5±9.5 years, mean±standard deviation (SD)). The cadavers were obtained from the donation program of the Department of Anatomy and Neurosciences of the VU University Medical Center (VUmc), Amsterdam, The Netherlands. The bodies were formalin-preserved with hip and knee joints in extended position. Extension in the joint was not forced. Therefore, knee and hip joints were in a slightly flexed position (i.e. hip: 8.8°±1.8; knee: 4.2°±5.8°). To measure knee and hip angles an extendable goniometer with two moveable arms was used (Model 01135, Lafayette Instrument, US). Upper leg length, defined as the linear distance between the trochanter major and the most prominent part of the lateral epicondyle of the femur, was 39.0±3.2 cm and measured by a 1 meter ruler with mm increments.
Chapter 3

US imaging and volume reconstruction

Prior to anatomical dissection, US imaging of ST muscle was performed freehand using a B-mode US apparatus with a 5 cm linear probe (Technos MPX, ESAOTE S.p.A., Italy). The scanning area was covered with a thick layer (≈5 mm) of ultrasound gel (Transsound, EF Medica Sri, Italy) to improve image quality. A 30-40 seconds sequence of transverse US images (i.e. axial plane of the ST muscle) were collected starting distally at the ST tendon (i.e. at the point that the tendon could be sufficiently visualized in the popliteal fossa) ending at the origin on the ischial tuberosity. None of the ST muscles of the cadavers was wider than 5 cm; therefore we could measure all muscles by a single sweep using a 5 cm linear probe. The US images were sampled at a rate of 25 Hz using a video card and capturing software (miroVIDEO DC30; Pinnacle Systems Inc.). The position of each US image in 3D space was recorded by tracking the US probe (based on three-markers that were rigidly attached to it) using an Optotrak motion capture system (type 3020; Northern Digital, Waterloo, Canada). Positions of selected bony anatomical landmarks (i.e. most prominent part of the ischial tuberosity, lateral and medial epicondyle) were recorded prior to scanning using an optically six marker rigid body optotrak-pointer (also referred to as optotrak-probe) (Optotrak, Northern Digital, Waterloo, Canada). All three bony landmarks were identified by palpation and confirmed by US. If this was not possible for the ischial tuberosity, the location was confirmed by pricking a needle. Prior to 3D US imaging of the cadavers, the transformation matrix from the probe frame to the US images was determined within the US images by identifying a cross-point of two intersecting wires at a known position in a water cube. The cross of the wire was scanned from different positions and with different tilt angles of the US probe, while the cross-wire was kept visible within the recorded ultrasound image sequence (Prager et al., 1998). The propagation speed of sound in muscle tissue at body temperature (i.e. 1570 m/s) is different from that in water due to density differences and is temperature dependent. The temperature of the water was measured using a digital thermometer (GTH 175/PT, Greisinger, Germany) and the speed of sound in water was set according to the corresponding temperature. The cadaveric bodies were leave to warm up to the temperature in the dissection room (about 18° Celsius) for 4-6 hours and propagation speed was set to 1532 m/s, according to a temperature of 18° Celsius (Straube and Arthur, 1994), in the reconstruction process. No corrections were made for possible differences in propagation speed due to formalin preservation of the cadavers, because effects have been shown to be very low (Bamber et al., 1979, Baldwin et al., 2006).

A custom made program in Matlab (version R2014A, the Mathworks Inc.) was used to fill a 3D voxel array with pixels from the transverse US images (Bénard et al., 2011) for later analysis (see below). Missing information was interpolated using nearest neighbourhood interpolation. The size of a voxel was 0.2x0.2 mm for the transverse direction and approximately 0.5 mm longitudinally (dependent on the sliding velocity of the probe and, hence, the number of images collected).
Assessing semitendinosus muscle morphology

**Anatomical dissection to measure morphological characteristics of cadaveric ST**

After ultrasound imaging skin, subcutaneous tissues, gluteus muscle and fasciae of ST, semimembranosus and biceps femoris muscles were removed (Figure 3.1A). The location, where the distal ST tendon passed the knee axis of rotation (i.e. defined as the line between medial and lateral epicondyles), was marked with a suture on the ST tendon. Then ST was carefully dissected from its origin at the ischial tuberosity and at its insertion at the tibia as close as possible to the bone.

The following morphological characteristics of ST muscle-tendon unit (MTU) were measured: muscle belly length ($\ell_m$), the distance from ischial tuberosity to distal end of muscle belly), distal tendon length ($\ell_{t_{\text{dist}}}$), the distance from the distal end of the most distal fascicle to the insertion at the pes anserinus; distal tendon length proximal to the knee axis ($\ell_{t_{\text{dist}_p}}$) (i.e. the length of the distal tendon from the distal end of the most distal fascicles to the knee axis); and the length of the portion of the tendinous inscription that was visible externally of the muscle belly ($\ell_{t_{\text{external}}}$). In a pilot study we measured $\ell_m$ in five cadavers before and after removal of ST from the skeleton. Measurements confirmed that $\ell_m$ after dissection did not differ from that in situ (0.02±0.53 cm; $p=0.944$).

For further measurements, the muscle belly was longitudinally cut in two halves. The blade was orientated perpendicularly to the apical surface of the oval-shaped distal aponeurosis. The muscle was cut towards the proximal end of the tendinous inscription (Figure 3.1B). The longitudinal sections were positioned on a table, aligned with the distal and proximal ends and left in the shape defined by formalin fixation within the body (Figure 3.1C). Within the plane of the longitudinal section of ST, the following anatomical length variables were measured using a ruler with mm increments: distal aponeurosis length ($\ell_{a_{\text{dist}}}$), proximal aponeurosis length ($\ell_{a_{\text{prox}}}$), length of tendinous inscription ($\ell_{t_{i}}$), within the distal muscle compartment the lengths of the most distal ($\ell_{f_{\text{asc}_{\text{dist}_d}}}$), intermediate ($\ell_{f_{\text{asc}_{\text{dist}_m}}}$) and most proximal fascicles ($\ell_{f_{\text{asc}_{\text{dist}_p}}}$), and within the proximal compartment lengths of the most distal ($\ell_{f_{\text{asc}_{\text{prox}_d}}}$), intermediate ($\ell_{f_{\text{asc}_{\text{prox}_m}}}$) and most proximal fascicles ($\ell_{f_{\text{asc}_{\text{prox}_p}}}$) (Figure 3.1D). In addition, by using a protractor we determined the angle of the most distal fascicles of the distal compartment with the muscle line of pull ($\alpha_{\text{dist}}$), the angle of the distal aponeurosis with the muscle line of pull ($\beta_{\text{dist}}$), the angle of the most proximal fascicles of the proximal compartment with the muscle line of pull ($\alpha_{\text{prox}}$), and the angle of the proximal aponeurosis with the muscle line of pull ($\beta_{\text{prox}}$). The muscle line of pull was defined as the line between the proximal end of the most proximal fascicles and the distal end of the most distal fascicle (Figure 3.1D). Angle gamma ($\gamma$) was calculated by summing $\alpha$ and $\beta$ ($\gamma_{\text{dist}}$, $\gamma_{\text{prox}}$). $\ell_m$ and $\ell_{t_{\text{dist}_p}}$ were summed to assess MTU length up to the estimated knee axis ($\ell_{mtu}$).
Figure 3.1 Typical example of semitendinosus muscle (ST) in situ, of a mid-longitudinal transection of semitendinosus muscle (ST) and illustration of morphological characteristics. A: The ST was made visible by removing the skin, subcutaneous tissues, gluteus muscle and the fasciae of ST. Posterior view, proximal of knee axis. B: Photographic image demonstrating the transection. Starting from the distal end of the most distal fascicle, the blade was perpendicularly orientated on the apex of the oval-shaped distal aponeurosis. The muscle was cut in the direction of the proximal end of the tendinous inscription. C: Photographic image of longitudinal section of ST. D: 2D planimetric model of the morphological variables measured in the mid-longitudinal plane: distal aponeurosis (ℓa <sub>dist</sub>), proximal aponeurosis (ℓa <sub>prox</sub>), most distal fascicle of distal compartment (ℓfasc <sub>dist</sub>), most proximal fascicles of distal compartment (ℓfasc <sub>dist</sub>), most distal fascicle of proximal compartment (ℓfasc <sub>prox</sub>), most proximal fascicles of proximal compartment (ℓfasc <sub>prox</sub>), intermediate fascicles (ℓfasc <sub>dist</sub> <sup>dist</sup> ℓfasc <sub>prox</sub> <sup>dist</sup> ℓfasc <sub>prox</sub> <sup>prox</sup> ℓfasc <sub>dist</sub> <sup>prox</sup> ℓfasc <sub>prox</sub> <sup>dist</sup>) and tendinous inscription (ℓti). Grey dotted line indicates the muscle line of pull, defined as the line between the proximal end of the most proximal fascicles and the distal end of the most distal fascicle. Blue dotted line indicates the location for the transversal section of ST for assessment of anatomical cross-sectional area (ACSA). TI=tendinous inscription; l=lateral; m=medial; d=distal; p=proximal.
After measuring the morphological characteristics within the mid-longitudinal plane, the muscle was cut perpendicularly to its length at the middle of the projection of the distal and proximal ends of the tendinous inscription. A photograph of the anatomical cross-section (ACSA) was taken while a millimeter scale was positioned in the image plane. The ACSA was measured from the image using the open source imaging software Fiji (http://fiji.sc) (Schindelin et al., 2012, Schneider et al., 2012). Subsequently, the general fascia, as well as the distal tendon were removed, leaving solely the muscle belly including the distal and proximal aponeuroses.

The proximal and distal compartments of ST were separated by carefully cutting the myotendinous connections of the fascicles from the distal side of the tendinous inscription. The volumes of the distal ($V_{\text{vol, dist}}$) and proximal compartments ($V_{\text{vol, prox}}$) were measured by submerging them in distilled water ($\approx 20^\circ\text{C}$) within a calibrated measuring cylinder. $V_{\text{vol, dist}}$ and $V_{\text{vol, prox}}$ were summed up to obtain the total muscle belly volume of ST ($V_{\text{vol}}$). The lengths of the most distal, intermediate and most proximal fascicles of distal and proximal compartment were summed. The mean of these summed fascicles at each location was calculated and represented the mean fascicle length of the whole muscle ($f_{\text{fasc, average}}$).

From each compartment two fascicle segments of at least 3 cm were carefully dissected from a medial and a lateral location and stored in a formalin and ethanol based solution for later assessment of sarcomere length. From each of these stored fascicles, small fibre bundles were dissected and placed on a microscopic slide. Every three millimetres photographs were taken using a digital camera (MikroCam 5 MP, Bresser, Rhede, Germany) mounted on a microscope (Ortholux II, Leitz, Wetzlar, Germany). The method used to assess sarcomere lengths has recently been described (Tijs et al., 2015). The distribution of grey scale values for a single fibre on each photograph was determined using the plot profile plugin in Fiji. The grey scale signal was filtered (Butterworth, 5th order) to exclude sarcomere lengths above 4 µm (cut-off frequency: 0.25 sarcomeres per µm) and below 1.5 µm (cut-off frequency: 0.67 sarcomeres per µm). A discrete Fourier transformation was performed in Matlab to determine the number of sarcomeres in series. Subsequently, fibre mean sarcomere lengths were calculated by dividing total fibre length by the number of sarcomeres. If the count of number of sarcomeres in series was not possible automatically due to noise in the sinusoidal wave, measurement of sarcomere lengths was performed using FFT plugin in Fiji. Fibre mean sarcomere lengths were first averaged across fibres from each location within the compartment, then across compartment locations and, subsequently, across both compartments ($f_{\text{sarc}}$). Fascicle length at estimated optimum sarcomere length ($f_{\text{fasc, average, optimum}}$) was calculated by the following equation.

$$f_{\text{fasc, average, optimum}} = f_{\text{fasc, average}} \times (2.7\ \mu\text{m} / f_{\text{sarc}}) \quad \text{(Equation 3.1)}$$

For humans a sarcomere length of 2.7 µm is in between the range that has been described for optimum sarcomere length, 2.67-2.81 µm (Rassier et al., 1999, Walker and
Schrodt, 1974) and 2.60-2.80 µm (Lieber et al., 1994). The physiological cross-sectional area (PCSA) of ST, defined as the cross-sectional area of all muscle fibres arranged in parallel, was calculated by dividing \( \text{Vol} \) by \( \ell_{\text{fasc}} \) (PCSA) and by \( \ell_{\text{fasc}} \) (PCSA\text{optimum}), respectively (Alexander and Vernon, 1975). PCSA and PCSA\text{optimum} were calculated for both compartments as well as for the whole muscle.

Figure 3.2 Typical example of segmented semitendinosus muscle (ST) based on transversal images. ST segmented into the proximal (ST\text{prox}) (red) and distal compartment (ST\text{dist}) (yellow), which are separated by the tendinous inscription (TI) (green) viewed from posterior-medial. Above three representative transversal US images used for segmentation. The concave TI is indicated by a green arrow, visible between the two compartments, the distal aponeurosis (apo\text{dist}) – also shaped concavely - by a white arrow. Segmentation was performed on transversal images every 5 mm along the muscle belly. Below, the image shows the TI (green) and the distal aponeurosis (white) which are orientated in parallel to each other, but opposite in orientation of their concave shapes. Note, that the image of this example for the method section was taken from a subject in vivo. In the voxel arrays of the cadavers a segmentation of the proximal compartment was not possible. SM=semimembranosus muscle; BF=Biceps femoris muscle; l=lateral; m=medial; p=posterior; a=anterior.
Assessing semitendinosus muscle morphology

Image analysis 3D US

We developed a 3D US protocol to measure the following subset of morphological characteristics taking into account the two compartments of the ST: ℓm, ℓt<sub>dist_p</sub>, ℓa<sub>dist</sub>, ℓfasc<sub>dist_d</sub>, ℓfasc<sub>dist_p</sub>, ℓfasc<sub>prox_p</sub>, Vol<sub>dist</sub>, Vol<sub>prox</sub> and ACSA.

Muscle structures (i.e. tendon, distal aponeurosis, tendinous inscription) were identified by visual inspection in three dimensions within the voxel array using open source software Chimera 1.9 (http://www.cgl.ucsf.edu/chimera) (Pettersen et al., 2004) (Figure 3.2). Segmentation of ST for assessment of muscle volume was performed using the Segmentation Editor Plugin (http://fiji.sc/Segmentation_Editor) in Fiji. The outline of ST was encircled in transverse images every 5 mm, interpolating the gaps between the segmented images (Weller et al., 2007). Muscle volume was measured using the volume measurement tool in Chimera 1.9. We planned to segment distal and proximal compartments of the ST and assess Vol<sub>dist</sub> and Vol<sub>prox</sub> (Figure 3.2). However for all cadavers full segmentation of the Vol<sub>prox</sub> was not feasible due to insufficient image quality of the most proximal part within the voxel array.

In order to measure the above specified variables, x, y, z coordinates of the following points were determined within the voxel array in combination with positions of the bony landmarks obtained from the optotrak registration (Figure 3.3): ischial tuberosity, most proximal and distal ends of the tendinous inscription, proximal end of the distal aponeurosis, the distal end of the most distal fascicle and the most distal point of the distal tendon (proximal of the knee axis). In addition, direction vectors were defined to describe a line between the distal end of the most distal fascicle (indicated as point 7 in Figure 3.3) and the most distal visible point of the distal tendon (indicated as point 8 in Figure 3.3) (i.e. ‘line of tendon’) and a line between the medial and lateral epicondyles (i.e. ‘line of estimated knee axis’) (Figure 3.3). The point along the ‘line of tendon’ from which the distance to the ‘line of estimated knee axis’ was smallest, was taken as estimate of the crossing of the distal tendon with the knee axis (indicated as point 9 in Figure 3.3). Distances between x, y, z coordinates of above mentioned points were used to define ℓm, ℓt<sub>dist_p</sub>, ℓa<sub>dist</sub>, ℓfasc<sub>dist_d</sub>, ℓfasc<sub>dist_p</sub>, ℓfasc<sub>prox_p</sub> (Figure 3.3).

Determination of the ischial tuberosity within the voxel array was not always possible. If the ischial tuberosity was not visible, the position obtained from the optotrak registration was used for further calculations. The ACSA was measured at the middle of the tendinous inscription, perpendicular to the muscle line of pull. PCSA<sub>dist</sub> was calculated by dividing Vol<sub>dist</sub> by the average of both measured distal fascicles (i.e. ℓfasc<sub>dist_d</sub>, ℓfasc<sub>dist_p</sub>).
Figure 3.3 Illustration of anatomical points used for analysing three-dimensional ultrasound (3D US) imaging of semitendinosus muscle (ST). Bony landmarks (blue) and points at the ST (red): 1: Ischial tuberosity; 2: Medial epicondyle; 3: Lateral epicondyle; 2-3: Estimated knee axis; 4: Proximal end of the tendinous inscription; 5: Distal end of the tendinous inscription; 6: Proximal end of the distal aponeurosis; 7: Distal end of the most distal fascicle (i.e. distal end of muscle belly); 8: Most distal point of ST tendon visible by ultrasound; 9: Point where tendon passes knee axis; Variables measured as distance between points: 1-7: Muscle belly length ($\ell_m$), 1-4: Most proximal fascicle of proximal compartment ($\ell_{fasc_{prox_p}}$), 4-6: Most proximal fascicle of distal compartment ($\ell_{fasc_{dist_p}}$), 5-7: Most distal fascicle of distal compartment ($\ell_{fasc_{dist_d}}$), 6-7: Distal aponeurosis ($\ell_a$) and 7-9: Distal tendon length till estimated knee axis ($\ell_{tt_{dist_p}}$). Note, that the image of this example for the method section was taken from a subject in vivo. In the voxel arrays of the cadavers, a segmentation of the proximal compartment was not possible.
Assessing semitendinosus muscle morphology

Assessment of the x, y, z coordinates of points at ST within the voxel array (i.e., points 4-8 in Figure 3.3) was performed five times by the same observer. Segmentations of the volumes and measurement of ACSA were repeated three times by the same observer. After five repetitions for assessment of x, y, z coordinates of points and three repetitions of analysing volume of distal compartment and ACSA, the increase of intraclass correlation coefficients (ICC) and the decrease of standard error of measurement (SEM) were minimal. Therefore, these numbers of repetitions were deemed sufficient. ICCs based on five and three repetitions, respectively, were higher than 0.90, SEMs were between 0.5-4 mm for the point assessment, 1.5 cm³ for the distal volume and 0.2 cm² for the ACSA. The means of x, y, z coordinates of five observations for the point assessment and of three repetitions of segmentations of the distal volume and measurement of ACSA were used for further calculations.

Treatment of data and statistics

Differences between morphological characteristics assessed by 3D US and after dissection were calculated and compared by Paired-Samples T-test. To assess how comparable morphological variables assessed by 3D US are to those measured after dissection, 95% limits of agreement (95% LoA) and ICCs were calculated. The 95% LoAs were calculated with the Bland and Altman method using the mean systematic difference between 3D US and dissection (\(\bar{d}\)) and the standard deviation of these difference (SD\(_{dif}\)) (Bland and Altman, 1986).

\[
95\% \text{ LoA} = \bar{d} \pm 1.96 \times \text{SD}_{\text{dif}} \quad \text{(Equation 3.2)}
\]

ICC for a single measurement was calculated using variance components of subject (i.e., cadaver) and method (i.e., 3D US and dissection) determined by a Restricted Maximum Likelihood Estimation approach (Vet et al., 2011).

For variables only assessed after dissection, two-way repeated measures ANOVAs were used to test for differences in fascicle length between compartments (proximal and distal) and between intra-compartmental locations (most distal, most proximal and intermediate). As a follow-up test for significant interaction effects, Paired sample T-tests with Bonferroni corrections were performed. The difference in ACSA and PCSA both assessed after dissection was determined by Paired-samples T-tests. Paired-samples T-tests were also used to test whether \(\alpha\), \(\beta\), \(\gamma\), \(Vol\), PCSA, \(\text{PCSA}_{\text{optimum}}\), \(t_a\), \(t_{sarc}\), \(t_{\text{fasc,average, optimum}}\) differed between proximal and distal compartments. The 95% confidence interval was calculated, along with Cohen’s \(d_z\) determined from the t-value and the sample size (\(n\)) as a measure of effect size (Lakens, 2013).

\[
\text{Cohen’s } d_z = \frac{t}{\sqrt{n}} \quad \text{(Equation 3.3)}
\]
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Power using Cohen’s dz and alpha=0.05 were calculated post hoc (G*Power, Germany) (Faul et al., 2007) for all variables where a proximal-distal difference was assessed. The level of significance was set at 0.05 for all statistical tests. Values are presented as mean±SD.

Results

Comparison of the morphological characteristics assessed using 3D US and after dissection

All morphological variables as described in the method section could be analysed by 3D US in four cadavers. In two cadavers assessment of x, y, z coordinates of the proximal end of the distal aponeurosis and/or the proximal end of the tendinous inscription was not feasible and therefore related variables could not be assessed (Table 3.1).

Table 3.1 Morphological characteristics of semitendinosus muscle (ST) of six cadavers obtained by three-dimensional ultrasound (3D US) and after dissection

<table>
<thead>
<tr>
<th>Morphological characteristics</th>
<th>n</th>
<th>3D US</th>
<th>Dissection</th>
<th>Difference between methods</th>
<th>ICC</th>
<th>95% upper LoA</th>
<th>95% lower LoA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ℓmtu</td>
<td>6</td>
<td>38.4±3.3 cm</td>
<td>37.7±2.5 cm</td>
<td>-0.7 cm (1.9%)</td>
<td>0.87</td>
<td>2.1 cm</td>
<td>-3.5 cm</td>
</tr>
<tr>
<td>Vol_dist</td>
<td>5</td>
<td>38.4±7.4 cm³</td>
<td>38.4±7.5 cm³</td>
<td>0.0 cm³ (0.0%)</td>
<td>0.96</td>
<td>4.6 cm³</td>
<td>-4.6 cm³</td>
</tr>
<tr>
<td>ℓm</td>
<td>6</td>
<td>31.6±2.6 cm</td>
<td>31.3±1.7 cm</td>
<td>-0.3 cm (1.0%)</td>
<td>0.83</td>
<td>2.4 cm</td>
<td>-3.0 cm</td>
</tr>
<tr>
<td>ℓt_dist_p</td>
<td>6</td>
<td>6.8±1.0 cm</td>
<td>6.4±1.2 cm</td>
<td>-0.4 cm (6.6%)</td>
<td>0.80</td>
<td>0.8 cm</td>
<td>-1.6 cm</td>
</tr>
<tr>
<td>ℓa_dist</td>
<td>5</td>
<td>14.0±2.7 cm</td>
<td>13.2±3.7 cm</td>
<td>-0.8 cm (6.2%)</td>
<td>0.91</td>
<td>1.6 cm</td>
<td>-3.2 cm</td>
</tr>
<tr>
<td>ℓfasc_dist_d</td>
<td>6</td>
<td>12.3±1.7 cm</td>
<td>11.3±1.7 cm</td>
<td>-1.0 cm* (8.9%)</td>
<td>0.75</td>
<td>0.8 cm</td>
<td>-2.8 cm</td>
</tr>
<tr>
<td>ℓfasc_dist_p</td>
<td>4</td>
<td>8.3±1.3 cm</td>
<td>8.3±1.2 cm</td>
<td>0.0 cm (0.4%)</td>
<td>0.99</td>
<td>0.4 cm</td>
<td>-0.3 cm</td>
</tr>
<tr>
<td>ℓfasc_prox_p</td>
<td>5</td>
<td>9.2±2.5 cm</td>
<td>8.9±1.0 cm</td>
<td>-0.3 cm (3.3%)</td>
<td>0.58</td>
<td>3.4 cm</td>
<td>-4.0 cm</td>
</tr>
<tr>
<td>ACSA</td>
<td>5</td>
<td>4.6±1.2 cm²</td>
<td>4.2±1.0 cm²</td>
<td>-0.4 cm² (9.5%)</td>
<td>0.91</td>
<td>0.1 cm²</td>
<td>-0.9 cm²</td>
</tr>
<tr>
<td>PCSA_dist</td>
<td>4</td>
<td>4.0±1.1 cm²</td>
<td>4.2±1.3 cm²</td>
<td>+0.2 cm² (6.0%)</td>
<td>0.90</td>
<td>1.3 cm²</td>
<td>-0.8 cm²</td>
</tr>
</tbody>
</table>

* significantly different between dissection and 3D US (p<0.05); Values in parenthesis are percentages of the dissection mean group values. ICC=intraclass correlation coefficients; LoA=limits of agreement; ℓmtu=length of muscle-tendon unit till estimated knee axis as the sum of ℓm and ℓt_dist_p; Vol_dist=muscle volume of the distal compartment; ℓm: length muscle belly: ischial tuberosity to distal muscle tendinous junction; ℓt_dist_p=length of distal tendon proximal of the estimated knee axis; ℓfasc_dist_d=most distal fascicle of distal compartment; ℓfasc_dist_p=most proximal fascicles of distal compartment; ℓfasc_prox_p=most proximal fascicles of proximal compartment; ACSA=anatomical cross-sectional area; PCSA_dist=physiological cross-sectional area of distal compartment; PCSA_dist=(Vol_dist/((ℓfasc_dist_d+ℓfasc_dist_p)/2));

3D US determined ℓmtu, Vol_dist, ℓm, ℓfasc_prox_p and ℓfasc_dist_p differed less than 5% from those assessed after dissection, while estimates of ACSA, PCSA_dist, ℓt_dist_p, ℓa_dist and ℓfasc_dist_d were less than 10% different (Table 3.1). For all variables assessed, 3D US obtained measures were not significantly different from those measured in the dissected muscle,
Assessing semitendinosus muscle morphology except for the most distal fascicles of the distal compartment of ST (Table 3.1). Fascicles lengths at that location were systematically overestimated by 3D US by a mean value of 1.0±0.9 cm (i.e. 8.9%, p=0.045). ICCs were higher than 0.75 for all variables except for fascicle length measured within the proximal compartment (ICC=0.58, Table 3.1).

**Morphology ST determined after dissection**

ST MTUs, that were assessed after cadaveric dissection, consisted for one-third of tendon (ℓₜ₉₀=15.0±2.0 cm) and for two-third of muscle belly (ℓₘ=31.3±1.75 cm). ACSA and PCSA did not differ (p=0.283, Table 3.2).

**Table 3.2 Morphological characteristics of semitendinosus muscle (ST) from cadaveric dissection from six cadavers for distal and proximal compartment (mean ± standard deviation, 95% confidence interval (CI), and effect size (Cohen’s d)).**

<table>
<thead>
<tr>
<th></th>
<th>Proximal compartment</th>
<th>Distal compartment</th>
<th>Whole muscle</th>
<th>Difference between compartments p-value</th>
<th>95% CI</th>
<th>Effect size</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol (cm³)</td>
<td>39.0±8.3</td>
<td>38.0±6.8</td>
<td>77.0±15.1</td>
<td>0.607</td>
<td>-3.7 to 5.7</td>
<td>0.2</td>
<td>0.07</td>
</tr>
<tr>
<td>ℓₚ (cm)</td>
<td>11.3±1.7</td>
<td>13.0±3.3</td>
<td>n/a</td>
<td>0.077</td>
<td>-3.7 to 0.3</td>
<td>0.9</td>
<td>0.44</td>
</tr>
<tr>
<td>ℓₕₚ(cm)</td>
<td>8.9±0.9</td>
<td>8.0±1.2</td>
<td>16.9±1.1</td>
<td>0.684</td>
<td>-0.9 to 2.8</td>
<td>0.6</td>
<td>0.20</td>
</tr>
<tr>
<td>ℓₕₚ(cm)</td>
<td>8.1±1.2</td>
<td>9.7±1.7</td>
<td>17.8±2.1</td>
<td>0.291</td>
<td>-3.8 to 0.4</td>
<td>0.8</td>
<td>0.38</td>
</tr>
<tr>
<td>ℓₕₚ(cm)</td>
<td>7.0±1.5</td>
<td>11.3±1.7</td>
<td>18.3±1.9</td>
<td>0.027</td>
<td>-7.0 to -1.7</td>
<td>1.7</td>
<td>0.91</td>
</tr>
<tr>
<td>ℓₕₚ(cm)</td>
<td>8.0±0.8</td>
<td>9.7±1.2</td>
<td>17.7±1.1</td>
<td>0.061</td>
<td>-3.4 to 0.1</td>
<td>1.0</td>
<td>0.49</td>
</tr>
<tr>
<td>ℓₕₚ(cm)</td>
<td>7.2±0.10</td>
<td>3.00±0.19</td>
<td>2.91±0.11</td>
<td>0.079</td>
<td>-0.40 to 0.03</td>
<td>0.9</td>
<td>0.43</td>
</tr>
<tr>
<td>ℓₕₚ(cm)</td>
<td>7.7±0.7</td>
<td>8.7±1.0</td>
<td>16.4±0.8</td>
<td>0.140</td>
<td>-2.6 to 0.5</td>
<td>0.7</td>
<td>0.30</td>
</tr>
<tr>
<td>α (○)</td>
<td>1.7±3.2</td>
<td>10.2±5.2</td>
<td>n/a</td>
<td>0.032</td>
<td>1.7 to 24.9</td>
<td>1.2</td>
<td>0.65</td>
</tr>
<tr>
<td>β (○)</td>
<td>18.0±10.4</td>
<td>4.8±3.2</td>
<td>n/a</td>
<td>0.036</td>
<td>-16.3 to -0.8</td>
<td>1.2</td>
<td>0.63</td>
</tr>
<tr>
<td>γ (○)</td>
<td>19.7±8.4</td>
<td>15.0±5.0</td>
<td>n/a</td>
<td>0.116</td>
<td>-1.7 to 11.2</td>
<td>0.8</td>
<td>0.34</td>
</tr>
<tr>
<td>ACSA (cm²)</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>PCSA (cm²)</td>
<td>4.9±0.9</td>
<td>4.0±1.1</td>
<td>4.4±1.0</td>
<td>0.009</td>
<td>0.3 to 1.3</td>
<td>1.7</td>
<td>0.91</td>
</tr>
<tr>
<td>PCSAoptimum (cm²)</td>
<td>5.1±0.9</td>
<td>4.4±1.0</td>
<td>4.7±0.9</td>
<td>0.041</td>
<td>0.4 to 1.3</td>
<td>1.1</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Vol=muscle volume; ℓₚ=length aponeurosis; ℓₕₚ=length most proximal fascicle ℓₕₚ=length intermediate fascicle; ℓₕₚ=length most distal fascicle; ℓₕₚ=average ℓₕₚ, ℓₕₚ, ℓₕₚ and ℓₕₚ; ℓₜₚ=mean length of sarcomeres; ℓₕₚ=average optimum calculated at optimum ℓₜₚ (i.e. 2.7 µm); α=angle of the muscle line of pull with fascicles; β =angle of the muscle line of pull with the aponeurosis; γ= α + β ; ACSA, anatomical cross-sectional area; PCSA=physiological cross-sectional area; PCSA=Vol/ℓₕₚ; PCSAoptimum = Vol/ℓₕₚoptimum².
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Repeated measures ANOVA neither revealed significant differences in fascicle lengths between compartments (i.e. proximal and distal) ($p=0.061$) nor between locations within the compartments (i.e. most distal, intermediate and most proximal fascicles) ($p=0.377$). However, a significant interaction effect between intra-compartmental location and compartment was shown ($p=0.001$). Follow-up analysis indicated a significant length difference between compartments only for the most distal fascicles of both compartments ($\ell_{\text{fasc}_d}$, Table 3.2).

Volumes of the proximal and distal compartments were not different ($p=0.607$). Also, for $\ell_{\text{sarc}}$ and $\ell_{\text{fasc}_{\text{average optimum}}}$ no differences between both compartments were found ($p=0.079$ and $p=0.140$, respectively). However, PCSA and $\text{PCSA}_{\text{optimum}}$ of the proximal compartment were significantly larger than those of the distal compartment ($p=0.009$ and $p=0.041$, Table 3.2). Measurements of angles $\alpha$ and $\beta$ revealed small differences between proximal and distal compartments (i.e. $\alpha_{\text{prox}} < \alpha_{\text{dist}}$, $p=0.036$ and $\beta_{\text{prox}} > \beta_{\text{dist}}$, $p=0.032$). Angle $\gamma$, did not differ between compartments ($p=0.116$). Effect sizes (Cohen’s $d_z$) were 0.6 or higher for all morphological variable between proximal and distal compartment, except for volume (Table 3.2).

Discussion

In this study, we introduce a novel 3D US protocol to assess morphology of ST in vivo. Comparison of morphological variables obtained after dissection and by 3D US revealed that the proposed 3D US protocol allows for reasonably accurate estimates of key morphological characteristics of ST muscle. ICCs for $\ell_{\text{mtu}}$, $\ell_m$ and $\ell_{\text{fasc}}$ were at least similar or higher than those reported previously for 2D ultrasound measurements of ST (Kellis et al., 2009), based on the same number of cadaveric muscles.

Our estimates of ST morphological variables obtained using 3D US imaging did not differ from those measured after cadaveric dissection, except for $\ell_{\text{fasc}_{\text{dist,d}}}$ which was systematically overestimated. $\ell_{\text{fasc}_{\text{dist,d}}}$ was defined as the distance between the distal end of the most distal fascicle and distal end of the tendinous inscription. The distal end of the most distal fascicle was also used for calculation of $\ell_m$ and $\ell_{\text{dist,p}}$, for which no significant difference between both types of measurements were found. Therefore, we conclude that the overestimation of $\ell_{\text{fasc}_{\text{dist,d}}}$ was caused by erroneously localization of distal end of the tendinous inscription proximal to its actual location. This may occur because the tendinous inscription is distally very closely aligned with the epimysium. Within the voxel array the tendinous inscription can only be visually distinguished from the epimysium when the tendinous inscription is clearly diverging from the epimysium.

For the proximal fascicle length ($\ell_{\text{fasc}_{\text{prox,p}}}$), the ICC was relatively low (0.58). Estimation of $\ell_{\text{fasc}_{\text{prox,p}}}$ was most likely affected by a lower quality of images from the most proximal region of ST compared to those from the middle and distal regions. This made it more difficult to assess $x$, $y$, $z$ coordinates of the points (i.e. proximal end of the tendinous inscription and
Assessing semitendinosus muscle morphology

within the voxel array. Also due to the limited image quality, we could not
determine the volume of the proximal compartment using 3D US. The lower image quality
proximally compared to distally was probably caused by a thicker layer of subcutaneous
tissue and by the m. gluteus. US image quality of muscle tissue in vivo is known to be higher
than in formalin-preserved cadavers (Tsui et al., 2007). In addition, estimates of positions
of bony landmarks (especially the location of the ischial tuberosity) in vivo is expected to be
more accurate than in cadavers, because in live subjects these landmarks can be identified
easier by palpation (i.e. due to more compliant tissues and flexibility of joints). Regarding the
estimation of the proximal ST volume, we expect that this can also be estimated accurately,
if the borders of the muscle are sufficiently visible and, thus, can be segmented within the
voxel array. This expectation is confirmed by results for the distal compartment and by other
studies, that have shown that if a muscle can be accurately segmented valid measurements
of muscle volume of different muscles are possible (Weller et al., 2007, Barber et al., 2009,
MacGillivray et al., 2009). Segmentation of the proximal volume of ST is expected to be
limited also in vivo in subjects with a very thick layer of subcutaneous tissue and/or a large
m. gluteus.

ACSA was measured perpendicular to the line of pull. Since the angle of the
distal fascicles with the line of pull was shown to be very low, ACSA of ST did not differ from
PCSAs in the cadavers using 3D US, we could only assess PCSA of the distal compartment. However, if the proximal volume of the ST
within the proximal compartment only the length of the most proximal fascicles (i.e. ℓfasc prox p) can be assessed by 3D US. Because of the non-uniformity of fascicle length within
compartments, ℓfasc prox p cannot be used to assess PCA prox. The observation that fascicle
lengths within a compartment are not uniform is in line with local differences in sarcomere
numbers in other human leg muscles (Huijing, 1985). When assessing fascicle lengths and
PCSAs of each ST compartment, this non-uniformity in length has to be considered.

No differences in in fascicle lengths between distal and proximal compartment were
found, except for most distal fascicles at both compartments (ℓfasc). Similar fascicles
lengths within the two compartments (Kellis et al., 2012, Woodley and Mercer, 2005,
Wickiewicz et al., 1983), as well as shorter fascicle lengths in the proximal compared to
the distal compartment (Markee et al., 1955) have been reported. However, comparison of
results between present data and those of previous studies regarding differences in fascicle
lengths within and between compartments are hampered by a lack of standardization of
the location of the measured fascicles or differences in the dissection plane. The absence
of significant proximal-distal differences in the current and above described studies may
have been the result of low statistical power. Based on the observation that no differences
were found between proximal and distal volumes, while PCSAs of both compartments were
significantly different, one would expect also a difference in fascicle length (ℓfasc average optimum)
between the compartments. Power calculations using the effect size (Table 3.2), alpha=0.05 and beta=0.80 (G*Power, Germany) (Faul et al., 2007) revealed that at least 19 subjects would have been required to confirm proximal-distal differences for $l_a$, $f_{fasc}$, $f_{fasc\text{-average}}$, and $f_{fasc\text{-average\_optimum}}$ (12, 15, 10 and 19 subjects, respectively).

Muscle belly length, tendon length, fascicle length as well as sarcomere length of our specimen were comparable to those reported previously (van der Made et al., 2013, Kellis et al., 2010, Kellis et al., 2009, Ward et al., 2009, Woodley and Mercer, 2005, Friederich and Brand, 1990, Wickiewicz et al., 1983, Markee et al., 1955, Cutts, 1988). Note that morphological variables of ST in the current study and in most of the previous studies were determined in cadavers which were at high age. This should be taken into account when absolute values are compared with subjects of younger age, but it does not bias the comparison between 3D US and dissection. Particularly, PCSA and muscle volumes of ST can be expected to be larger for younger people (Nishino et al., 2006). Assuming that aging effects are comparable at different locations and compartments of ST, the presented relative differences and similarities between and within compartments of ST are representative in all age groups.

**Possible applications**

The proposed 3D US protocol yields promising results to measure important morphological variables of ST in vivo. Multiple freehand 3D US measurements can be easily performed, for example at different knee and hip joint angles (Haberfehlner et al., 2015). Measurements of lengths of muscle and tendon may provide insight in differences in their stiffness. This information is relevant to understand the effects of alterations of ST muscle on movement limitations around the knee in patients (e.g. cerebral palsy or patients after an anterior cruciate ligament reconstruction using the ST tendon).

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CHAPTER 4

Knee moment-angle characteristics and semitendinosus muscle morphology in children with spastic paresis selected for medial hamstring lengthening

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ABSTRACT

To increase knee range of motion and improve gait in children with spastic paresis (SP), the semitendinosus muscle (ST) amongst other hamstring muscles is frequently lengthened by surgery, but with variable success. Little is known about how the pre-surgical mechanical and morphological characteristics of ST muscle differ between children with SP and typically developing children (TD).

The aims of this study were to assess (1) how knee moment-angle characteristics and ST morphology in children with SP selected for medial hamstring lengthening differ from TD children, as well as (2) how knee moment-angle characteristics and ST morphology are related.

In nine SP and nine TD children, passive knee moment-angle characteristics and morphology of ST (i.e. fascicle length, muscle belly length, tendon length, physiological cross-sectional area, and volume) were assessed by hand-held dynamometry and freehand 3D ultrasound, respectively.

At net knee flexion moments above 0.5 Nm, more flexed knee angles were found for SP compared to TD children. The measured knee angle range between 0 and 4 Nm was 30% smaller in children with SP. Muscle volume, physiological cross-sectional area, and fascicle length normalized to femur length were smaller in SP compared to TD children (62%, 48%, and 18%, respectively). Sixty percent of the variation in knee angles at 4 Nm net knee moment was explained by ST fascicle length.

Altered knee moment-angle characteristics indicate an increased ST stiffness in SP children. Morphological observations indicate that in SP children planned for medial hamstring lengthening, the longitudinal and cross-sectional growth of ST muscle fibres is reduced. The reduced fascicle length can partly explain the increased ST stiffness and, hence, a more flexed knee joint in these SP children.
Introduction

Children with spastic paresis (SP) who are walking with a flexed knee gait pattern are frequently treated by single-event multilevel surgery (SEMLS) (McGinley et al., 2012, Rutz et al., 2012). In such a surgery, several bony and soft-tissue procedures are combined in a single session. One commonly used surgical soft-tissue procedure is medial hamstring lengthening (Beals, 2001, Dagge et al., 2012, Abel et al., 1999, Chang et al., 2004, De Mattos et al., 2014, Dreher et al., 2012b, Kay et al., 2002, Zwick et al., 2002). In a high number of children with SP, SEMLS including medial hamstring lengthening seems successful in correcting the flexed knee gait pattern (Rodda and Graham, 2001, Gage, 1990, Ounpuu et al., 2015, Dreher et al., 2012b). Side effects on gait, however, due to weakening of the hamstring muscles and overcorrections leading to hyperextension of the knee, increased anterior pelvic tilt, and lumbar lordosis, are frequently reported (Adolfsen et al., 2007, Chang et al., 2004, Dreher et al., 2012b, Dhawlikar et al., 1992, Zwick et al., 2002). Also, persistence of flexed knee gait or even recurrence after initial success can be a problem (Dhawlikar et al., 1992, Dreher et al., 2012b, Chang et al., 2004). Several strategies have been proposed to improve the selection safety of patients for surgical medial hamstring lengthening to reduce side effects. Botulinum toxin test injections have been recommended to assess possible negative effects of muscle weakening on gait (Rutz et al., 2010). Also, musculoskeletal modeling, used to estimate muscle length changes during gait, has been proposed to assist decision making by orthopedic surgeons (Arnold et al., 2006, Laracca et al., 2014, Hicks et al., 2011). However, with musculoskeletal modeling generally only origin-insertion length of a muscle (i.e. length of the muscle-tendon unit (MTU)) is estimated based on joint angles and moment arms. This does not provide full insight in morphological alterations that may potentially underlie the reduced range of motion (ROM) around the knee in children with SP. Assessment of tendon length, fascicle length, and physiological cross-sectional area (PCSA) by ultrasound enables the comparison of alterations in muscle morphology to limitations in knee ROM and to increased joint stiffness (e.g. a smaller PCSA will decrease while shorter fascicles will increase passive MTU stiffness). Such information may provide indications for the magnitude of effects to be achieved by hamstring lengthening. To our knowledge, such measurements of fascicle length, PCSA and tendon length of hamstring muscles have not been performed in children with SP indicated for medial hamstring lengthening. Insight in morphological variables that affect knee-joint mechanics in children with SP prior to such surgery is the first step to identify factors that explain the side effects.

One of the targeted muscles for medial hamstring lengthening is the biarticular semitendinosus muscle (ST). The ST is divided by a tendinous inscription into two in series arranged compartments which are separately innervated (Van Campenhout and Molenaers, 2011, Woodley and Mercer, 2005, Rab et al., 1997, Seidel et al., 1996, Christensen, 1959, Markee et al., 1955). Because of its low degree of pennation, the ST exerts force over a large ROM in hip and knee joints (Markee et al., 1955). Low muscle belly volume and short
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muscle belly length of ST have previously been shown for SP children (Oberhofer et al., 2010, Noble et al., 2014, Lampe et al., 2006, Handsfield et al., 2016). We have recently described a freehand three-dimensional ultrasound method (3D US) to assess morphological variables of ST (e.g. muscle belly length, tendon length, fascicle length and whole muscle volume, and volumes and fascicle length of both compartments) (Haberfehlner et al., 2016b) and a method to reliably measure knee moment-angle characteristics in children with SP (Haberfehlner et al., 2015), which provides quantitative measures of knee ROM and stiffness.

The aims of this study were to assess how (1) knee moment-angle characteristics and ST morphology in children with SP selected for medial hamstring lengthening differ from those in age-gender matched typically developing (TD) children, as well as (2) how knee moment-angle characteristics and ST morphology are related.

We hypothesized that in children with SP knee moment-angle curves are steeper and shifted to more flexed knee angles in comparison to TD children. Furthermore, we expected that these differences are explained by differences in morphological properties of ST.

Methods

The study was approved by the Medical Ethics Committees of the VU University Medical Center (VUmc), Amsterdam (The Netherlands) and of the University of Basel Children’s Hospital (UKBB), Basel (Switzerland). All children and their parents gave written informed consent.

Study population

We recruited children with SP at the pediatric rehabilitation and orthopedic departments of the VUmc and the pediatric orthopedic department of the UKBB who were selected for SEMLS to improve gait. Six children with SP were recruited and assessed at the VUmc and three children with SP at the UKBB. All TD children were recruited and assessed at the VUmc. Included patients had: (1) a clinical diagnosis of SP due to cerebral palsy or hereditary spastic paresis (Rosenbaum et al., 2007, SCPE, 2002), (2) were selected for ST lengthening within a SEMLS or as a single procedure - indications for surgery were (a) a fixed knee flexion contracture of ≥15° and/or a popliteal angle of ≥60° and (b) a gait pattern with flexion of the knee in midstance and endorotation-adduction movement of the hips in terminal swing, (3) Gross Motor Function Classification System (GMFCS) (Palisano et al., 2008) level I, II (walking without walking aids) or III (walking with a walking aid), and (4) were between 6 and 20 years old. Patients were excluded if they had interfering treatment and/or had a co-morbidity that could affect walking ability and tissue properties of the hamstring muscles. We considered as interfering treatment: (1) medication that affected neuromuscular properties, (2) treatment with Botulinum toxin A, or (3) serial casting within three months prior to the measurements, (4) selective dorsal rhizotomy, (5) any preceding
hamstring muscle surgery, or (6) intrathecal baclofen treatment. The control group consisted of age and gender matched TD children. Table 4.1 shows the subject characteristics. Each group consisted of 5 females and 4 males with a mean age of 14 years/1 month. Seven children with cerebral palsy and two children with hereditary spastic paresis were included in the patient group. Children with hereditary spastic paresis: one female: 11 years/2 month, GMFCS II and one male: 12 years/6 month, GMFCS III. Eight of the nine children had at least one previous treatment with Botulinum Toxin A in ST and semimembranosus muscle in the past (Table 4.1).

**Table 4.1 Anthropic and subject data ± standard deviation (range) and number of previous treatments with Botulinum toxin A**

<table>
<thead>
<tr>
<th>Group</th>
<th>SP (n=9)</th>
<th>TD (n=9)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year/month)</td>
<td>14/1±2/8 (10/7-18/2)</td>
<td>14/1±3/2 (10/0-18/5)</td>
<td>0.977</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>5/4</td>
<td>5/4</td>
<td></td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>150.0±11.4 (136-176)</td>
<td>162.6±11.8 (147-182)</td>
<td>0.036</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>43.1±11.1 (27.0-61.0)</td>
<td>51.2±9.9 (37.8-66.0)</td>
<td>0.122</td>
</tr>
<tr>
<td>Femur length (cm)</td>
<td>34.2±3.4 (31.3-41.0)</td>
<td>37.1±2.8 (33.3-40.8)</td>
<td>0.064</td>
</tr>
<tr>
<td>BMI</td>
<td>18.9±3.0 (13.9-23.2)</td>
<td>19.2±1.9 (17.1-22.3)</td>
<td>0.772</td>
</tr>
<tr>
<td>GMFCS (I-III)</td>
<td>II (3), III (6)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Popliteal angle (degree)</td>
<td>71±6 (60-80)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Maximal knee extension (passive) (degree)</td>
<td>27±10 (15-45)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Number of previous treatment Botulinum toxin A (all longer than 6 month ago):</td>
<td>n/a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. Semitendinosus</td>
<td>0x(1), 1x(4), 2x(1), 3x(1), 6x(1), 8x(1)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>M. Semimembranosus</td>
<td>0x(1), 1x(4), 2x(1), 3x(1), 6x(1), 8x(1)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>M. Biceps femoris</td>
<td>0x(8), 1x (1)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>M. Gracilis</td>
<td>0x(1), 1x(4), 3x(2), 6x(1), 8x(1)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>M. Psoas</td>
<td>0x(5), 1x (2), 4 (1), 6x(1)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>M. Rectus femoris</td>
<td>0x (6), 2x(2), 3x(1)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>M. Gastrocnemius</td>
<td>0x(4), 1x(2), 3x(1), 4x(1), 8x(1)</td>
<td>n/a</td>
<td></td>
</tr>
</tbody>
</table>

*TD=typically developing children, SP=spastic paresis; BMI=Body mass index, GMFCS=Gross Motor Function Classification System*
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Measurements

Clinical measurements

Body mass and body height were measured and body mass index (BMI) was calculated (kg/m²). The popliteal angle was measured according to Reimers (Reimers, 1974). The passive maximal knee extension was measured using the neutral zero method (Cave and Roberts, 1936). For all knee angle measurements full knee extension is defined as 0° with an increasing angle towards knee flexion.

Measurements for knee moment angle characteristics at rest

The experimental protocol and setup have been described in detail previously (Haberfehlner et al., 2015). Subjects were positioned on an examination bed on their left side, with the hip of the measured (right) leg at 70° flexion (Figure 5.1). To prevent pelvic tilt and hip movements during measurements, pelvis and upper leg were tightly secured to the bed. The subjects stayed in this position for the entire measurement session. The lower leg was moved on a low-friction moveable plate. Changes in knee angle were measured using a Twin Axis digital goniometer (model SG150; Biometrics Ltd, UK). Anatomical bony landmarks (trochanter major, lateral femoral epicondyle, caput fibulae, and lateral malleolus) measured by an optically six marker rigid body Optotrak-probe (VUmc: Optotrak type 3020; Northern Digital, Waterloo, Canada) or 14 mm diameter-reflective VICON markers (UKBB: VICON MX20 System Oxford Metrics, Oxford, UK) defined the lengths of femur and fibula as well as the absolute knee angles. The latter were used for calibration of the goniometer, this was done to enhance the validity of the goniometer. Force applied at the lower leg was measured using a custom-made hand-held device instrumented with a bi-directional force transducer with an accuracy of 0.5 N (HBM Darmstadt, Germany). The lever arm was measured on the line between lateral femoral epicondyle and lateral malleolus, as the distance from the point of application of the force measurement device to the lateral femoral epicondyle. The lateral femoral epicondyle was used as an estimate of the location of the knee joint flexion/extension axis.

Activity levels of biceps femoris, gastrocnemius medialis, rectus femoris, and vastus lateralis muscles were assessed using surface electromyography (EMG). The skin was prepared and EMG electrodes placed according to SENIAM guidelines (Hermens et al., 1999). At the VUmc, force, knee angle, and EMG activity were sampled at 1000 Hz by a Mobi system (TMSI, The Netherlands). At the UKBB, force and knee angle were sampled at 100 Hz by a GSV-3USBx2-amplifier (ME-measuring systems GmbH, Germany) and EMG at 1000 Hz by a Biovision-EMG-system (Wehrheim, Germany).

The child watched a movie during the measurements for distraction and relaxation. Prior to the assessment of net knee moment-angle characteristics, the subject was asked to fully relax the leg for ten seconds to assess EMG activity at rest. To get accustomed to the measurements, three knee flexion-extension cycles from 110° of flexion to knee
extension of maximal 20° were performed. The leg was moved only within a knee angle range that could be imposed without clearly detectable EMG bursts, as determined by visual inspection, and/or without discomfort experienced by the child. After these cycles, the lower leg was placed into a 110° knee flexion position and then slowly released till the plate stopped (i.e. zero knee moment). From that position, the knee was extended in steps of 5°. The subject was instructed to relax and neither to resist nor assist the movement. At each step of knee extension, the position was maintained for ten seconds to allow for effects of stress-relaxation. When the maximal knee extension angle was reached, the leg was slowly released and pulled towards flexion again. This procedure was repeated three times.

**Measurements of ST morphology using 3D US**

3D US imaging was performed at three knee angles (i.e. the angles corresponding to a knee flexion moment of 0 and 4 Nm, as well as at a knee angle of 65°). The angles at 0 and 4 Nm were selected during the three flexion-extension repetitions of the knee moment-angle measurements by determining the knee angle during every repetition and taking the mean of these three angles.

Then the subject’s lower leg was fixed by three suction cups around the low-friction moveable plate at knee angle corresponding to 0 Nm, while the bandage that stabilized the upper leg was released to allow for US scanning of ST. The order of US scanning at different knee angles was (1) angle corresponding to 0 Nm, (2) 65°, and (3) angle corresponding to 4 Nm. Two US scans were performed at each of the three knee angles. If during US scanning an EMG burst was detected and/or movement of the child was observed, the scan was discarded and an additional scan was obtained.

US imaging of ST was performed freehand using a B-mode US apparatus with a 5 cm linear probe 12.5 MHz (VUmc: Technos MPX, ESAOTE S.p.A., Italy; UKBB: Philips HD II). A 30-40 seconds sequence of transverse US images (i.e. axial plane of the ST) was collected starting distally at the ST tendon (i.e. at the point that the tendon could be sufficiently visualized in the popliteal fossa) to the origin on the ischial tuberosity (Figure 4.1). The US images were sampled at a rate of 25 Hz using an AD-video converter (Canopus ADVC-330, Grass valley) connected via a FireWire cable to the PC. The position of each US image in 3D space was recorded by tracking the US probe (based on three markers that were rigidly attached to it) using a motion capture system (VUmc: Optotrak type 3020; Northern Digital, Waterloo, Canada; UKBB: VICON MX20 System Oxford Metrics, Oxford, UK) (Figure 4.1). Positions of four bony anatomical landmarks (i.e. most prominent part of the ischial tuberosity, lateral and medial femoral epicondyles, and insertion of the ST tendon at the tibia) were recorded prior to scanning using an optically six marker rigid body Optotrak-probe (VUmc) or 14 mm diameter-reflective VICON markers (UKBB). All four bony landmarks were identified by palpation.
Figure 4.1 Setup of freehand three-dimensional ultrasound to measure semitendinosus (ST) muscle morphology. Subjects were positioned on an examination bed on their left side, with the hip of the measured (right) leg at 70° flexion. At knee angles corresponding to a knee moment of 0 and 4 Nm and at a knee angle of 65°, a 30-40 seconds video sequence of transverse US images was collected by a conventional 2D ultrasound apparatus, starting distally at the ST tendon to the ischial tuberosity (white arrow on the thigh indicates scan direction). The position of each ultrasound image in space was recorded by tracking the ultrasound probe (based on three markers that were rigidly attached to it – indicated by markers probe) using a motion capture system (tracking device). The images from the ultrasound video sequence were combined with the probe position data an reconstructed to a voxel array that was used for further analysis.

Prior to 3D US imaging the transformation matrix from the probe frame to the US images was determined by identifying a cross-point of two intersecting wires at a known position in a water cube within the US images (i.e. using the settings in scaling and resolution of the used ultrasound apparatus). The cross of the wire was scanned from different positions and with different tilt angles of the ultrasound probe, while the cross-wire was kept visible within the recorded ultrasound image sequence (Prager et al., 1998). A custom made program in Matlab (version R2014A, the Mathworks Inc.) adapted from a previous version (Bénard et al., 2011, Weide et al., 2015) was used to fill a 3D voxel array with the US pixels (Figure
4.1). This algorithm consists of a distribution step and an additional gap filling step (Solberg et al., 2007). The size of a voxel was 0.2x0.2x0.2 mm.

**Data analysis**

**EMG data analysis**

Since artefacts were present below 100 Hz, EMG data were high-pass filtered at 100 Hz (Potvin and Brown, 2004, Staudenmann et al., 2010), rectified, and low-pass filtered at 5 Hz. Means and standard deviations (SD) of smoothed, rectified resting EMG were calculated. The threshold level for muscle activity was set at mean resting EMG + 2 SD.

**Analysis knee-moment angle data**

The analyses and reliability have been described in detail previously, reporting a standard error of the mean for these measurements of about 5º (Haberfehlner et al., 2015). In brief, joint angle and force data were low-pass filtered at 1 Hz. For each knee angle (i.e. in steps of 5º) force and joint angle data were time averaged over the last three seconds of every ten seconds measurement interval. These last three seconds were taken for analysis because at this instance stress-relaxation of the muscles has attenuated and a steady state is reached. The net knee moment was calculated by multiplying the force measured at the force transducer by the lever arm.

Data were excluded from further analysis when the mean EMG envelope during the last three seconds of the ten second measurement interval exceeded the 2 SD threshold for one of the four muscles. The included data points from the three repetitions were fitted by a third order polynomial function. This equation was retrieved based on a stepwise regression analysis in a previous study (Haberfehlner et al., 2015):

\[ y = ax^3 + bx^2 + cx + d \] (Equation 4.1)

In this equation, \( y \) represents the net knee moment, \( x \) represents the knee angle, and constants \( a, b, c, \) and \( d \) were determined by the fitting procedure. The minimal requirements to fit a function were defined as at least four data points (i.e. after the exclusion of data points due to the above described EMG threshold), at least one data point lower than 0.5 Nm and at least one data point higher than 3 Nm. Knee angles at 0, 0.5, 1, 2, 3 and 4 Nm (\( \theta_{0Nm}, \theta_{0.5Nm}, \theta_{1Nm}, \theta_{2Nm}, \theta_{3Nm}, \theta_{4Nm} \)) were derived from the fitted curves. Range of knee motion between 0 and 4 Nm was calculated (\( \text{ROM}_{0-4Nm} \)), \( \theta_{0Nm}, \theta_{0.5Nm}, \theta_{1Nm}, \theta_{2Nm}, \theta_{3Nm}, \theta_{4Nm}, \) \( \text{ROM}_{0-4Nm} \)) as well as maximum measured moment (\( M_{\text{max}} \)) and maximum measured angle (\( \theta_{\text{max}} \)) were used for statistical analysis.

**Image analysis 3D US**

For US scanning the recorded EMG activity was checked to verify whether it did not exceed the 2 SD threshold. The following morphological characteristics of ST were
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determined (see for details of the method and validation (Haberfehlner et al., 2016b)): length of the muscle-tendon unit (ℓmtu), muscle belly length (ℓm), distal tendon length (ℓt_dist), total fascicle length (ℓfasc), fascicle length of the most proximal fascicle of the distal compartment (ℓfasc_dist_p), fascicle length of the most distal fascicle of the distal compartment (ℓfasc_dist_d), fascicle length of the most proximal fascicle of the proximal compartment (ℓfasc_prox_p), whole muscle volume (Vol), muscle volume of the distal compartment (Vol_dist), muscle volume of the proximal compartment (Vol_prox) and, PCSA, defined as the cross-sectional area of all muscle fibers arranged in parallel including the intramuscular connective tissues.

Segmentation of ST for assessment of muscle volume was performed using Fiji (http://fiji.sc) (Schindelin et al., 2012, Schneider et al., 2012). The outline of ST was encircled in transverse images every 5 mm along the length of the muscle (Weller et al., 2007). The gaps between the encircled images were interpolated to obtain a fully segmented volume by Segmentation Editor Plugin (http://fiji.sc/Segmentation_Editor). Muscle volume of each compartment was measured using the volume measurement tool in Chimera 1.9 (http://www.cgl.ucsf.edu/chimera) (Pettersen et al., 2004). Vol_dist and Vol_prox were summed to calculate Vol. Volume measurements were performed using the voxel array obtained at the 4 Nm knee angle. The coordinates in x, y, z directions of the following points were determined within the voxel array using Chimera 1.9: (1) most proximal and (2) distal ends of the tendinous inscription, (3) proximal end of the distal aponeurosis, (4) the distal end of the most distal fascicle (i.e. distal muscle-tendinous junction) and (5) ischial tuberosity. Determination of the ischial tuberosity was not always possible within the voxel array. When the ischial tuberosity was not visible within the voxel array, the position from the bony landmark registration was used for further calculations. Linear distances between coordinates of above mentioned points were used to define ℓm (point: 4-5), ℓfasc_dist_p (1-3), ℓfasc_dist_d (2-4), and ℓfasc_prox_p (1-5).

For estimation of distal tendon length the following procedure was performed. First, the most distal point of the distal tendon (proximal to the knee joint axis) was assessed within the voxel array. Subsequently, the following direction vectors were defined: (a) a line between the distal end of the most distal fascicle and the most distal visible point of the distal tendon (i.e. ‘line of tendon’) and (b) a line between the medial and lateral femoral epicondyles (i.e. ‘line of estimated knee joint axis’). The point along the ‘line of tendon’ from which the distance to the ‘line of estimated knee joint axis’ was smallest, was taken as estimate of the crossing of the distal tendon with the knee joint axis (6) (Haberfehlner et al., 2016b). The distance between the distal end of the most distal fascicle and the crossing of the distal tendon with the knee joint axis (6) (Haberfehlner et al., 2016b). The distance between the distal end of the most distal fascicle and the crossing of the distal tendon with the knee joint axis was calculated to estimate the distal tendon proximally of the knee axis (4-6). With the calculated crossing point and the registered coordinates of the insertion of the distal tendon on the tibia (7), the length of the distal tendon distally to the knee axis was assessed (6-7). The two tendon segments (i.e. proximally and distally of the knee axis) were summed to ℓt_dist.

Voxel arrays were anonymized for group as well as for measurement condition (i.e.
Knee moment angle characteristics and semitendinosus muscle morphology

0 Nm, 4 Nm, 65°) and were analyzed in randomized order. Assessment of the x, y, z coordinates of the seven above defined points was performed three times by the same observer (HH). Distances between the three identified points were calculated, yielding nine length measures per variable per subject. If the coefficient of variation (CV) of these nine values exceeded 10% of the mean of the length variable, the selected point related to this variable with the highest deviation with respect to the mean position was checked to verify whether the x, y, z, position of this point was misinterpreted. If this was the case, a new assessment for this specific point was made. When after these procedures the CV of the length measure still exceeded 10%, this length measure was excluded. For further calculations and statistical analyses, mean values of nine length measures were used.

To assess total fascicle length of ST (ℓfasc), ℓfasc_{\text{dist}_p} and ℓfasc_{\text{dist}_d} were summed. Length of the muscle-tendon unit (ℓmtu) was calculated by the sum of ℓm and ℓt_{\text{dist}}. PCSA of ST was calculated by dividing Vol by ℓfasc at 4 Nm. Differences in length of total fascicle and tendon length between the 0 and 4 Nm condition were calculated (i.e. Δℓ_{\text{fasc}} and Δℓ_{\text{t}}) and expressed relative to the length measured at 0 Nm (i.e. Δℓ_{\text{fasc}}^{\text{rel}} and Δℓ_{\text{t}}^{\text{rel}}).

All length variables and differences herein were expressed as percentages of femur lengths (variablename_{\text{nom}}). Volume measures and PCSA were expressed as absolute values.

Statistics

Independent t-tests were used to test for differences between SP and TD children in anthropometric parameters, \( M_{\text{max}} \), \( \theta_{\text{max}} \), \( \text{ROM}_{0-4\text{Nm}} \), morphological characteristics at 65° knee angle, muscle volumes, PCSA, and differences in total fascicle and tendon length. Within each group, paired t-tests were performed to test for differences in proximal and distal volume.

Differences in knee moment-angle characteristics and morphological characteristics (at 0 and 4 Nm) between SP and TD were tested with repeated measures ANOVA (factors: group x moment). Knee angles were tested at six knee flexion moments (\( \theta_{0\text{Nm}} \), \( \theta_{0.5\text{Nm}} \), \( \theta_{1\text{Nm}} \), \( \theta_{2\text{Nm}} \), \( \theta_{3\text{Nm}} \), \( \theta_{4\text{Nm}} \)), while morphological characteristics were assessed at two flexion moments (knee angles corresponding to 0 and 4 Nm). For post hoc comparisons, independent t-tests with Bonferroni-Holm corrections were performed, accordingly the p-values were corrected. For all variables normality was tested by the Shapiro-Wilk test. If data was not normally distributed (which was the case for ℓt_{\text{dist}}^{\text{65deg norm}} and ℓmtu_{4\text{Nm norm}}) the non-parametric Mann-Whitney-U Test was used. For repeated measures ANOVA, the Greenhouse Geisser correction was used when the assumption of sphericity was violated. If homogeneity of variance was violated, Welch’s t-tests were used instead of independent t-tests.

To assess whether muscle morphological parameters can predict the mechanical properties at the joint level, multiple regression was used, including the data points of all subjects, to test the relationship between three predictor variables (i.e. PCSA, ℓt_{\text{dist}}^{0\text{Nm norm}}, and
\( \ell_{\text{fasc}}^{0\text{Nm}} \) and \( \theta_{4\text{Nm}} \). In addition, for the combined group, correlations between \( \ell_{\text{fasc}}^{0\text{Nm}} \) and \( \theta_{4\text{Nm}} \) were calculated by Pearson correlation coefficient (Pearson’s r).

Data were presented as means ± SD and differences were presented as means ± standard error of difference. The level of significance was set at 0.05 for all statistical tests.

**Results**

SP and TD children did not differ in body mass, BMI or femur length (Table 4.1). Body height was lower in children with SP (150.0±11.4 cm) compared to TD children (162.6±11.8 cm) (\( p=0.036 \); Table 4.1).

![Figure 4.2 Knee moment-angle characteristics of children with a spastic paresis (SP) and typically developing (TD) children. The curve of SP children was shifted towards more flexed knee angles compared to the curve of TD children and has a steeper slope (i.e. higher stiffness). Black line: SP children; Grey dashed line: TD children. Values are mean ± SD.](image)
Knee moment-angle characteristics

Figure 4.2 shows for both SP and TD children net knee flexion moments as a function of knee angle. Repeated measures ANOVA for knee angle revealed an effect of group (p=0.004) and an interaction effect of group and net knee flexion moment (p=0.010, Figure 4.2). In TD children, knee angles measured at 0 Nm and 4 Nm were 78.6±5.6° and 37.6±7.7°, respectively. In the imposed posture (i.e. hip in 70° flexion), the maximal extended knee angle was 31.5±6.8°. For SP children, knee angles for the different moments were more flexed (θ_0Nm: 84.9±9.5°, θ_4Nm: 55.5±10.1°, and θ_max: 54.1±11.8°). Maximum knee moment (M_max) did not differ between groups (SP: 5.2±1.9 Nm; TD: 5.8±1.3 Nm, p=0.446). Post-hoc t-tests revealed that in SP children knee angles corresponding to 0.5 Nm and higher were significantly more flexed than those of TD children (θ_0Nm: p=0.104; θ_0.5Nm: p=0.036; θ_1Nm: p=0.030; θ_2Nm: p=0.012; θ_3Nm: p=0.005; θ_4Nm: p=0.006; θ_max: p<0.001). Differences in knee angles between SP and TD children increased with larger knee moments ranging from 8.9±3.4° for θ_0.5Nm to 17.9±4.2° for θ_4Nm and 22.7±4.5° for θ_max. These results show that the knee moment-angle curve in children with SP was shifted towards more flexed knee angles in comparison to that in TD children. The significant interaction indicates also a steeper slope of the curve. This was also confirmed by a smaller ROM_0-4Nm in children with SP (29.4±5.4°) compared to TD children (41.0±8.1°) (p=0.003) and, hence, a higher increase of knee angle per Nm in SP (SP: 0.14±0.03 Nm/°; TD: 0.10±0.02 Nm/°; p=0.007).

Morphological characteristics

The 3D US measurements for the 0 Nm condition were performed at a knee angle of 82.6±14.4° (SP) and 75.5±4.5 (TD) (p=0.203) and for the 4 Nm condition at a knee angle of 55.6±11.9° (SP) and 38.3±7.5 (TD) (p=0.004). The actual knee angle of 65° angle did not differ between the groups (64.3±6.5° for SP and 68.5±3.5° for TD, p=0.127).

To characterize ST morphology, ℓmtu, ℓm, ℓfasc_dist, Vol_prox, and Vol_dist were analyzed in eight of the nine included children with SP and their matched TDs. In one child with SP, ultrasound measurements could not be performed due to anxiety and unrest. For this SP child, 3D US results of the matched TD child were also excluded from further analysis. In addition, fascicle length was excluded from analysis in a few children with SP due to inaccurate identification of x, y, and z coordinates of the required points (see method & Table 4.2).

Comparison of origin and insertion distance (ℓmtu) of the ST at a knee angle of 65° should reveal the same values for both groups unless joint and/or bone morphology differ between groups. We found no difference in ℓmtu_65deg_norm between SP and TD (Table 4.2). In SP children, both normalized fascicle lengths of the distal compartment (ℓfasc_dist_p_65deg_norm, ℓfasc_dist_dist_65deg_norm) and normalized total fascicle length (ℓfasc_65deg_norm) assessed at 65° were 30.2±7.3%, 23.4.2±7.8%, and 21.8±5.0%, respectively lower than those in TD children. All other normalized length variables (ℓm_65deg_norm, ℓfasc_dist_65deg_norm and ℓfasc_prox_p_65deg_norm) did not differ between SP and TD children (Table 4.2).
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Table 4.2 Morphological characteristics of semitendinosus muscle (ST) in children with a spastic paresis (SP) and typically developing (TD) children at 65° knee angle and knee angles corresponding to 0 Nm and 4 Nm net knee flexion moments. P-value shows the difference between children with SP and TD children.

<table>
<thead>
<tr>
<th>Morphological characteristics</th>
<th>SP</th>
<th>TD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ℓMTU&lt;sub&gt;65°&lt;/sub&gt;</td>
<td>121.2±5.7%</td>
<td>123.1±6.9%</td>
<td>0.553</td>
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<tr>
<td>ℓm&lt;sub&gt;65°&lt;/sub&gt;</td>
<td>74.6±6.6%</td>
<td>81.6±8.3%</td>
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<tr>
<td>ℓt&lt;sub&gt;dist&lt;/sub&gt;&lt;sub&gt;65°&lt;/sub&gt;</td>
<td>46.6±7.1%</td>
<td>41.5±5.9%</td>
<td>0.161</td>
</tr>
<tr>
<td>ℓfasc&lt;sub&gt;65°&lt;/sub&gt;</td>
<td>38.2±5.1%</td>
<td>48.9±4.4%</td>
<td>0.001</td>
</tr>
<tr>
<td>ℓfasc&lt;sub&gt;dist_p&lt;/sub&gt;&lt;sub&gt;65°&lt;/sub&gt;</td>
<td>19.9±3.7%</td>
<td>28.5±3.6%</td>
<td>0.002</td>
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<tr>
<td>ℓfasc&lt;sub&gt;dist_d&lt;/sub&gt;&lt;sub&gt;65°&lt;/sub&gt;</td>
<td>26.8±6.0%</td>
<td>35.1±4.6%</td>
<td>0.010</td>
</tr>
<tr>
<td>ℓfasc&lt;sub&gt;prox_p&lt;/sub&gt;&lt;sub&gt;65°&lt;/sub&gt;</td>
<td>19.5±3.4%</td>
<td>20.4±2.3%</td>
<td>0.586</td>
</tr>
<tr>
<td>ℓMTU&lt;sub&gt;0&lt;/sub&gt;</td>
<td>118.4±8.3%</td>
<td>121.7±5.2%</td>
<td>0.574</td>
</tr>
<tr>
<td>ℓMTU&lt;sub&gt;4&lt;/sub&gt;</td>
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<td>130.1±7.6%</td>
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</tr>
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<td>ℓm&lt;sub&gt;0&lt;/sub&gt;</td>
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<td>78.9±7.8%</td>
<td>0.177</td>
</tr>
<tr>
<td>ℓm&lt;sub&gt;4&lt;/sub&gt;</td>
<td>77.6±7.1%</td>
<td>86.8±8.5%</td>
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</tr>
<tr>
<td>ℓt&lt;sub&gt;0&lt;/sub&gt;</td>
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<td>41.8±5.1%</td>
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<td>43.2±7.3%</td>
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<td>30.8±3.9%</td>
<td>0.024</td>
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<td>28.2±6.8%</td>
<td>37.1±5.4%</td>
<td>0.024</td>
</tr>
<tr>
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<td>19.2±3.7%</td>
<td>0.543</td>
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<td>21.7±3.7%</td>
<td>22.7±4.1%</td>
<td></td>
</tr>
</tbody>
</table>

ℓMTU= length of muscle-tendon unit; ℓm= length muscle belly; ℓt = length of distal tendon ℓfasc= fascicle length; all length variables were expressed as % of femur length<sub>(norm)</sub>.

No differences between groups were shown for ℓMTU<sub>norm</sub>, ℓm<sub>norm</sub>, and ℓt<sub>dist_nor</sub> when assessed at similar net knee moments (i.e. 0 and 4 Nm) (Table 4.2). Total fascicle lengths (ℓfasc<sub>norm</sub>) at 0 Nm and 4 Nm were 17.8±5.0% and 18.1±6.1% lower in SP than in TD children. This difference in ℓfasc<sub>norm</sub> was mainly due to shorter fascicles in the distal ST compartment of SP children (ℓfasc<sub>dist_p_norm</sub>). The proximal fascicle length (ℓfasc<sub>prox_p_norm</sub>) did not differ between groups (Table 4.2).

For the length variables, no significant interaction effects were shown between factors group and knee moment. This suggests that when the knee was extended from a knee angle corresponding to 0 Nm to a knee angle corresponding to 4 Nm, length variables in SP and TD children increased similarly. Absolute and relative fascicle and tendon length
Knee moment angle characteristics and semitendinosus muscle morphology

Changes between 0 and 4 Nm did not differ significantly between groups (Δℓ_{fasc\_norm}; p=0.372; Δℓ_{fasc\_norm, rel}; p=0.851; Δℓ_t_{dist\_norm}; p=0.518; Δℓ_t_{dist\_norm, rel}; p=0.450; Figure 4.3).

Figure 4.3 A: Absolute and relative (rel) length changes (Δ) of the fascicles between knee angles corresponding to 0 Nm and 4 Nm net knee moment. B: Absolute and relative length changes of the distal tendon between these two knee angles. Fascicle length and tendon length are normalized to femur length (ℓ_{fasc\_norm}, ℓ_{t\_dist\_norm}). Absolute as well as relative length changes of fascicles and tendons did not differ significantly between children with a spastic paresis (SP) and typically developing (TD) children. Data are presented as means ± SD.

A typical example of a longitudinal view is shown in Figure 4.4. Total ST volume (Vol) was 62.0±10.5% smaller in children with SP (36.6±17.8 cm³) than in TD children (96.0±22.3 cm³) (p<0.001). This was also the case for each of the volumes of the proximal compartment (SP: 18.5±10.2 cm³, TD: 45.0±10.5 cm³; p<0.001) and of the distal compartment (SP: 18.1±8.0 cm³, TD: 51.0±12.6 cm³; p<0.001). Paired t-tests revealed that in TD children volumes of proximal and distal compartments (Vol_{prox} and Vol_{dist}) differed (volume of distal compartment was 11.7±4.4% larger, p=0.032). However, such a proximal-distal difference was not shown for the SP group (p=0.802). PSCA was 47.7±12.1% smaller in SP compared to that in the TD children (p=0.003, Figure 4.5).

Relationship between muscle morphology and θ_{0Nm} and θ_{4Nm}

Multiple regression analyses revealed that PCSA, ℓ_t_{dist\_0Nm\_norm}, and ℓ_fasc_{0Nm\_norm} predicted θ_{4Nm} (R²=0.57, p=0.033; θ_{4Nm}=85.49-(1.30×PCSA)+(0.46×ℓ_t_{dist\_0Nm\_norm})-(1.31×ℓ_fasc_{0Nm\_norm})). However, only ℓ_fasc_{0Nm\_norm} significantly added to the prediction (p=0.037). When PCSA and ℓ_t_{dist\_0Nm\_norm} were removed as predictor variables, ℓ_fasc_{0Nm\_norm} predicted θ_{4Nm} r²=0.49, p=0.006; θ_{4Nm}=114.20-(1.63×ℓ_fasc_{0Nm\_norm}). (Figure 4.6A). These results indicate that 49% of the variation θ_{4Nm} was explained by ℓ_fasc_{0Nm\_norm}. Changing the prediction variable from ℓ_fasc_{0Nm\_norm} into ℓ_fasc_{4Nm\_norm} resulted in an explained variation of 60% for θ_{4Nm} (r²=0.60, p=0.001; θ_{4Nm}=116.03-(1.41×ℓ_fasc_{4Nm\_norm}), Figure 4.6B). These results indicate that the slope of the knee moment-angle curve was largely determined by the fascicle length.
Figure 4.4 Typical example of 3D ultrasound images and segmentation of muscle volume of a child with a spastic paresis (left A1-C1) and typically developing child (right A2-C2). A: longitudinal view of semitendinosus muscle (ST) (proximal on the left side); B: transversal view of ST at three locations (most proximal on left side; orientation of images: medial (left), lateral (right)); yellow: distal compartment of ST; red: proximal compartment of ST; C: Proximal (red) and distal (yellow) compartments after segmentation.
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**Figure 4.5** Muscle volume and physiological cross sectional area (PCSA) of semitendinosus muscle (ST) of children with a spastic paresis (SP) and typically developing children (TD). Muscle volume of ST (proximal, distal and total muscle volume) and PCSA are substantially smaller in SP children. PCSA was calculated by dividing muscle volume by fascicle length at 4 Nm. Data are presented as means ± SD; *p<0.01.

**Figure 4.6** Knee angle at 4 Nm (θ4Nm) plotted as a function of normalized fascicle length at 0 Nm (fasc0Nm) (A) and at 4 Nm (fasc4Nm) (B). Variation in fasc0Nm and fasc4Nm explained a substantial part of variation in θ4Nm (49% and 60%, respectively). Lines indicate the regression lines for the combined group. Separate symbols are used to indicate data points for SP (spastic paresis) and TD (typically developing).
Chapter 4

Discussion

This is the first study describing differences in knee moment-angle characteristics and ST morphology between children with SP, who were indicated for medial hamstring lengthening surgery, and TD children. The net knee moment-angle curve of children with SP in rest was shifted towards more flexed knee angles and showed a steeper slope. Muscle volume, PCSA, and fascicle length normalized for femur length of ST were all lower in SP compared to those in TD children. No differences in normalized muscle belly length of ST could be shown in SP compared to TD children.

Relationship between knee moment-angle characteristics and ST morphology

The observed steeper slope and shift of the knee-moment angle curve in SP indicates a higher stiffness and decreased slack length of ST and/or other knee flexor muscles (i.e. m. semimembranosus, m. biceps femoris and m. gracilis). A stiffer MTU can theoretically be the result of an increase in the number of sarcomeres in parallel and connective tissues content (i.e. higher PCSA), a decrease in the number of sarcomeres in series, and/or decreased tendon length (Gajdosik, 2001, Zajac, 1989). In the SP group, PCSA of ST was substantially lower compared to that in TD, which suggests that in SP children, trophy of ST muscle fibers (i.e. cross-sectional growth of fibers) was attenuated. The substantial lower PCSA and the lower proximal and distal muscle volumes in children with SP is supported by previous studies reporting a lower ST muscle volume (Oberhofer et al., 2010, Noble et al., 2014, Handsfield et al., 2016). Note that the volume reduction in the current study (about 60%) exceeds that reported in previous studies (with about 35% volume reduction) (Noble et al., 2014, Handsfield et al., 2016). A reduced PCSA would decrease, rather than increase muscle stiffness. The substantially lower PCSA may be explained by a smaller cross-sectional area of muscle fibers (fCSA) or a lower number of muscle fibers in SP (Gough and Shortland, 2012, Herskind et al., 2015). Using ST biopsies, fCSA was shown to be 32% lower in children with SP compared to TD children (Smith et al., 2011). This would partly explain the 48% PCSA reduction we observed, but suggest an additional reduction of muscle fiber number. The length of the distal ST tendon did not differ between groups in the current study and, hence, cannot explain the steeper slope of the knee-moment angle curve. In addition, shorter ST fascicles may have contributed to the observed changes in moment-angle characteristics. Assuming that for both groups at θ_{0Nm} the ST fascicles were at slack length, shorter fascicles at θ_{0Nm} in children with SP indicate fewer sarcomeres in series. This implies that at a given knee angle sarcomeres in fascicles of SP children will be more strained than those in TD. Thereby, passive fascicle stiffness will be higher. This may explain that the slope of the moment-angle curve in SP was steeper than in TD children. Shorter ST fascicles and fewer sarcomeres in series in ST of SP children are in agreement with the results of a previous study, in which in vivo sarcomere length in children with SP was increased (by 16% longer) compared that predicted by a musculoskeletal model for TD children (Smith et al., 2011). Note however, that in the latter study the patient group...
Knee moment angle characteristics and semitendinosus muscle morphology

consisted of a higher number of non-ambulatory children (Smith et al., 2011). Therefore, a higher fascicle stiffness may be more likely to be found in this group compared to the SP children in the current study, who were all ambulant.

As our results indicate that fascicle length cannot explain all variation in \( \theta_{4Nm} \), other mechanisms should have contributed to the steeper knee-moment knee angle curve in children with SP. An increased fiber stiffness due to changes in the composition of the sarcomeres (i.e. change in titin isoform expression) has not been found for ST in SP (Smith et al., 2011). However, dissected muscle fascicles of ST in SP children were less compliant at sarcomere lengths above about 3.5 \( \mu m \) compared to TD (Smith et al., 2011), suggesting altered mechanical properties of fascicles in SP children. However, in the current study relative changes in fascicle length between 0 and 4 Nm in the SP and TD group were not different, indicating no differences in fascicle stiffness between the groups. With knee angles measured at 4 Nm knee flexion moment, sarcomeres may not have been stretched above 3.5 \( \mu m \) and as such it is not likely that altered mechanical properties of SP muscle fascicles explain our observed changes in the knee moment-ankle characteristics.

Alternatively, enhanced resistance to stretch may be caused by increased stiffness due to changes in intramuscular connective tissues. Differences in collagen content were reported from muscle biopsies of ST (Smith et al., 2011) and other muscles of children with SP (Booth et al., 2001, de Bruin et al., 2014). Accumulation of connective tissue was reported to occur mainly around the neurovascular tract (de Bruin et al., 2014). A higher ultrasound echo intensity was reported within the medial gastrocnemius of SP children (Pitcher et al., 2015). It was shown that such echo intensity was related to the amount of fibrous tissue (i.e. collagen) and fatty tissue (Pillen et al., 2009, Pillen et al., 2007). Collagen content, as mentioned above, as well as fat content were reported to be increased in children with SP (Smith et al., 2011, Booth et al., 2001, de Bruin et al., 2014, Johnson et al., 2009). In this study we did not quantify echo intensity, but observed major differences between images of SP and TD children with more white pixels and less contrast in images of SP children (see for typical example Figure 4.4). This suggests differences in connective tissue composition of ST in our group of children with SP compared to that of TD children, which may enhanced MTU stiffness by increasing the intramuscular connective tissue arranged in parallel of the muscle fibers. Note however, that quantifying echo intensity does not allow differentiation between connective and fatty tissue (Pillen et al., 2009). Therefore, muscle biopsies studies in combination with mechanical and morphological measurements in vivo are required to assess the impact of tissue composition on MTU stiffness.

Besides MTU stiffness of ST, or other knee flexor muscles, altered epimuscular myofascial interactions (Smeulders and Kreulen, 2007, Smeulders et al., 2004b, Huijing, 2007) as well as differences in other structures around the knee (e.g. articular capsule, ligaments, nerves, blood vessels, and connective tissues) may also contribute to the steeper slope of knee-moment angle curve in children with SP.
When measuring net knee flexion moments, this includes contributions from agonistic and antagonistic muscles, as well as above mentioned non-muscular structures around the joint (Wu et al., 2012). Shorter fascicle lengths of rectus femoris muscle, measured at the resting angle of the knee, of SP children have been reported (Moreau et al., 2009). It is likely that, besides knee flexion muscles, also knee extension muscles in our group of SP children were affected. As the measured net knee moment is based on the net mechanical effects of agonist and antagonist muscles, shorter fascicles of a knee extensor muscle (i.e. rectus femoris muscle) would shift the knee angle at which a net zero knee moment (equilibrium) was attained to a more extended knee angle. This would imply that for these children at $\theta_{0\text{Nm}}$ ST was lengthened beyond its resting length and that at 0 Nm fascicles of ST were not at their passive slack length, indicating that the difference in actual resting fascicle length between SP and TD children was even more pronounced.

*Morphological differences between ST compartments*

Our results suggest that there are local differences in the effects of SP on ST morphology. The effects were greater for the distal compartment than for the proximal compartment, particularly regarding the shorter fascicle length. Shorter fascicles within the distal compartment in children with SP may be secondary to the joint position in which ST is predominantly used during movement. Flexed knee gait is characterized by excessive hip and knee flexion during stance (Rodda and Graham, 2001, Becher, 2002, Gage et al., 2009) and is often associated with a lack of knee extension in terminal swing (Cooney et al., 2006). Therefore, during terminal swing ST is unstrained due to a lack of knee extension (Cooney et al., 2006). In addition, strain applied to ST during gait may not be equally distributed between proximal and distal compartments of ST. This means that the hip flexion may stretch proximal fascicles of ST, while fascicles of the distal compartment may be at relatively shorter length due to knee flexion. However, this conclusion regarding differences in altered fascicle lengths between compartments due to a flexed gait pattern may only occur if effects of ST over hip and knee are to some extent independent (Markee et al., 1955). This independency of compartments is plausible because it is known that the compartments have separate innervations (Van Campenhout and Molenaers, 2011, Woodley and Mercer, 2005, Rab et al., 1997, Seidel et al., 1996, Christensen, 1959, Markee et al., 1955) and there are possible enhanced (Smeulders and Kreulen, 2007, Smeulders et al., 2004b, Huijing, 2007) epimuscular myofascial linkages between adjacent muscles or extramuscular connective tissues (Maas and Sandercock, 2010, Huijing and Jaspers, 2005).

*Clinical implications*

Given the relation between PCSA and force generation (Brand et al., 1986), the reduced PCSA observed in this study and the reduced muscle volume of ST (present study and (Noble et al., 2014, Oberhofer et al., 2010, Handsfield et al., 2016)) suggest that, compared to TD children, in a majority of children with SP ST is weak. As lengthening of the ST tendon
is presumed to induce even more ST weakness (Rutz et al., 2010), a low preoperative PCSA of ST (i.e. more than 60% lower than in TD) may be a risk factor for side effects of the surgery such as increased anterior pelvic tilt during standing and walking. In addition, fascicles that are short before surgery may further decrease in length after surgery if due to lengthening of the tendon the muscle belly is not sufficiently strained. Consequently the active length range of force exertion of the muscle will decrease. Therefore, caution should be taken before indicating hamstring lengthening. Surgical lengthening of ST tendon in SP may result in a shift of the knee-moment angle curve towards more normal knee extension. In addition, the longer and more compliant tendon may also result in a decrease in slope of the knee moment-angle characteristics. These effects may be beneficial for knee movement during gait, but may also increase hip flexion and anterior pelvic tilt, which has been reported to occur after SEMLS surgery (Adolfsen et al., 2007, Dreher et al., 2012b, Dhawlikar et al., 1992, Zwick et al., 2002). To what extent the morphological and mechanical alterations in children with SP prior medial hamstring lengthening contribute to the treatment outcome needs to be evaluated in follow-up studies.

Limitations

The number of subjects in the current study was rather low and consisted of a selected group of children with SP (i.e. selected for surgery and all ambulant, GFMCS I-III). Therefore, results cannot be generalized to the whole population of children with SP. However, as our study group is suspected to be limited by hamstring muscles during gait (and thus indicated for surgery), the current results can be assumed to be representative for the pre-surgical situation of hamstring lengthening in children with SP.

The children with SP were included and assessed in two medical centers. The positioning of the subjects in the measurement set-up was identical in the two centers. However, measurements were performed with different ultrasound apparatus’, and motion tracking, force measurement and EMG systems. Although all systems are high-end and commonly used in clinical practice and for research, this could have resulted in additional variance. To reduce variation, the same person (HH) performed all but one measurements and analyzed all data.

Ultrasound measurements do not allow measurement of sarcomere length and/or number of sarcomeres in series within muscle fibers. Although fascicle length measured at standardized knee flexion moments may indirectly provide information about sarcomere length and number, for accurate assessment of these parameters fascicle length as well as sarcomere length should be measured in the same child (Mathewson et al., 2015).
Chapter 4

Conclusions

Knee moment-angle characteristics and ST morphology in children with SP differ from those in TD children. Overall, our results indicate a strongly reduced ST cross-sectional area in children with SP (i.e. 48% smaller PCSA) which is likely due to a reduced rate of muscle trophy. The 18% shorter fascicle length in children with SP suggests a reduced number of sarcomeres in series. Shorter fascicles of ST explain a substantial part of the limited knee ROM in children with SP. Regarding their effects on ST stiffness, shortening of fascicles and reduction in PSCA are canceling each other and may not fully explain the increased slope of the net knee flexion moment–angle characteristics in children with SP.

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CHAPTER 5

Outcome of medial hamstring lengthening in children with spastic paresis: a biomechanical and morphological observational study

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ABSTRACT

To improve gait in children with spastic paresis due to cerebral palsy or hereditary spastic paresis, the semitendinosus muscle is frequently lengthened amongst other medial hamstring muscles by orthopaedic surgery. Side effects on gait due to weakening of the hamstring muscles and overcorrections have been reported. How these side effects relate to semitendinosus morphology is unknown.

This study assessed the effects of bilateral medial hamstring lengthening as part of single-event multilevel surgery (SEMLS) on (1) knee joint mechanics (2) semitendinosus muscle morphology and (3) gait kinematics.

All variables were assessed for the right side only. Six children with spastic paresis selected for surgery to counteract limited knee range of motion were measured before and about a year after surgery.

After surgery, in most subjects popliteal angle decreased and knee-moment angle curves were shifted towards a more extended knee joint, semitendinosus muscle belly length was approximately 30% decreased, while at all assessed knee angles tendon length was increased by about 80%. In the majority of children muscle volume of the semitendinosus muscle decreased substantially suggesting a reduction of physiological cross-sectional area. Gait kinematics showed more knee extension during stance, but also increased pelvic anterior tilt.

In most subjects, surgical lengthening of semitendinosus tendon contributed to more extended knee joint movement during static measurements as well as during gait, whereas extensibility of semitendinosus muscle belly was decreased. Post-surgical treatment to maintain muscle belly length and physiological cross-sectional area may improve treatment outcome of medial hamstring lengthening.
Outcome of medial hamstring lengthening in children with spastic paresis

Introduction

Medial hamstring lengthening in children with spastic paresis (SP) is commonly performed to increase the range of motion (ROM) of the knee, and thereby the ability of the child to extend the knee during walking (Novacheck, 2009). Medial hamstring lengthening includes lengthening of the distal semitendinosus (ST) and gracilis tendons, as well as aponeurotomy of the semimembranosus muscle (Rutz et al., 2013, Kay et al., 2002, Dreher et al., 2012b). Medial hamstring lengthening as part of single-event multilevel surgery (SEMLS) is thought to contribute to correction of flexed knee gait (Dreher et al., 2012b, Rodda and Graham, 2001, Gage, 1990, Ounpuu et al., 2015). However, the success rate is limited due to side effects of surgery (e.g. increased anterior pelvic tilt, and lumbar lordosis as well as hyperextension of the knee during gait) (Adolfsen et al., 2007, Dreher et al., 2012b, Dhawlikar et al., 1992, Zwick et al., 2002). These side effects are thought to be a consequence of over-lengthening and weakening of hamstring muscles (Dreher et al., 2012b, Dhawlikar et al., 1992, Zwick et al., 2002). In addition, persistence of flexed knee gait or even recurrence after initial success have been described (Dhawlikar et al., 1992, Dreher et al., 2012b, Chang et al., 2004). Until now, little is known about the effects of hamstring lengthening on muscle morphology (i.e. muscle belly length, tendon length and muscle volume). Detailed insight in post-surgery adaptation and function of hamstring muscles around the knee may help to increase our understanding how effects of surgery on gait, and underlying musculoskeletal structures and functions are related. Such knowledge may provide indications for improvement of surgical intervention in children with SP.

The ST, one of the hamstring muscles, is presumed to contribute to ROM limitation of the knee in children with SP and its tendon is, therefore, frequently lengthened (Rutz et al., 2013, Kay et al., 2002, Dreher et al., 2012b). In children with SP, ST has been shown to have a shorter muscle belly and lower volume than in typically developing children (Oberhofer et al., 2010, Noble et al., 2014, Lampe et al., 2006, Handsfield et al., 2016, Haberfehlner et al., 2016a). Also stiffer fascicles, sarcomeres that operate at higher length, and lower muscle fiber cross-sectional area compared to typically developing children have been reported (Smith et al., 2011). For medial gastrocnemius muscle it has been shown that one year after aponeurotomy, fascicles as well muscle belly length were shorter, without a loss of muscle volume (Fry et al., 2007, Shortland et al., 2004). How morphology of ST changes in response to surgical lengthening of the distal tendon is yet unknown.

The aims of this study were to assess the effects of medial hamstring lengthening on (1) knee joint mechanics (i.e. popliteal angle, minimal knee angle towards extension and knee moment-angle characteristics) (2) ST morphology and (3) gait kinematics. We hypothesized that a longer and more compliant tendon of ST after surgery would lead to a shift in knee moment-angle curve and a decreased popliteal angle, as well as a decrease in the slope of curve. As a consequence, the knee angle in mid-stance and terminal swing of gait would be more extended.
Chapter 5

Methods

The study was approved by the Medical Ethics Committees of the VU University Medical Center (VUmc), Amsterdam (The Netherlands) and of the University of Basel Children’s Hospital (UKBB), Basel (Switzerland) and was registered in the Dutch and German trial register (NTR3042; DKRS00004723). All children and their parents gave written informed consent.

Study design

In this prospective observational cohort study, we included children with SP who were scheduled for orthopaedic surgical lengthening of the muscle-tendon unit (MTU) of the medial hamstrings. Surgery was performed to increase the knee passive ROM and thereby improve gait function. Inclusion and exclusion criteria have been described recently (Haberfehlner et al., 2016a) (for details, see Additional file 1). All measurements were planned before first surgery and 12 months after hamstring lengthening surgery. Knee-moment angle characteristics and muscle morphology measurements were planned at six weeks, six months and 24 months after first surgery.

Medial hamstring lengthening

All children included were planned to undergo medial hamstring lengthening within a SEMLS or as a single procedure. Medial hamstring lengthening was performed distally by lengthening the ST and gracilis tendons by Z-plasty and the semimembranosus muscle by aponeurotomy (Bleck, 1987). In one of the two centers participating in the study (UKBB), it is common practice to perform the surgical medial hamstring lengthening three month prior to final SEMLS procedure (Rutz et al., 2013)

Participants

Children were included between September 2011 until May 2015 in the VUmc and UKBB. Initially, nine children (five females and four males; six children in the VUmc and three children in the UKBB) with a mean age of 14.1±2.7 years were included in the study (Haberfehlner et al., 2016a). From these nine children, three children (two females (UKBB) and one male (VUmc) were not included in the follow-up measurements and thus in the analysis of this study. The reasons were: one child eventually was not treated by surgically lengthening of the medial hamstring but was treated by distal femoral anterior guided growth (hemi-epiphysiodesis) combined with Botulinum toxin A injections into the hamstring muscles. Another child did not tolerate ultrasound measurements due to anxiety and unrest. For the third child there were planning issues. For three of the six children included in the follow-up measurements medial hamstring lengthening of the right leg was combined with hemi-epiphysiodesis, in two others with supracondylar extension osteotomy and one child did not have additional bony procedures of the right femur. For two of the six children, serious adverse events occurred after the surgery: (1) neuropathic pain after
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epidural pain management (subject 1) and (2) stage IV pressure ulcer (subject 6). The exact surgical procedures, adverse events as well as the rehabilitation program are described in supporting information (Additional file 2).

**Measurements**

**Patient characteristics and anthropometrics**

Functional mobility level was classified by the Gross Motor Function Classification System (GMFCS) (Palisano et al., 2007). Body mass and body height were measured and body mass index (BMI) was calculated.

**Knee joint characteristics**

Knee joint characteristics were determined for the right leg. The popliteal angle was measured according to Reimers (Reimers, 1974). The passive knee angle with the hip in 0° was measured using the neutral zero method (Cave and Roberts, 1936). For knee angle measurements, neutral position was defined as 0° with increasing knee angle towards knee flexion.

Knee moment-angle characteristics were measured at rest by instrumented handheld dynamometry. The experimental setup and procedures have been described in detail previously (Haberfehlner et al., 2015, Haberfehlner et al., 2016a). In brief, children were positioned on their left side, with the hip of the right (measured) leg at 70° flexion. The right lower leg was positioned on a low-friction moveable plate. The knee moment was assessed at various knee angles in steps of 5°. Knee moment and knee angles were measured only when muscle activity levels measured by surface electromyography of biceps femoris, gastrocnemius medialis, rectus femoris, and vastus lateralis muscles were absent or very low (Haberfehlner et al., 2015, Haberfehlner et al., 2016a). Data were fitted by third order polynomial functions. Knee angles at 0, 0.5, 1, 2, 3 and 4 Nm were derived from the fitted curves (Haberfehlner et al., 2015, Haberfehlner et al., 2016a). Range of knee angles between 0 and 4 Nm was calculated and referred to as range of motion (ROM<sub>0-4Nm</sub>).

**Measurements and analysis of morphological characteristics**

Muscle morphology of right ST was determined by freehand three-dimensional ultrasound (3D US). 3D US measurements, reconstruction and analysis method have previously been described in detail (Haberfehlner et al., 2016a, Haberfehlner et al., 2016b). US imaging was performed at three knee angles (i.e. at 65° and angles corresponding to 0 and 4 Nm knee moment).

Voxel arrays were anonymized for subjects, follow-up moment as well as for measurement condition (i.e. 0 Nm, 4 Nm, 65°) and analyzed in randomized order. To characterize ST morphology, (1) muscle-tendon unit length (ℓ<sub>mtu</sub>), (2) muscle belly length (ℓ<sub>m</sub>), (3) tendon length (ℓ<sub>dist</sub>) and (4) muscle belly volume were assessed. All length variables have been expressed as percentage of femur length (ℓ<sub>norm</sub> %femur). Changes in ℓ<sub>m_norm</sub> and
\( \ell_{\text{dist\_norm}} \) from 0 to 4 Nm knee flexion moment were calculated \((\Delta \ell_{\text{m\_norm}}, \Delta \ell_{\text{dist\_norm}})\).

**Gait kinematics**

Kinematic parameters were obtained by three-dimensional instrumented gait analysis. For gait analysis in VUmc, the methods have been described in detail previously (Kerkum et al., 2016). In brief, a technical clusters of three markers were rigidly attached to the body segments and anatomically calibrated by probing bony landmarks. Segment movements were tracked using an optoelectronic motion capture system (Optotrac 3020, Northern Digital, Canada). The strides were analyzed using custom-made software (Bodymech, www.bodymech.nl). Joint and segment kinematics were calculated according to International Society of Biomechanics (ISB) anatomical frames (Cappozzo et al., 1995). One postoperative gait analysis was analyzed by a custom-made, open-source software package to simultaneously observe gait parameters and video recordings (the MoXie Viewer®, http://moxie.smalll.eu/). Details have been described previously (Grunt et al., 2010). For UKBB, reflective markers (14 mm diameter) were attached bilaterally to bony landmarks on the skin. The Helen Hayes Marker set (Kadaba et al., 1990) was used to model the lower body. Movements of the subjects were tracked by a VICON motion capture system (twelve MXT20 cameras, 200 Hz; Vicon. Oxford, UK). VICON-software was used for the pre-processing of the data.

Joint angles were time normalized to a gait cycle, defined as the time between two consecutive foot strikes of the same leg. The parameters of interest were segment/joint angles in the sagittal plane at mid-stance (30% gait cycle) and at terminal swing (99% gait cycle): pelvic tilt, hip angle and knee angle of the right leg.

**Data treatment and statistics**

Follow-up measures – scheduled six weeks after surgery – could only be performed in four of the six children and were delayed. Also other scheduled measurements were postponed or canceled (for details see Additional file 3). Due to these missing data, follow-up measurements were grouped in two time windows: (1) short-term follow-up (11-20 weeks after surgery) and (2) medium-term follow-up (8-20 months after surgery). Short-term follow-up for knee moment-angle characteristics and muscle morphology measurements were only assessed in four children. As subject 1 could not walk after surgery, no gait kinematics of this subject were included.

Paired T-tests were used to test for differences between baseline and the medium-term follow-up measures of anthropometric parameters, morphological characteristics at 65° knee angle, \( \Delta \ell_{\text{m\_norm}}, \Delta \ell_{\text{dist\_norm}} \), muscle volumes and gait kinematics. To test for differences in morphological characteristics (at 0 and 4 Nm) at baseline and medium-term follow-up, repeated measures two-way ANOVA (factors: time x moment) was used. Differences in knee moment-angle characteristics (0, 0.5, 1, 2, 3 and 4 Nm) at the different time points were tested using repeated measures two-way ANOVA (factors: time x moment) with knee angle
as independent factor. Differences in ROM\textsubscript{0.4Nm} were tested by paired T-tests. Correlations were calculated by Pearson correlation coefficient (Pearson’s r). Normal distribution was tested by Shapiro-Wilk test. If data were not normally distributed, which was the case for ℓ\textsubscript{dist}\textsubscript{65deg} and muscle volume, non-parametric Related Samples Wilcoxon Signed Rank tests were used. For ANOVA, Greenhouse Geisser correction was used when the assumption of sphericity was violated. Data were presented as mean±standard deviation (SD). The level of significance was set at 0.05.

Results

**Patient characteristics and anthropometrics**

Three boys and three girls were included in the follow-up measures with a mean age of 13 years and 10 months (Table 1). From baseline to the medium-term follow-up measurements, body height and body mass increased by 6.0±4.3 cm and 4.2±2.5 kg, respectively (p=0.019, p=0.008, Table 1), while BMI and femur length of the right leg did not change (p=0.631, p=0.580, Table 1). After surgery, in all children GMFCS level remained unchanged except for one child. In this child (subject 1), GMFCS increased from level III to IV.

<table>
<thead>
<tr>
<th>Table 5.1 Clinical characteristics before and 8-20 months after medial hamstring lengthening. Values are mean±standard deviation (range).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td><strong>Gender (female/male)</strong></td>
</tr>
<tr>
<td><strong>Body height (cm)</strong></td>
</tr>
<tr>
<td><strong>Body mass (kg)</strong></td>
</tr>
<tr>
<td><strong>Femur length (cm)</strong></td>
</tr>
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<td><strong>BMI</strong></td>
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</table>

*BMI=Body mass index*

**Knee joint characteristics**

Popliteal angle of the right leg decreased from 72±8° (range 60-80°) at baseline to 47±15° (range 30-70°) at medium-term follow-up (p=0.017). Minimal passive knee angle towards extension of the right leg measured with the hip at 0° decreased from 29±12° (range 15-45°) at baseline to 10±11° (range 0-25°) at medium-term follow-up (p=0.024).
Figure 5.1 Individual net knee moments as a function of knee angle of all six subjects. A: Subject 1; B: Subject 2; C: Subject 3; D: Subject 4; E: Subject 5; F: Subject 6; Squares: Baseline; Triangle: short-term follow-up 11-20 weeks after surgery (for 4 subjects A-D); Dots: Follow-up 8-20 months after surgery.
Outcome of medial hamstring lengthening in children with spastic paresis

For the four subjects (subject 1-4) for whom short-term follow-up measurements (i.e. 11-20 weeks after surgery) were obtained, ANOVA of the knee angle did not reveal any effect of time ($p=0.065$). However, a significant interaction effect between time and net knee flexion moment was found ($p=0.046$), indicating a change in the shape of the curve. This is most evident at low knee moments (between 0 and 1 Nm) at which the slope was less steep (Figs 1A-D). The $\text{ROM}_{0-4\text{Nm}}$ was $29\pm2^\circ$ (range 27-31°) at baseline and $40\pm8^\circ$ (range 28-46°) at short-term follow-up, but this difference was not significant ($p=0.100$).

Medium-term follow-up measurements (8-20 months post-surgery) of knee-moment angle were assessed for the whole group of children. At baseline, knee angles ranged from $83\pm11^\circ$ at 0 Nm knee flexion moment to $56\pm3^\circ$ at 4 Nm. After surgery, knee angles ranged from $63\pm12^\circ$ at 0 Nm net knee flexion moment to $41\pm17^\circ$ at 4 Nm. Note that the variation in effect of surgery on the knee moment-angle curve was substantial (Figure 5.1). Repeated measures ANOVA revealed for the medium-term follow-up no effect of time on the knee angle ($p=0.080$) and no interaction effect of time and net knee flexion moment ($p=0.768$). The lack of interaction effect was supported by the observation that pre and post-surgery knee $\text{ROM}_{0-4\text{Nm}}$ did not differ (Baseline: $28\pm5^\circ$; medium-term follow-up: $22\pm7^\circ$; $p=0.180$). At medium-term follow-up, knee moment-angle curves for all subjects, except for one (subject 1) were shifted towards lower knee angles (i.e. a more extended knee, Figure 5.1). The largest curve shifts occured in children who had more flexed knees (i.e. higher knee angles) prior to the surgery (i.e. subject 2, Figure 5.1B; subject 3, Figure 5.1C and subject 6, Figure 5.1F).

For four subjects (subject 1-4), we could make comparisons between short-term and medium-term effects. No effect of time ($p=0.367$), but a significant interaction effect between time and net knee flexion moment was found ($p=0.001$). The knee $\text{ROM}_{0-4\text{Nm}}$ in these four subjects changed from $40\pm8^\circ$ at short- term follow-up to $24\pm9^\circ$ at medium-term follow-up ($p=0.033$), thus, back to baseline levels. These results indicate that at short-term follow-up, mean slope of the knee moment-angle curve was decreased. However, at medium-term follow-up mean slope returned back towards baseline values.

**Morphological characteristics of semitendinosus muscle**

At baseline, we measured $\ell_{\text{mtu}}$, $\ell_m$, $\ell_{\text{tdis}}$, muscle volume, fascicle length and volumes of the both ST compartments (Haberfehlner et al., 2016a). After surgery, however, fascicle length and proximal and distal muscle volume could only be estimated in two subjects because enhanced ultrasound echo intensity was leading to an inaccurate identification of tendinous inscription (see Figure 5.2 for an example of decreased image quality after lengthening of ST tendon).
Figure 5.2 Typical example of 3D ultrasound images and segmentation of muscle volume of a child with a spastic paresis before medial hamstring lengthening. Images before surgery (left, A1-C1) and 12 months after surgery (right, A2-C2). A: longitudinal view of semitendinosus muscle (ST) (proximal on the left side); B transversal view of ST at three locations (most proximal on left side; orientation is medial-lateral). Yellow: distal compartment of ST; red: proximal compartment of ST. C: Proximal (red) and distal (yellow) compartments after segmentation. After surgery, this child showed a reduction of muscle volume by 26%, muscle belly length decreased by 32% and tendon length increased by 62%, measured at a knee angle corresponding to 4 Nm knee moment. Note the post-surgical increase in ultrasound echo intensity (cf. A2 and A1), which complicated exact identification of structures, in particular the distal and proximal ends of the tendinous inscription. The tendinous inscription is indicated by a red arrow in A1 and A2.
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Origin and insertion distance of ST muscle at a knee angle of 65° ($l_{mtu\;65deg\;norm}$) increased by 11±8% between baseline and medium-term follow-up measurement (Table 2). This increase was likely due to effects of bone surgery (i.e. hemi-epiphysiodesis and supracondylar extension osteotomy). Despite this increase in MTU length, muscle belly length at 65° knee angle ($l_{m\;65deg\;norm}$) was decreased by 28±7%. In contrast, $l_{t\;dist\;65deg\;norm}$ was increased by 77±26% after surgery (Table 2, Figure 5.3 E&F).

After surgery, $l_{mtu\;norm}$ measured at net knee flexion moments of 0 Nm and 4 Nm were increased (i.e. 12±9% longer at 0 Nm and 10±7% at 4 Nm, Table 2). Muscle belly lengths ($l_{m\;norm}$) at 0 Nm and 4 Nm were decreased after surgery by 28±7% and by 33±6%, respectively (Table 2; Figure 5.3A&C). Tendon length at 0 Nm ($l_{t\;dist\;norm}$) was increased by 83±43% and by 85±30% at 4 Nm (Table 2; Figure 5.3B&D). For $l_{m\;norm}$, a significant interaction effect was found between factors time (i.e. baseline and medium-term follow-up) and knee moment (p=0.008). However, no such effect was found for $l_{mtu\;norm}$ and $l_{t\;dist\;norm}$ (p=0.719 and p=0.216, respectively). These interaction effects were also indicated by a smaller $\Delta l_{m\;norm}$ at medium-term follow-up compared to that at baseline, while $\Delta l_{mtu\;norm}$ and $\Delta l_{t\;dist\;norm}$ did not differ significantly (Table 2).

Table 5.2 Morphological characteristics of semitendinosus muscle before and 8-20 months after medial hamstring lengthening (length variables as percentage of femur length (%femur) at 0 Nm ($0Nm$) and 4 Nm ($4Nm$) knee moment and 65 degree ($65deg$) knee angle.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Medium- term follow-up (8-20 months)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>$l_{mtu;0Nm;norm}$</td>
<td>115.6±7.1 %femur</td>
<td>129.0±13.3 %femur</td>
<td>0.020</td>
</tr>
<tr>
<td>$l_{mtu;4Nm;norm}$</td>
<td>123.3±6.8 %femur</td>
<td>135.8±11.6 %femur</td>
<td></td>
</tr>
<tr>
<td>$\Delta l_{mtu;norm}$</td>
<td>7.7±3.4 %femur</td>
<td>6.8±4.4 %femur</td>
<td>0.719</td>
</tr>
<tr>
<td>$l_{mtu;65deg;norm}$</td>
<td>118.9±4.3 %femur</td>
<td>131.8±13.4 %femur</td>
<td>0.025</td>
</tr>
<tr>
<td>$l_{m;0Nm;norm}$</td>
<td>73.4±8.5 %femur</td>
<td>52.8±7.1 %femur</td>
<td>0.000</td>
</tr>
<tr>
<td>$l_{m;4Nm;norm}$</td>
<td>78.0±8.1 %femur</td>
<td>52.8±7.7 %femur</td>
<td>0.008</td>
</tr>
<tr>
<td>$\Delta l_{m;norm}$</td>
<td>4.6±3.0 %femur</td>
<td>-0.1±4.4 %femur</td>
<td></td>
</tr>
<tr>
<td>$l_{m;65deg;norm}$</td>
<td>75.2±6.8 %femur</td>
<td>54.4±7.6 %femur</td>
<td>0.000</td>
</tr>
<tr>
<td>$l_{t;dist;0Nm;norm}$</td>
<td>42.2±4.3 %femur</td>
<td>76.2±13.8 %femur</td>
<td>0.001</td>
</tr>
<tr>
<td>$l_{t;dist;4Nm;norm}$</td>
<td>45.3±3.4 %femur</td>
<td>83.0±9.6 %femur</td>
<td></td>
</tr>
<tr>
<td>$\Delta l_{t;dist;norm}$</td>
<td>3.1±3.1 %femur</td>
<td>6.8±7.8 %femur</td>
<td>0.216</td>
</tr>
<tr>
<td>$l_{t;dist;65deg;norm}$</td>
<td>43.7±3.9 %femur</td>
<td>77.3±12.9 %femur</td>
<td>0.028</td>
</tr>
</tbody>
</table>

$\Delta l_{m\;norm}$ = length of muscle-tendon unit as the sum of $l_{m}$ and $l_{t\;dist}$; $l_{m}$=length muscle belly: ischial tuberosity distal muscle tendinous junction; $l_{t\;dist}$=length of distal tendon; all length variables were expressed as % of femur length ($\%femur$).
Figure 5.3 Individual effects of hamstring surgery on ST morphology. Muscle belly length and tendon length were measured at knee angles corresponding to 0 Nm (A, B), 4 Nm (C, D) and at 65° knee flexion angle (E, F). Muscle belly decreased after surgery, while tendon length increased. Time points used for statistical analysis are highlighted by circles.
In four of the six subjects muscle volume of ST decreased substantially between baseline and medium-term follow-up measurements. In one subject muscle volume decreased slightly and in one subject muscle volume was slightly increased (Additional file 3). Overall an average 44% decrease was found, however this decrease did not reach significance (p=0.075, Table 2). The more pronounced decrease in muscle volume after medial hamstring lengthening compared to the decrease in muscle belly length, suggests that at medium-term follow up the physiological cross-sectional area (PCSA) of ST had reduced.

In successive follow-ups, for ℓm or ℓt, no changes towards values measured before surgery could be shown (Figure 5.3). Therefore, the observed recurrence of the steepness of the knee moment-angle curve between short-term and medium-term follow-up (Figure 5.1) does not seem to be explained by changes in ST morphology.

Figure 5.4 Effects of hamstring muscle surgery on gait kinematics. Presented data were measured before (Baseline, black) and 8-20 months (Medium follow-up, grey) after medial hamstring lengthening. In white the reference data of a group of typically developing children (TD) are presented. Values are mean±standard deviation. MSt=Mid-stance; TSw=Terminal Swing. **p<0.01; *p<0.05.
Figure 5.5 Individual gait kinematics before and after hamstring lengthening surgery. Pelvic tilt at mid-stance and terminal swing (A, B), hip angle at mid-stance and terminal swing (C, D) and knee angle at mid-stance and terminal swing (E, F) at baseline and 8-20 months after surgery. Pelvic tilt changed towards more anterior tilt and knee joint was more extended both at mid-stance and terminal swing, while effects on hip angles were variable. The grey area represents the mean and two standard deviations of a group of typically developing children.
Gait kinematics

At baseline and medium-term follow-up, sagittal plane joint kinematics could be assessed in five out of six children (Additional file 4). The knee was more extended during mid-stance and terminal swing, but pelvic anterior tilt was increased (Figure 5.4). While in all children, during mid-stance and terminal swing the knee was more extended and pelvic anterior tilt was increased, effects on the hip angle were variable. The hip was more extended in one child (subject 3), slightly more flexed (about 10º) in three children (subject 2, 4 and 5) and substantially more flexed in one child (subject 6) (Figure 5.5). A more extended knee joint during mid-stance and terminal swing correlated positively with a decrease in hip angle (i.e. more extended hip joint) ($r=0.954$; $p=0.012$ and $r=0.920$; $p=0.027$), suggesting that children with less improvement towards knee extension showed a higher increase in hip flexion (Figure 5.6).

![Figure 5.6](image)

**Figure 5.6 Relation between change in knee angle at terminal swing and change in hip angle at terminal swing.** A: Regression analysis showed a significant relation. The more extended knee joint during terminal swing and the change in hip angle ($r=0.920; p=0.027$), indicating that the children with larger improvements of knee angles (i.e. more extended knee joint) showed larger decreases in hip flexion. B: Typical stick diagram of two subjects for hip and lower leg joint angles before and after surgery. Yellow represents the baseline (pre-surgical) of joint angles and pelvic tilt during terminal swing and red represents medium follow-up. Anterior pelvic tilt increased in both subjects after surgery. In subject 3, hip angle flexion decreased by 10º and knee joint angle towards extension increased substantially (47º), while in subject 6, hip flexion angle increased substantially (i.e. 38º) and knee joint angle towards extension increased only slightly (7º).
Chapter 5

Discussion

This study shows that after medial hamstring lengthening popliteal angle and minimal passive knee joint angle towards extension were both improved. In most subjects, knee-moment angle curves initially showed a shift towards more extended knee angles, while knee ROM between 0 and 4 Nm knee flexion moment increased, but recurred to pre-surgery values at medium-term follow-up. Muscle belly length decreased while tendon length increased and the change in muscle belly length from 0 to 4 Nm decreased. In gait, knee joint angles measured at medium-term follow-up were more extended in mid-stance and terminal swing, but pelvic anterior tilt increased also. There was no consistent change in hip angle. Individual data indicate that less improvement of the knee joint angles towards extension angles during gait correlated with more increase of hip flexion.

Changes in knee joint characteristics and ST morphology

The decreased popliteal angle and improved passive knee angle at medium-term follow-up in five of the six subjects were as expected. Also the marked shift towards more extended knee angles measured by knee moment-angle characteristics in four of the six subjects was expected. Medium-term improvement in knee angle could not be shown for subject 1 in whom a major adverse event occurred (see S2 Table). Our data and those of previous studies suggest that a more extended knee joint measured by both clinical examination and instrumented hand-held dynamometry can be expected after medial hamstring lengthening (Chang et al., 2004, Dreher et al., 2012b, Dhawlikar et al., 1992), at least when no major adverse events complicate recovery and rehabilitation.

Assuming that the ST largely contributes to the knee extension limitations before surgery (Haberfehlner et al., 2016a), the improved knee angles towards extension after surgery can theoretically be the result of an increase in ST muscle belly length, which given the parallel fibered architecture of ST implies longer fascicles, and/or increases ST tendon length. However, instead of an increase in ST muscle belly length we showed a reduction in muscle belly length. This reduction is most likely due to fascicle shortening by a reduction in the number of sarcomeres in series. A decrease in number of sarcomeres in series after surgery would reduce extensibility of fascicles, which was confirmed by the decrease in muscle belly length change from 0 and 4 Nm. In contrast, ST tendon length was substantially increased, which exceeded the shortening of ST muscle belly length and therefore ST MTU length was increased. It seems that the increased ST tendon length after surgery likely contributed to the increase in knee angle towards knee extension.

At short-term follow-up, the mean slope of the knee moment-angle curve was decreased, suggesting a decrease in stiffness of the structures spanning the knee joint. However, this was only temporary. At medium-term follow-up, there was no difference in slope. As there were no changes in ST muscle belly and tendon lengths back to pre-surgical values, the recurred steeper slope of knee moment-angle curve must be the result of other
changes such as (1) length changes of (other surgically) treated muscles (i.e. gracilis and semimembranosus), (2) changes in mechanical properties (i.e. increased stiffness) of hamstring MTUs and/or (3) increased stiffness of extramuscular connective tissue structures (e.g. due to scar tissue formation). Scar tissue of hamstring tendons has also been observed at repeated hamstring lengthening during surgery (Dhawlikar et al., 1992). The formation of scar tissue may counteract the initial decrease in stiffness and may cause the recurrent stiffness at medium-term follow-up.

**Effect of surgery on joint position during gait**

After ST lengthening surgery, all children showed improved knee angles in mid-stance and terminal swing during gait, while effects on hip flexion were variable. The magnitude of increase in knee extension in mid-stance is similar to that reported in previous studies (Dreher et al., 2012b, Abel et al., 1999). In addition, the effects of surgery on knee angle in terminal swing are similar to those of previous studies (Dreher et al., 2012b, Abel et al., 1999). The biarticular ST is maximally stretched in terminal swing with the hip in maximal flexion and the knee extended (Cooney et al., 2006). Therefore, lengthening of the biarticular ST may lead to a reduction in passive resistance of ST against hip flexion and knee extension. In both terminal swing and mid-stance, subjects with larger improvements of knee angles (i.e. more extended knee joint) showed larger decreases in hip flexion (subject 3) or unchanged hip flexion angles (subject 2 and subject 4), while subjects with less improvement in knee angles towards knee extension showed an increase in hip flexion (subjects 5 and 6, Figure 5.6). These relations indicate that the effect of medial hamstring lengthening can differ for knee and hip, with a greater change around the knee (i.e. more change towards knee extension) in most children.

The observation that all subjects in the current study showed an increased anterior pelvic tilt after medial hamstring lengthening, was higher (i.e. 100%) than the percentage that has been reported previously (i.e. 43%) (Dreher et al., 2012b). During the stance phase of gait, an increased anterior tilt occurs when knee joint angles improve towards extension while hip flexion remains unchanged or is increased. Therefore, a decrease in hip flexion (i.e. a more extended hip) would be necessary to compensate for the effects of increased anterior pelvic tilt due to hamstring lengthening. However, after medial hamstring lengthening, ST may not sufficiently contribute to stabilize the hip during stance, because its capacity to generate active force is likely reduced, due to a decrease of PCSA of ST Also, walking aids may influence pelvic tilt (Krautwurst et al., 2016). Three subjects walked with different support during gait analysis after surgery than before (subject 3, subject 4 and subject 6) (Additional file 4). These three subjects were the only ones with an anterior tilt greater than normal reference values during mid-stance (Figure 5.5A). Possibly the change in support affected the pelvic tilt in these subjects in addition to changes in active force generation.
Clinical implications

A decrease in muscle belly length of ST after surgical lengthening indicates a decrease in length range of active force exertion of ST, while a decrease in ST muscle PCSA suggests a decrease in its force generating capacity. However, previous research has shown that while after hamstring lengthening strength of knee flexor muscles declined initially, it returned back to pre-surgical values nine months after hamstring lengthening (Damiano et al., 1999). This indicates that other knee extensor and hip flexor muscles (i.e. m. biceps femoris, m. semimembranosus) might compensate for the decrease in PCSA of ST.

Optimal treatment to improve knee angles towards extension in mid-stance should increase knee extension movement and maintain ST muscle belly length as well as PCSA. A longer ST MTU without shortening of muscle belly may be obtained when the ST tendon is lengthened without break-down of of sarcomeres in series. Muscle activation simultaneous with stretch has been suggested to maintain the number of sarcomeres (Van Dyke et al., 2012). Resistance training (Seniorou et al., 2007) in joint positions in which muscles are strained and/or active-movement training in a stretched position (Zhao et al., 2011) may therefore be required to counterbalance the decrease in muscle belly length and PCSA. In addition, the method of lengthening the ST (Z-lengthening of the tendon vs. tenotomy or aponeurotomy) (Dagge et al., 2012) and the magnitude of lengthening may contribute to the extent by which the muscle belly is strained during stretching exercises as well as during daily activities. Compared to aponeurotomy (Dagge et al., 2012), Z-lengthening of ST tendon, as performed in the current study, may result in less extension of the muscle belly due to a longer and more compliant distal ST tendon. Experimental aponeurotomy in rat m. gastrocnemius medialis has shown to increase optimum muscle length without a decrease in optimal force (Brunner et al., 2000). Future research should investigate the effects of different lengthening procedures of ST (i.e. z-lengthening of the tendon, (percutaneous) tenotomy and aponeurotomy) on its morphology and relate these to knee joint mechanics, gait and functional outcome in order to improve treatment outcome of SEMLS including medial hamstring lengthening. In addition, different rehabilitation protocols comprising resistance training, stretching and immobilization should be investigated for their effects to counteract atrophy and a shortening of the ST muscle belly. When considering hamstring lengthening surgery to improve gait, it is important to realize the effects that this may have not just on knee kinematics, but also on kinematics of the pelvis and hip. After medial hamstring lengthening, additional treatments to improve hip movement towards extension may be necessary to counterbalance the increased anterior pelvic tilt after medial hamstring lengthening. An increased resistance to stretch of hip flexor muscles (i.e. m. rectus femoris and m. psoas) as well as weakness of hip extensors may contribute to the anterior pelvic tilt. Surgical strategies to reduce hip flexion, such as proximal m. rectus femoris lengthening and/or m. psoas lengthening, have been suggested (Park et al., 2009), however with variable success (Delp et al., 1996, Morais Filho et al., 2006, Mallet et al., 2016). Note, that none of the children included in the current study were indicated for a hip
flexor procedure (Additional file 2). Next to hip flexor procedures also strengthening of hip extensors (Seniorou et al., 2007) and abdominal muscles by training may help to reduce the increased anterior tilt after surgery. In addition, the impact of walking devices on pelvic tilt should be considered.

Limitations

The number of subjects in this study was low, but this was the maximum number that could be included from both medical centers during the inclusion period of 3.5 years. Assessments took about three hours and could only be performed on the days that children had to visit the hospital for preliminary or control examinations for surgery to burden children and their parents as less as possible. Therefore, subjects were not measured at all initially scheduled time points. A larger study group would be needed for more comprehensive conclusions about the effects of medial hamstring lengthening on overall treatment outcome. However effects of z-lengthening of the ST tendon on muscle morphology seem to be quite consistent and can be noticed reliably even in a small group of children as assessed in the current study.

We assessed morphology of only one muscle that was surgically treated, while the whole intervention included procedures on multiple muscles and sometimes bones. Due to the duration of measurements it was not feasible to additionally assess morphology of other muscles (e.g. semimembranosus or gastrocnemius muscle). However, muscles with different morphologies will respond differently to surgical lengthening of the tendon (as described by a modeling approach (Delp and Zajac, 1992)) or to aponeurotomy and these effects should be studied in future as they might influence treatment outcome.

In two of the six children, serious adverse events occurred after surgery. Previous studies have shown that peripheral neurological complications and skin problems (as in the current study), as well as other adverse events (e.g. infection, respiratory problems and pain) occur frequently during or after SEMLS in children with SP (Inan et al., 2015, Lee et al., 2015, Stout et al., 2008, Karol et al., 2008). These adverse events need to be taken into account when SEMLS are indicated and in the interpretation of outcome of surgery.

In the current study two etiologies of SP (i.e. cerebral palsy and hereditary spastic paresis) have been included. Etiology may impact treatment outcome of medial hamstring lengthening, however the sample size was too low to investigate the effect of etiology. Future studies with a larger sample size are warranted to investigate such effects.
Chapter 5

Conclusion

Medial hamstring lengthening leads to a longer ST tendon, but a shorter and smaller ST muscle belly. The longer tendon seems to contribute to a more extended knee joint during static measurement as well as reduced knee flexion in mid-stance and terminal swing during gait. The extensibility of ST muscle belly after surgery is decreased, likely by a shorter muscle belly. Maintaining of muscle belly length and PCSA may improve treatment outcome of medial hamstring lengthening.

Acknowledgements

We wish to thank Danny Koops, Léon Schutte, and Guus Baan for designing and engineering the setup. Andrea Spierenburg, Rozemarijn Dekker, Guido Weide and Marjolein Piening, Beat Göpfert, Christine Seppi, and Katrin Pua are acknowledged for their assistance with measurements. We are very grateful to Guido Weide whose Matlab programs for reconstruction of the voxel array were used in this study.
Appendix

Additional file 1

Inclusion and exclusion criteria

Inclusion criteria for the current study were: (1) a clinical diagnosis of SP due to cerebral palsy or hereditary spastic paresis (Rosenbaum et al., 2007, SCPE, 2002, Fink, 2013), (2) being selected for ST lengthening within a SEMLS or as a single procedure. Indications for surgery were (a) a fixed knee flexion limitation of ≥15° and/or a popliteal angle of ≥60° and (b) a gait pattern with flexion of the knee in midstance and endorotation-adduction movement of the hips in terminal swing, (3) Gross Motor Function Classification System (GMFCS) (Palisano et al., 2008) level I, II (walking without walking aids) or III (walking with a walking aid), and (4) an age between 6 and 20 years. Patients were excluded if they had interfering treatment and/or had a co-morbidity that could possibly affect walking ability and the tissue properties of the hamstring muscles. We considered as interfering treatment: (1) use of medication that affected neuromuscular properties, (2) treatment with Botulinum toxin A and/or (3) serial casting within 3 months before measurements, as well as any preceding (4) selective dorsal rhizotomy, (5) hamstring muscle surgery, or (6) intrathecal baclofen treatment.
**Additional file 2**

**Individual patient information, surgery, adverse events and rehabilitation program**

<table>
<thead>
<tr>
<th>Subjects 1 - 3: Gender Age Etiology Length / Weight GMFCS</th>
<th>Surgery</th>
<th>Adverse events</th>
<th>Rehabilitation / additional treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: female 10.6 years cerebral palsy 146 cm / 46 kg GMFCS III</td>
<td>Both legs distal medial hamstring lengthening, adductor and psoas release; left leg: hemi-epiphysiodesis</td>
<td>Neuropathic pain after epidural pain management; morphine pump GMFCS III =&gt; GMFCS IV</td>
<td>First 6 weeks: immobilization in knee extension brace left leg (23 hours per day); first half year three to four times a week physiotherapy including strengthening, mobilization, pain rehabilitation and hydrotherapy; second half year after surgery two to three times per week physiotherapy including training on parallel bars, transfers training and biking</td>
</tr>
<tr>
<td>2: male 12.7 years hereditary spastic paresis 136 cm / 27 kg GMFCS III</td>
<td>Both legs distal medial hamstring lengthening and hemi-epiphysiodesis; right leg: exorotating femur osteotomy, endorotating tibia osteotomy and aponeurotic lengthening of triceps surae (Vulpius); left leg: aponeurotic lengthening of triceps surae (Vulpius) and triple arthrodesis foot; After one week of first SEMLS: triple arthrodesis right foot</td>
<td></td>
<td>First 6 weeks: no-weight bearing; immobilization in knee extension brace both legs (23 hours per day); Till half year after SEMLS four times a week physiotherapy including gait training, mobilization, and strengthening; after 12 weeks therapy included two times training in water; Eight months after SEMLS: Botuline toxin A treatment: psoas, adductors, rectus femoris, flexor dig. brevis and flexor dig. longus;</td>
</tr>
<tr>
<td>3: female 17.2 years cerebral palsy 154 cm / 47 kg GMFCS III</td>
<td>Both legs distal medial hamstring lengthening and supracondylar extension osteotomy; Right leg: aponeurotic lengthening of triceps surae (Vulpius)</td>
<td></td>
<td>First 8 weeks immobilization in knee extension brace both legs (23 hours per day); inpatient rehabilitation nine weeks including gait training, mobilization, and strengthening; Till half year after SEMLS two times a week physiotherapy, then one time.</td>
</tr>
</tbody>
</table>
### Outcome of medial hamstring lengthening in children with spastic paresis

<table>
<thead>
<tr>
<th>Subjects 4 - 6: Gender Age Etiology Length / Weight GMFCS</th>
<th>Surgery</th>
<th>Adverse events</th>
<th>Rehabilitation / additional treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>4: male 15.8 years cerebral palsy 151 cm / 53 kg GMFCS II</td>
<td>Both legs distal medial hamstring lengthening three month prior to SEMLS; SEMLS: supracondylar extension osteotomy; patellar tendon shortening; both feet: fusion of calcaneocuboid joints</td>
<td>First 9 weeks: immobilization in knee extension brace both legs (23 hours per day); one hour mobilization and standing exercises; followed by knee extension brace for 2-3 hours and three sessions of physiotherapy every week, including gait training, mobilization and strengthening. After SEMLS: 12 weeks of inpatient rehabilitation. Two years after SEMLS: Rectus release both legs</td>
<td></td>
</tr>
<tr>
<td>5: female 11.2 years hereditary spastic paresis 151 cm / 46 kg GMFCS II</td>
<td>Both legs distal medial hamstring lengthening and distal femoral anterior guided growth (hemi-epiphysiodesis)</td>
<td>Pain at growth plate at location of fixation plates till half year after surgery</td>
<td>First 12 weeks: immobilization in removable knee extension brace both legs (8 hours per night); combined with five times a week physiotherapy including gait training, mobilization, and strengthening. Till half year after SEMLS two times a week physiotherapy, then one time. Biking at five weeks after SEMLS.</td>
</tr>
<tr>
<td>6: male 15.2 years cerebral palsy 176 cm / 61 kg GMCS III</td>
<td>Both legs distal medial hamstring lengthening; right leg hemi-epiphysiodesis, exorotating femur osteotomy, endorotating tibia osteotomy; Left leg: hemi-epiphysiodesis, triple arthrodesis foot; After four weeks of first SEMLS: triple arthrodesis right foot</td>
<td>Stage 4 pressure ulcer surgically treated</td>
<td>First 8 weeks: immobilization in knee extension brace both legs (23 hours per day); 6 weeks below-knee cast for mobilization, 8 hours per night knee extension braces, inpatient rehabilitation 12 weeks including gait training, mobilization, and strengthening; Till half year after SEMLS three times a week physiotherapy, then one time. Wheelchair sports training four times a week.</td>
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## Additional file 3

### Individual data knee joint mechanics and muscle morphology (subject 1 - 3)

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<tr>
<th>Subject</th>
<th>Time before / after surgery</th>
<th>Baseline</th>
<th>T1 10 - 20 wk</th>
<th>T2 5 - 9 mo</th>
<th>T3 10 – 20 mo</th>
<th>T4 &gt; 20 mo</th>
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<td>70</td>
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<td>20</td>
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<td>22.4 / 28.4</td>
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<td>26.2 / 18.3</td>
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<td>22.4 / 28.4</td>
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### Outcome of medial hamstring lengthening in children with spastic paresis

**Individual data knee joint mechanics and muscle morphology (subject 4 - 6)**

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<th>Baseline</th>
<th>T1 10 - 20 wk</th>
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<th>T3 10 - 20 mo</th>
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<tbody>
<tr>
<td><strong>4</strong></td>
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<tr>
<td>Maximal knee extension (º)</td>
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<td>θ₀Nm (º)</td>
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<td>28</td>
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<td>ℓm₀Nm / ℓt₈₀Nm (cm)</td>
<td>26.8 / 12.3</td>
<td>19.5 / 21.1</td>
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<td>ℓm₄Nm / ℓt₈₄Nm (cm)</td>
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<td>Maximal knee extension (º)</td>
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<td>20.3 / 28.2</td>
<td>21.5 / 32.4</td>
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<td>21.5 / 30.1</td>
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<td>23.0 / 31.2</td>
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ℓ_femur = femur; θ₀Nm, θ₄Nm = knee angle corresponding to 0 Nm and 4Nm net knee moment; ℓm = length of muscle belly: ischial tuberosity to distal muscle tendinous junction; ℓt₈₀ = length of distal tendon. ℓm, ℓt₈₀ are measured at three knee angles (i.e. θ₀Nm, 65 degree knee flexion θ₄Nm); wk = weeks; mo = month.
### Additional file 4

**Individual data gait kinematics**

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<th>Baseline</th>
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<td>TSw Pelvic tilt</td>
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<td>MST Knee angle</td>
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<td>TSw Knee angle</td>
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<td>MST Hip angle</td>
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<td></td>
<td>TSw Hip angle</td>
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<td>Walking device*</td>
<td>posterior walker</td>
<td>posterior walker</td>
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<td></td>
<td>TSw Hip angle</td>
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<td>MST Knee angle</td>
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<td>TSw Knee angle</td>
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<td>MST Hip angle</td>
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<td>Walking device*</td>
<td>posterior walker</td>
<td>walking stick with four legs</td>
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*MSt=mid-stance; TSw=terminal swing; *during gait analysis*
Epilogue
Thesis summary

The general aim of this thesis was to investigate effects of medial hamstring lengthening in children with spastic paresis (SP) on knee joint mechanics, morphological characteristics of the semitendinosus (ST) muscle and gait and, thereby, to identify factors that may contribute to a favourable or unfavourable outcome of the surgery.

In Chapter 2, we presented a modified dynamometer approach to measure knee joint mechanics. We aimed (1) to test the standard errors of measurement (SEM) and the smallest detectable differences (SDD) of knee joint mechanics in repeated measurements, (2) to determine the correlation between knee angle measurements at 4 Nm knee flexion moment by instrumented hand-held dynamometry and popliteal angle and (3) to compare knee joint mechanics between children with SP to typically developing (TD) children. We showed with instrumented hand-held dynamometry it is possible to measure knee moment-angle curves between days with a SEM of about 5º and SDD of below 14º at knee angles corresponding to 1-4 Nm net knee flexion moments. In children with SP, the knee angle measured at a standardized knee moment (i.e. 4 Nm) did not correlate with popliteal angle. The knee moment-angle curve of children with SP was not shifted compared to the curve of TD children, while the slope of the knee-moment angle curve at 4 Nm was steeper in children with SP compared to TD children (i.e. knee angles did not differ between groups at low net knee moments). In conclusion, we have shown that the presented method to measure knee joint mechanics can be used to assess clinically relevant changes in knee moment-angle characteristics.

The aims in Chapter 3 were (1) to present a newly developed analysing protocol for measuring muscle morphology of ST muscle by freehand three-dimensional ultrasound (3D US) and (2) to compare morphological characteristics of ST determined by 3D US with those measured on dissected cadaveric muscles. Mean differences between morphological characteristics (e.g. muscle belly length, muscle fascicle length and muscle volume) measured by 3D US and after dissection were smaller than 10%. Intra-class correlation coefficients (ICCs) were higher than 0.75 for all variables except for the length of proximal fascicles (ICC=0.58). We concluded that the presented 3D US method allows for reasonably accurate measurements of key morphological characteristics of ST muscle.

In Chapter 4, we aimed to determine how knee joint mechanics and ST morphology differ between children with SP selected for medial hamstring lengthening and TD, as well as to assess how knee joint mechanics and ST morphology are related. At net knee flexion moments above 0.5 Nm, more flexed knee angles at equal knee moments were found for SP compared to TD children (Figure 6.1). The mean slope of the knee-moment angle curve was increased in children with SP, indicating an increased stiffness of the structures spanning the knee joint. This increased stiffness around the knee joint suggests an increased muscle-tendon unit (MTU) stiffness of the hamstring muscles. Muscle volume, physiological cross-sectional area (PCSA), and fascicle length of ST normalized to femur length were smaller
Chapter 6

in SP compared to those in TD children (62%, 48%, and 18%, respectively). Tendon length normalized to femur length did not differ between groups. The shorter fascicle length shown in children with SP partly explains the knee angle reached at 4 Nm knee moment and, hence, a more flexed knee joint in children with SP. Other factors that might have contributed to limited knee extension in children with SP are altered tissue composition of fascicles due to increased collagen content, alterations in morphology of other knee flexor muscles and/or increased stiffness of other structures around the knee (i.e. articular capsule, ligaments, nerves, blood vessels and connective tissues).

In Chapter 5, the aim was to evaluate longitudinal effects of medial hamstring lengthening on knee joint characteristics (i.e. knee moment-angle characteristics, popliteal angle and minimal knee angle towards extension with the hip extended), ST muscle morphology and gait kinematics. At short-term follow-up (i.e. 11-20 weeks after surgery), the mean slope of the knee moment-angle curve was decreased, suggesting a decrease in stiffness of the structures spanning the knee joint. However, this was only temporary. At medium-term follow-up (i.e. about one year after medial hamstring lengthening), there was no difference in slope compared to the pre-surgical slope of the knee moment-angle curve. The observed recurrence of the steepness of the knee moment-angle curve between short-term and medium-term follow-up could not be explained by changes in ST morphology. At medium-term follow-up, in most subjects a shift of the knee-moment angle curve towards more knee extension was observed (Figure 6.1), as well as an increase in maximum knee extension during static knee angle measurements (i.e. popliteal angle and minimal knee angle towards extension with the hip extended) as well as during gait, but also pelvic anterior tilt increased. About a year after medial hamstring lengthening ST belly length was more than 28% decreased and tendon length more than 77% increased. These results show that in most subjects, lengthening of ST tendon contributed to an increased knee extension during gait.

Combining the results of the studies on muscle morphology and knee joint mechanics of chapter 4 and 5, shows that after medial hamstring lengthening, muscle belly length and muscle volume of children with SP differ to a larger extent from those of TD children compared to the pre-surgical situation (i.e. shorter length and smaller volume), while the knee angle corresponding to 4 Nm is shifted towards more knee extension and was more comparable to that op TD children. However, after medial hamstring lengthening at medium-term follow-up the slope of the knee-moment angle curve (i.e. the range of knee angles between 0 and 4 Nm) remained unchanged in children with SP (Figure 6.1). This implies that the more extended knee angle at 4 Nm in most subjects was reached by a shift of the whole curve. Due to this shift the 0 Nm measured for children with SP knee angle was also shifted towards a more extended angle compared to TD children and to the pre-surgical situation. This shift of knee angle at 0 Nm may negatively affect the movement towards knee flexion after surgery.
Figure 6.1 Effect of spastic paresis (SP) and surgical intervention on knee moment-angle curve. Black dots: Children with SP before medial hamstring lengthening; Triangles: Age and gender matched typically developing (TD) children; White dots: Children with SP about a year after medial hamstring lengthening. Note that before surgery the knee-moment angle curve was slightly shifted and the slope differed between children with SP and TD children. After surgery the curve was shifted towards more knee extension (black arrow), while the slope did not change. After surgery, the knee angle at 4 Nm was similar compared to that of TD (dotted circle), while the range of knee angles between 0 and 4 Nm was still reduced due to a more extended knee angle at 0 Nm (black arrow).

In summary, the surgery results to a change in knee angle at 4 Nm comparable to knee angles shown for TD children, while the knee angle at 0 Nm was more extended compared to TD children. As a consequence, the range of knee joint angles between 0 and 4 Nm was not increased, but knee angles at measured moment were shifted towards more knee extension. These changes resulted in more knee extension during gait in the majority of children, which is most likely a functional advantage during walking.
Chapter 6

General discussion

The following section considers methodological limitations of the studies in this thesis and reflects on the outcomes of the measurements before and after surgery. Furthermore, clinical implications of the findings are discussed and recommendations for future research are made.

Methodological considerations to measure knee joint mechanics and muscle morphology

Standardization of measurements

Measurement of knee joint mechanics and muscle morphology in vivo was challenging because (1) measurements needed to be performed at low levels of muscle activation to maintain the muscle in a “passive state”, (2) a valid, non-invasive method was required to measure muscle morphology and (3) muscle morphology needed to be measured in standardized conditions (i.e. comparable knee moments and/or angles) to allow comparisons between different groups and allow pre-post intervention comparisons - which possibly alters muscle mechanical properties. In order to meet these requirements, we developed a method that combines freehand 3D US and instrumented hand-held dynamometry, while muscle activation is assessed by surface electromyography (EMG).

We aimed to assess knee joint mechanics and muscle morphology in a passive state and how both these are related. To achieve this, knee-moment angle measurements were performed while the leg was moved only within the range that was possible without obvious activity bursts of surrounding synergistic and antagonistic muscles of ST. EMG-activity was offline-analysed to determine whether the threshold level for considering the signal as a muscle activity burst, i.e. mean + two standard deviations of the envelope data from resting EMG, was exceeded. When mean EMG activity exceeded this threshold for one of the assessed muscles, data obtained for the corresponding knee joint angle was excluded from further analysis. The same threshold was used during the measurements of muscle morphology using 3D US. In none of the cases, both measured 3D US trials had to be excluded. For the knee moment-angle measurements, a significant number of data points were excluded because the threshold was exceeded. However, the number of excluded data points did not differ between TD and children with SP (percentage exclusion of data points: SP: 26±28%; TD: 30±25%; p=0.748). In a few individual cases, more than 70% of data points were excluded (for examples see Figure 6.2). However, comparison of curves above 0.5 Nm with and without exclusion of data points did not reveal differences in the curve characteristics suggesting that the levels of EMG activity present during the measurements did not have a substantial effect on the knee moment and knee angle (knee angles at 4 Nm knee moment, based on all measured data: 43.1±11.6º; based on data that met EMG-criterion: 42.7±11.8º; p=0.107). Only knee angles corresponding to 0 Nm knee moment differed slightly after excluding of data points: Based on all measured data points,
knee angle at 0 Nm yielded 80.9±8.9º, whereas based on data that met EMG-criterion this angle was 78.4±8.7º (p=0.041). This difference (2.5±11.6º) is low compared to the observed shift in knee moment-angle curve after surgery (changes in knee angle at 0 Nm after surgery at medium-term follow-up yielded 19.9±15.6º, Figure 6.1). The observed small differences in knee- moment-angle curves based on data with and without EMG bursts suggest that it may be possible to reliable assess knee moment-angle curves using the method described in chapter 2 without EMG-assessment or with EMG activity measured only on one of the synergistic and antagonistic muscles. This would be useful for children who are distressed by the removal of the EMG-electrodes. However, more research would be necessary to define which criteria are needed to be fulfilled (e.g. no perceivable movement during measurements) when knee-moment angle measurement trials and 3D US measurements are performed without EMG.

Figure 6.2 Typical example of a knee moment-angle curve of a child with spastic paresis. Knee moment-angle curves derived by all measured data comparison with knee moment-angle curves derived after data exclusion due to elevated muscle EMG activity levels. Left graph: Black stars represent all measured data, red stars represent data points that are left after exclusion of data points due to elevated EMG levels. Blue lines are fitted curves. Note that exclusion of data points due to elevated EMG levels mainly occurred at knee moments higher than 4 Nm. Right graph: Fitted curves of the same child in blue. Blue stars represent the extracted angles at 0, 0.5, 1, 2, 3 and 4 Nm from the curves derived by all measured data, red stars represent the extracted angles after data exclusion due to elevated muscle activation. Extracted angles are used for statistical analysis. Note that the differences between extracted angles are very small.
Figure 6.3 Difference in image quality of semitendinosus muscle (ST) between a typically developing child and a child with spastic paresis before and after medial hamstring lengthening. A: ST of typically developing child; B: pre-surgical ST of child with spastic paresis (age-matched to typically developing child of A); C: ST of same child one year following lengthening the tendon of ST. Note the differences in echo intensity (i.e. whiter appearance) between a typically developing child and spastic paresis, and between the pre-surgical muscle of a child with spastic paresis compared to the muscle after surgery.
Another challenge was to measure muscle morphology at standardized conditions. We would ideally measure muscle morphology at a known mean sarcomere length and at known muscle-tendon forces. However, this was not feasible in the current experiments as it would involve invasive measurements (cf. advanced methods to measure sarcomere length (Mathewson et al., 2015, Young et al., 2017, Llewellyn et al., 2008)) and/or a force transducer attached to the tendon (Smeulders et al., 2004a, Smeulders and Kreulen, 2007)). We chose, therefore, to measure at two externally applied knee flexion moments (i.e. 0 and 4 Nm), assuming that at 0 Nm knee moment mean ST sarcomere length was similar (i.e. sarcomere passive slack length) and that the 4 Nm knee flexion moment represents a condition in which the resistance to stretch of the target muscle is similar between conditions. Under these assumptions conclusion on absolute and relative extensibility of the target muscle can be drawn which provide indications of elastic properties of ST. In addition, we measured muscle morphology also at a predefined knee joint angle (i.e. 65º) representing a similar relative origin-insertion distance (i.e. MTU length). The predefined knee angle makes it possible to conclude on relative alterations of muscle belly length, tendon length and fascicle length as well as on possible effects of bony differences between children with SP and TD and between before and after surgery. When bony structures do not differ between groups or are not affected by surgery, the origin-insertion distance are expected to be similar.

**Analysing 3D US - Image quality**

In Chapter 3, we describe in detail the newly developed freehand 3D US method to analyse ST muscle morphology. We used the 3D orientation of several anatomical landmarks to assess muscle belly length, tendon length and fascicle length of the proximal and distal compartments of the ST (Figure 3.3, Chapter 3). The rationale for using this approach to analyse ST was the complex structure of the muscle, consisting of two compartments divided by a concave tendinous inscription orientated parallel to the distal aponeurosis but being opposite in shape (Figure 3.2, Chapter 3). Our approach allows length measurements of different fascicles as distances between well-defined landmarks within the muscle (i.e. distal muscle tendinous junction, distal and proximal end of the tendinous inscription and ischial tuberosity). A drawback of the current 3D US approach is that the assessment of morphological variables by using this method highly depends on the possibility to exactly locate the anatomical landmarks within the muscle on the US images. This is relevant for morphological measurements in patients, as US imaging of muscles in patients with neuromuscular disorders are known to provide images with relatively low quality due to a whiter appearance of muscle tissue and low contrast compared to images obtained in muscles of TD children (Pillen et al., 2009, Pillen et al., 2007, Pitcher et al., 2015) (Figure 6.3A&B). The quality of images seems to even further decrease after medial hamstring lengthening (Figure 6.3C). A higher resolution of the voxel array, especially in the longitudinal direction, might increase image quality – this would be possible with a
higher image collection frequency (currently 25 Hz) or with longer sampling time of images (currently 40-50 seconds). In addition, the use of advanced algorithms such as total variation minimization during construction of the voxel array might also improve the visibility of structures within the voxel array (Afonso and Sanches, 2013, Sidky and Pan, 2008).

Despite the above described problems regarding image quality after surgery, in the pre- and post-intervention studies muscle belly length, tendon length and volume could be assessed in all subjects. Selection of anatomical landmarks with the voxel array was optimized by moving through the voxel array using the sequence of the transversal images in combination with a simultaneous view of the longitudinal plane. By moving through a sequence of images, structures can be identified more easily as they appear and disappear within the image sequence. In conclusion, the presented 3D US approach allows assessment of ST morphology non-invasively in children with SP, even after interventions.

*Knee joint mechanics and morphological differences due to SP*

One of the major morphological differences of ST between children with SP and TD children was the shorter fascicle length. Fascicle length of ST normalized to femur length was 18% shorter in SP compared to that in TD children. Since our approach to measure muscle morphology was non-invasive, we do not have an estimate of the number of sarcomeres in series. However, as we measured at the same net knee joint moment (i.e. 0 Nm) - and assume that this implies a similar mean sarcomere length (see above) - the shorter fascicles are most likely due to fewer sarcomeres arranged in series. A lower number of sarcomeres arranged in series affects the passive force-length characteristics of a muscle resulting in: (1) shorter passive slack length of muscle fibres which shifts the passive length-force curve to lower lengths and (2) an increased slope of the passive length-force curve. Knee joint mechanics of children with SP differed from that of TD children - the slope of knee moment-angle curve was increased and the knee moment-angle curve was slightly shifted at knee moments higher than 0.5 Nm knee moment in children with SP prior to surgery (Chapter 4). It is therefore very likely that the reduced length of ST fascicles contributed to the impaired knee-moment angle curve in children with SP. No differences in tendon lengths were found between SP and TD children, this suggest that tendon length has less impact on the knee angle-moment curve. It has been assumed that longitudinal muscle fibre growth within ST as well as within other muscles in children with SP is delayed (Graham et al., 2016, Kinney et al., 2016). This reduced potential to add sarcomeres in series during growth has been hypothesized to be related to a reduced number of satellite cells within spastic muscles (as shown for the ST muscle) (Smith et al., 2013, Dayanidhi and Lieber, 2014). Under normal circumstances, the serial sarcomere number in human muscle seems to be tightly adjusted to longitudinal bone growth, suggesting that sarcomeres are added in series in response to length change of the bone. In vastus lateralis muscle for instance, fascicles of an adolescent typically developing girl increased from 9 cm to a length of 18 cm after a
year of femoral lengthening (sarcomere length was measured by laser diffraction and an increase of approximately 350 sarcomeres per day was calculated) (Boakes et al., 2007). In addition, in young as well as adult rodents, immobilization at a shortened or lengthened joint position can result in rapid reduction or increase in the number of sarcomeres arranged in series (Williams and Goldspink, 1971, Williams and Goldspink, 1973, Heslinga et al., 1995). As the number of sarcomeres arranged in series adapts to the joint positions in which a muscle is mostly active (Herring et al., 1984), early positioning (e.g. standing, sitting with one’s legs straight in front of them) and stretching of lower leg muscles in children with SP might be effective to facilitate addition of sarcomeres.

Another important difference between children with SP and TD, is the smaller PCSA and volume of ST. Although theoretically a smaller PCSA will result into a decrease of the slope of the passive length-force curve the consequences for active force generating capacity may be even more relevant for children with SP. The 60% lower PCSA of ST, as reported in Chapter 4, suggests that already before surgery the active force generating capacity of the ST to flex the knee and extend the hip is substantially reduced in children with SP compared to that in TD children. PCSA growth during typical muscle development during embryogenesis is accomplished by addition of muscle fibres (i.e. hyperplasia) and radial area growth of muscle fibres (i.e. hypertrophy) (Montgomery, 1962, Stickland, 1981). In the human m. sartorius, it has been shown that hyperplasia mainly occurs till a foetal crown-rump length of about 35 cm (i.e. 26 weeks of gestation age) - after that time point hyperplasia contributes to the increase in PCSA by approximately 6% whereas hypertrophy contributes to PCSA growth by 94% (Stickland, 1981). However, the contribution of hyperplasia may not stop completely before birth (Stickland, 1981) and/or different developmental patterns may exist between morphologically different muscle in humans, as it has been shown in mice (Li et al., 2015). Therefore, multiple factors may contribute to the lower PCSA in children with SP: (1) Children with SP are possibly born with a lower number of muscle fibres compared to TD children depending on the timing of the brain lesion in cerebral palsy and/or reduced intra-uterine nutrition (Gough and Shortland, 2012) and have thereby a reduced capacity for hypertrophy after birth; (2) trophy of the muscle fibres seems to be attenuated in children with SP compared to that in TD children due to altered muscle activation patterns (Dayanidhi and Lieber, 2014) leading to disuse or altered functional use of muscles and (3) atrophy may occur during development due to a decrease of functional activities (e.g. walking, standing) when a child is growing older and gaining weight. Insight in muscle fiber size and number in children with SP is important. The question is whether and to what extent it is be possible to induce hypertrophy of muscle fibres of children with SP and how atrophy can be prevented when these children grow older.
**Effect of medial hamstring lengthening on knee joint mechanics, morphology and gait**

The results of the study described in chapter 5 show that surgical medial hamstring lengthening causes a substantial reduction of ST muscle belly length. We attributed this to a decrease of fascicle length during the follow up after surgery. For ST, conclusions regarding effects of surgery on fascicle length, based on muscle belly length measurements, seem to be reasonable as we found for children with SP before surgery and TD children a consistent proportional relation between fascicle length and muscle belly length measured at 0 and 4 Nm knee moment (Figure 6.4). The reduced fascicle length in ST muscle after surgery is likely the result of a loss in sarcomeres in series after surgery. To prevent this loss, additional treatment or changes in surgical procedures are required (for possible options see paragraph below on Clinical implications and future research). Note, that the loss of sarcomeres in series theoretically leads to a shift of the passive length-force curve to the shorter lengths (i.e. more flexed knee positions) and an increase in its slope compared to the pre-surgical curve. These changes are in a direction opposite to the aim of surgery. Interestingly, the effects of reduced fascicle length after surgery are not apparent from the measured knee moment-angle curve. In contrast, after surgery the knee moment-angle curve shifted towards more knee extension without a change in slope of the curve. The lack of effect of shorter fascicles on the knee moment-angle curve after surgery (i.e. a shift at 0 Nm towards more flexed angels and an increased slope), is likely due to counterbalancing effects on the passive length-force curve due to ST atrophy and lengthening of the distal tendon. The more pronounced decrease in muscle volume compared to the decrease in muscle belly length after medial, suggest a reduction of PCSA compared to pre-surgical measurements.

The results presented in this thesis show that the effects of lengthening of the ST tendon on knee joint mechanics and muscle morphology were quite consistent among subjects: (1) longer ST tendon and shorter ST muscle belly length and (2) both popliteal angle as well as knee angle during passive measurements improved. However, the results concerning gait were more heterogeneous (i.e. increased knee extension in mid-stance and terminal swing, variable effects on hip flexion and an increase in anterior pelvis tilt). More knee extension is considered to be positive for walking performance, while increased anterior pelvis tilt may lead to an increased lumbar lordosis or forward leaning. Both of the latter are considered as unwanted side effects. Compensation for the increase in anterior pelvis tilt requires an increase in hip extension, but apparently after medial hamstring lengthening not all of the children were able to extend the hip sufficiently. Possible causes for the limited hip extension after surgery are (1) a poor selective voluntary motor control of hip extensor muscles (Goldberg et al., 2011) as well as (2) an insufficient strength of hip extensors already before surgery (Seniorou et al., 2007) and/or (3) a loss of hip extensor strength after hamstring lengthening. These factors may limit the ability of children with SP to take full advantage of the increased knee extension.
Figure 6.4 Relationship between muscle belly and fascicle length of semitendinosus muscle. Muscle belly length as a function of fascicle length, both measured at different knee angles (i.e. knee angle corresponding to 0 and 4 Nm knee moment) in children with spastic paresis before surgery (SP, black) and typically developing children (TD, white).

Clinical implications and future research

We aimed to identify factors that may contribute to a favourable or unfavourable outcome of medial hamstrings lengthening. A favourable outcome is an increased knee extension during gait whereas an unfavourable outcome is persistence of flexed knee gait or recurrence after initial success as well as unwanted side effects such as increased anterior pelvic tilt, and lumbar lordosis as well as hyperextension of the knee during gait. Although our group of children with SP was too small (n=6) to reliably recognize success factors for a positive or negative treatment outcome, still some ideas, derived from our results, may be considered when children with SP are selected for medial hamstring lengthening and the implications for treatment may be studied in the future.

Low pre-surgical ST PCSA

Pre-surgical hip extensor weakness is a possible risk factor for an unfavourable outcome, as it may inhibit movement towards more hip extension after surgery. Therefore, it seems
useful to assess hip extensor strength (i.e. hamstring and gluteus muscles) before surgery, as well as the PCSAs of both lateral and medial hamstrings. Based on the results of such pre-surgical assessment hamstring and gluteus muscles may be exercised prior to surgery. Pre-surgical assessment of PCSA of the biceps femoris muscle will provide an additional estimate of the force generating capacity of the biceps femoris muscle to compensate for loss of PCSA of the ST, while assessment of PCSA of the semimembranosus, gracilis and ST muscles yields the possibility to compare between PCSAs of the different surgically treated muscles before surgery and how these muscle eventually adapt after surgical procedures. This knowledge may contribute to further fine-tune rehabilitation protocols to counterbalance hip extensor weakness and may be used in the selection procedure for medial hamstring lengthening.

Effects of muscle activation

In the studies described in this thesis, we did not focus on muscle activity during pathologic gait (e.g. due spasticity, muscle weakness and selectivity of motor control). We found the change in muscle morphology and knee joint restrictions were fairly similar in all children, while outcome on gait concerning hip angle was highly variable. This suggests that impaired muscle activity may have played an important role in the overall treatment outcome on gait. Thus, in addition to the assessment of passive structures, methods for measurement of the contribution of abnormal muscle activity (e.g. assessment of spasticity (Sloot et al., 2017, van der Krogt et al., 2016), muscle weakness (Willemse et al., 2013) and selectivity (Zwaan et al., 2012)) should be further developed and related to treatment outcome of medial hamstring lengthening.

Surgical lengthening methods

For surgical lengthening of triceps surae muscles, variable approaches have been developed (i.e. different techniques of Achilles tendon lengthening (Graham and Fixsen, 1988, Baker, 1956, Frost, 1971, Grabe and Thompson, 1979, Gaines and Ford, 1984) and different techniques of gastrocnemius-soleus intramuscular aponeurotomy (Strayer, 1950, Vulpius and Stoffel, 1913, Baumann and Koch, 1989, Baker, 1954)). Gastrocnemius-soleus intramuscular aponeurotomy has been shown to be associated with a lower risk for overcorrection in the long-term compared to Achilles tendon lengthening and is thereby favoured for treatment of equinus in children with SP (Dreher et al., 2012a). For medial hamstring lengthening also different combination of surgical interventions have been described (Novacheck, 2009, Bleck, 1987, Miller, 2005). The ST has been described to be surgical lengthened by (1) Z-lengthening (as used in the current study), (2) a transection of the ST tendon (i.e. tenotomy) or (3) lengthened by one or more incisions of the aponeurosis (i.e. aponeurotomy). With Z-lengthening it is generally assumed that it allows some control over the amount of lengthening of the tendon compared to tenotomy and in addition that tenotomy increases the risk of overcorrection (Bleck, 1987). On the other hand some authors advise to perform aponeurotomy of the ST to preserve muscle strength (Dagge et
However, effects of the different methods on risk factors and recurrence rate have not yet been investigated and described in detail for medial hamstring lengthening. Nevertheless, also for the ST the surgical intervention and the magnitude of lengthening of the distal ST tendon are likely contributing to the treatment outcome (Dagge et al., 2012). To prevent a reduction in muscle belly length it will be important to maintain sufficient strain on the muscle belly. Thereby, the effects of different surgical interventions on remaining strain on the muscle may effect the loss of muscle belly length reduction. Fine-tuned lengthening of the tendon and/or aponeurosis (i.e. lengthening by aponeurotomy) such that within a functional range of motion the muscle is still strained may improve the functional outcome of the surgical interventions.

Rehabilitation protocol after surgery

Intensive training after SEMLS has been reported to stimulate recovery of muscle strength after surgery, whereby progressive resistance training using free weights showed some advantages over active strengthening only by exercises against gravity (Seniorou et al., 2007). Therefore, immobilization after surgery should be as short as possible and an adequate rehabilitation program including resistance training is recommended. Currently, post-surgical immobilization and rehabilitation vary in time and intensity; further investigations to assess the best protocol are necessary. Improvement of post-surgical treatment will likely contribute to a favourable outcome with regard to gait and sustainability hereof.

Summary and conclusions

The work presented in this thesis shows that the developed measurement approaches of knee joint mechanics and morphological characteristics are reliable and valid. ST morphology differs substantially between children with SP and TD children. In particular in children with SP fascicle lengths are shorter than those in TD. This lack of longitudinal fascicle growth in the children with SP contributes to the higher passive resistance to knee extension during static measurements and during gait. The increase in ST tendon length after surgical lengthening of the medial hamstrings contributes to a more extended knee angle. However, due to surgery, ST muscle belly length is substantially decreased and atrophy of ST muscle occurs in the majority of children. The reduction in muscle belly length after surgery most likely contributes to a decrease in extensibility of the ST muscle belly, which possibly contributes to recurrence of knee extension limitations in the long-term (especially when children grow), whereas atrophy reduces the force generating capacity of the ST muscle, possibly leading to an increase in excessive hip flexion during gait. Based on these results, we advise (1) to consider the indication of medial hamstring lengthening very carefully and (2) to try to prevent above mentioned muscle adaptations by fine-tuning the magnitude of tendon lengthening and by intensive post-surgical rehabilitation. Following these advices may improve treatment outcome of medial hamstring lengthening in children with SP.
ABBREVIATIONS

General terms

BMI:       Body mass index
CV:        Coefficient of variation
EMG:       Electromyogram
GMFCS:     Gross Motor Function Classification System
ICC:       Intraclass correlation coefficients
ICF-CY:    International Classification of Functioning, Disability and Health for Children and Youth
LoA:       Limits of agreement
MTU:       Muscle-tendon unit
MSt:       Mid-stance
ROM:       Range of motion
SD:        Standard deviation
SDD:       Smallest detectable difference
SEM:       Standard error of measurement
SEMLS:     Single event multi-level surgery
SCP:       Spastic cerebral palsy
SP:        Spastic paresis
ST:        Semitendinosus
TD:        Typically developing
TSt:       Terminal Stance
TSw:       Terminal Swing
3D US:     Three-dimensional ultrasound
Abbreviations

Morphological variables

ACSA anatomical cross-sectional area
PCSA physiological cross-sectional area;
Vol muscle volume
ℓ Length
mtu muscle-tendon unit
m muscle belly
a aponeurosis
ti tendinous inscription
t tendon
fasc fascicle
sarc sarcomeres
m intermediate
d most distal
p most proximal
prox proximal compartment
dist distal compartment
α angle of the muscle line of pull with fascicles
β angle of the muscle line of pull with the aponeurosis
γ α + β
rel relative to length at 0Nm
optimum at optimum sarcomere length (2.7 µm)
norm normalized to femur length
0Nm measured at knee angel corresponding to 0 Nm knee moment
4Nm measured at knee angel corresponding to 4 Nm knee moment
65deg measured at knee angel of 65 degree
Δ difference
Abbreviations

Variables knee joint mechanics

$\theta_{0\text{Nm}}$  Knee angles at 0 Nm knee moment
$\theta_{0.5\text{Nm}}$  Knee angles at 0.5 Nm knee moment
$\theta_{1\text{Nm}}$  Knee angles at 1 Nm knee moment
$\theta_{2\text{Nm}}$  Knee angles at 2 Nm knee moment
$\theta_{3\text{Nm}}$  Knee angles at 3 Nm knee moment
$\theta_{4\text{Nm}}$  Knee angles at 4 Nm knee moment
$\theta_{\text{max}}$  Maximum measured angle
$M_{\text{max}}$  Maximum measured moment


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Spastische parese (SP) als een gevolg van cerebrale parese en hereditaire (erfelijke) spastische paraplegie leidt vaak tot beperkingen in dagelijkse activiteiten zoals lopen. Bij sommige kinderen met SP wordt het loopvermogen nog verder beperkt door een beperkte kniestrekking. Over het algemeen wordt aangenomen dat verkorting en/of verstijving van de hamstringspier, ook wel hamstringcontractuur genoemd, kan bijdragen aan bewegingsbeperkingen rond de knie. Om deze bewegingsbeperking te verhelpen en daarmee het lopen te verbeteren wordt bij sommige kinderen een chirurgische verlenging van de mediale hamstringspieren (waaronder de semitendinosus spier (ST)) uitgevoerd. Chirurgische verlenging van deze spieren leidt echter niet altijd tot een verbetering van het lopen. Om het resultaat van deze behandeling te verbeteren is meer kennis nodig over de onderliggende mechanisme die leiden tot deze bewegingsbeperking rond de knie.

Het doel van dit proefschrift was de mechanische eigenschappen van de knie en de morfologie van de ST bij kinderen met SP, evenals de effecten van chirurgische verlenging op de spier en op het lopen te onderzoeken. Hiermee wilden wij factoren proberen te identificeren die kunnen bijdragen aan een gunstig of ongunstig uitkomst van deze chirurgische ingreep.

**Hoofdstuk 2** beschrijft een meetmethode waarbij met een dynamometer de mechanische eigenschappen van het kniegewricht gemeten kunnen worden. Onze doelen voor dit onderzoek waren:

1. de standaard meetfouten en de kleinste detecteerbare verschillen van de mechanische eigenschappen van de knie in herhaalde metingen te testen;
2. de correlatie tussen de knie-hoek corresponderend bij een netto knie-flexie-moment van 4 Nm en de popliteale hoek (dit is een hoek die klinisch bepaalt is en de indicatie geeft over de lengte van de hamstringspieren, zie Figure 1.3 in hoofdstuk 1) te bepalen en
3. de hoek-moment relatie van de knie tussen kinderen met SP en typisch ontwikkelende kinderen te vergelijken.
We hebben gevonden dat het met de dynamometer methode mogelijk is om tussen verschillende dagen de knie-moment-hoekcurve betrouwbaar te meten: De standaard meetfout is ongeveer 5° en het kleinst detecteerbaar verschil is minder dan 14° bij kniehoeken die gerelateerd zijn aan 1-4 Nm netto knie-flexie-momenten. Bij kinderen met SP was de kniehoek gemeten op één gestandaardiseerd kniemoment (4 Nm flexie-moment) niet gecorreleerd aan de popliteale hoek. De helling van de knie-moment-hoekcurve op 4 Nm was steiler bij kinderen met SP in vergelijking met typisch ontwikkelende kinderen, terwijl de knie-moment-hoekcurve van kinderen met SP niet verschoven was in vergelijking met de curve van typisch ontwikkelende kinderen. Dit betekent dat er geen verschil in kniehoeken was tussen groepen bij lage netto knie-flexie-momenten. Ten slotte hebben wij aangetoond dat de gepresenteerde dynamometer methode gebruikt kan worden om klinisch relevante mechanische eigenschappen van de knie te meten.

Naast het meten van de mechanische eigenschappen van de knie was het ook belangrijk dat de spiermorfologie nauwkeurig bepaald kon worden. De doelstellingen van hoofdstuk 3 waren daarom:

1. een nieuw protocol voor het meten van de morfologie van de ST door middel van driedimensionale echografie (3D US) te presenteren en
2. de morfologische kenmerken van ST van een gebalsemd lichaam gemeten met 3D US en na dissectie te vergelijken.

De gemiddelde verschillen tussen morfologische kenmerken (bijv. spierbuiklengte, spierbundel lengte en spiervolume) gemeten door middel van 3D US en in het uitgeprepareerde spier waren kleiner dan 10%. Intra-klass correlatiecoëfficiënten (ICC’s) waren hoger dan 0.75 voor alle variabelen, behalve de lengte van de proximale spierbundels (ICC=0.58). Wij hebben geconcludeerd dat met de gepresenteerde 3D US methode redelijk nauwkeurige metingen van de belangrijkste morfologische eigenschappen van ST spier mogelijk zijn.

In hoofdstuk 4 is de combinatie van dynamometrie en 3D US toegepast bij kinderen met SP en typisch ontwikkelende kinderen om

1. te bepalen hoe mechanische eigenschappen van het kniegewricht en ST morfologie verschillen tussen kinderen met SP geselecteerd voor mediale hamstringverlenging en typisch ontwikkelende kinderen en
2. te bekijken hoe de mechanische eigenschappen van het kniegewricht en ST morfologie aan elkaar gerelateerd zijn.
Bij netto knie-flexiemomenten boven 0.5 Nm, vonden we grotere kniehoeken bij gelijke kniemomenten voor kinderen met SP in vergelijking met typisch ontwikkelende kinderen, dit betekent een beperking van de kniestrekking. De gemiddelde helling van de knie-moment-hoekcurve was steiler bij kinderen met SP, wat aangeeft dat de stijfheid van structuren over het kniegewricht hoger is. Deze verhoogde stijfheid rond het kniegewricht suggereert een verhoogde stijfheid van de spierbuiken en/of pezen van de hamstringspieren. Spiervolume, fysiologisch dwarsdoorsnede en de lengte van de ST spierbundel genormaliseerd naar femurlengte waren kleiner in SP dan in typisch ontwikkeldende kinderen (respectievelijk 62%, 48% en 18%). Peeslengte genormaliseerd naar femurlengte verschilde niet tussen groepen. De gevonden spierbundellengte was gerelateerd aan de kniehoek die bij 4 Nm kniemoment bereikt kan worden. De kortere spierbundellengte bij kinderen met SP verklaart dus deels het meer gebogen kniegewricht in deze groep. Andere factoren die kunnen bijdragen aan beperkte kniestrekking bij kinderen met SP zijn een veranderde spierweefselsamenstelling (b.v. verhoogde collageengehalte), veranderingen in morfologie van andere knieflexiespieren en/of verhoogde stijfheid van andere structuren rond de knie zoals de gewrichtcapsule, ligamenten, zenuwen, bloedvaten en tussenliggend bindweefsels.

In hoofdstuk 5 was het doel om de longitudinale effecten van mediale hamstring verlenging op de mechanische eigenschappen van het kniegewricht (zoals knie-moment-hoekrelatie en popliteale hoek), ST morfologie en kinematica van het lopen te evalueren. Bij korte termijn follow-up (11-20 weken na de operatie) was de gemiddelde helling van de knie-moment-hoekcurve minder stijl dan ervoor. Dit suggereert een afname in stijfheid van de structuren die over het kniegewricht lopen. Het effect was echter tijdelijk. Bij middellange follow-up (ongeveer een jaar na mediale hamstring verlenging) was er geen verschil in helling ten opzichte van de pre-operatieve steilheid van de knie-moment-hoekcurve. De terugkerende verhoogde steilheid van de knie-moment-hoekcurve tussen de korte en de middellange follow-up kon niet verklaard worden door veranderingen in de ST morfologie. Wel werd er tijdens de middellange follow-up meting bij de meeste proefpersonen een verschuiving van de knie-moment-hoekcurve naar meer kniestrekking waargenomen, evenals een toename van de maximale kniestrekking gemeten met de popliteale hoek. Deze toename in kniestrekking was ook te zien tijdens het lopen, maar hierbij was ook de anterior tilt van het bekken toegenomen, wat een negatief effect voor het lopen betekent. Ongeveer een jaar na mediale hamstring verlenging was de ST spierbundellengte met ongeveer 30% afgenomen en de lengte van de pees ruim 80% toegenomen, waardoor het totale spier-pees-complex in lengte was toegenomen. Overal tonen deze resultaten aan dat de verlenging van de ST bij de meeste patiënten tot meer kniestrekking tijdens het lopen leidt.
Als de resultaten van de studies betreffende de morfologie van ST en mechanische eigenschappen van het kniegewricht uit hoofdstuk 4 en 5 gecombineerd beschouwd worden, blijkt dat na het verlengen van de mediale hamstring de spierlengte en het spiervolume van kinderen met SP in een nog grotere mate verschillen van die van typisch ontwikkelende kinderen vergeleken met de pre-chirurgische situatie van kinderen met SP (dat wil zeggen een nog kortere lengte en een nog kleiner volume na de mediale hamstring verlenging). Echter de kniehoek op 4 Nm was na de ingreep vergelijkbaar met de kniestrekking die normaal ontwikkelde kinderen op 4 Nm bereiken (dat wil zeggen meer kniestrekking). De helling van de knie-moment-hoekcurve (dat wil zeggen het bereik van kniehoeken tussen 0 en 4 Nm) was een jaar na mediale hamstring verlenging hetzelfde als voor de operatie. Dit impliceert dat de meer gestrekte kniehoek bij 4 Nm bij de meeste proefpersonen is bereikt door een verschuiving van de hele curve. Dat de bewegingsbereik alleen maar verschoven was maar niet vergroot, betekend meer kniestrekking bij 4 Nm een bewegingsbeperking richting kniebuiging.

Samengevat leidt de operatie tot meer kniestrekking bij een netto knie-flexie-moment van 4 Nm. De bereikte kniehoeken waren daarmee een jaar na de ingreep vergelijkbaar met kniehoeken gemeten in typisch ontwikkelende kinderen. Deze veranderingen leiden tot een meer gestrekt kniegewricht tijdens het lopen in de meeste kinderen. Daarnaast vonden wij echter ook een negatief effect van de operatie: tijdens het lopen kantelt het bekken meer naar voren. Voor het nemen van een goede behandelbesslisisng is het belangrijk om deze factoren mee te laten wegen.
Helga Haberfehlner (March 10th, 1978) was born in Amstetten, Austria. In 1996, she graduated from high school (Ostarrichi-Gymnasium, Amstetten) and started studying occupational therapy in Baden, Austria. In 1999, she obtained her bachelor’s degree in occupational therapy. The following years Helga worked in pediatric rehabilitation as occupational therapist in Austria and the Netherlands. In 2007, she decided to start research education by studying Human movement science at the Vrije Universiteit in Amsterdam, where she obtained her master’s degree 2010 cum laude. Helga performed her master research internship in the rehabilitation center Reade (formerly Jan van Breemeninstitute) on handwriting difficulties in children with juvenile idiopathic arthritis. In November 2010 she started her PhD project described in this thesis at the department of Human Movement Sciences (Vrije Universiteit, Amsterdam). The project was performed in cooperation with the Department of Rehabilitation Medicine and the Department of Orthopaedic Surgery of VU University Medical Center in Amsterdam and the Pediatric Orthopaedic Department of the University Children’s Hospital Basle (Switzerland). The PhD project aimed to investigate effects of medial hamstring lengthening in children with spastic paresis on knee joint mechanics, morphological characteristics of the semitendinosus muscle and gait. Helga was twice nominated for the Best Paper Award of the European Scientific Society for Clinical Gait and Movement Analysis (ESMAC) (Heidelberg, 2015; Seville, 2016). In 2017 she received the Scientific Award of the Society for Pediatric Orthopaedics of Germany, Switzerland and Austria (VKO) and was nominated for the Best Oral Presentation Award of the Annual Meeting of the European Academy of Childhood Disability (EACD), Amsterdam. Since 2016 Helga works again as occupational therapist for the Kindercentrum Zwanenburg and as researcher for the Amsterdam University of Applied Sciences (HvA). Helga is married to Norbert Hinterleitner. Together they have three children: Jana and Lena (2012) and Simon (2017).
 Peer-reviewed journals


Publications

Conference Abstracts


Book chapter
In this last section I want to say thank you to everyone who has contributed to my dissertation, especially to:

Alle kinderen die mee hebben gedaan aan de metingen en hun ouders

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Children with spastic paresis due to cerebral palsy and hereditary spastic paraplegia walking in a flexed knee gait pattern are frequently treated by surgical lengthening of medial hamstring muscles including the semitendinosus muscle to improve walking. However, surgery is only partly successful in restoring gait. To improve the outcome of surgical interventions requires a detailed understanding of knee joint mechanics, underlying mechanical and morphological muscle characteristics and their relation to gait as well as knowledge of muscle adaptation after surgical lengthening. In this thesis a method to assess knee joint mechanics and semitendinosus muscle morphology is described. With this method the effects of medial hamstring lengthening on knee joint mechanics and morphological characteristics of the semitendinosus muscle were investigated and related to gait. Thereby, we aimed to identify factors that may contribute to a favourable or unfavourable outcome of medial hamstring lengthening.