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Summary

The aim of the present thesis was to examine the effects of long-term stretching training on hamstrings and triceps surae muscle-tendon morphology, mechanics and function. Elite rhythmic gymnasts (study I) and professional ballet dancers (study II), who had undertaken years of systematic stretching, were compared to control subjects with no history of stretching. In a within-subjects randomized controlled trial, healthy, recreationally active adults underwent 24 weeks of stretching training of one leg, while the contralateral leg served as control (study III).

Altogether, the studies demonstrate that joint range of motion (ROM) is associated with a number of morphological, mechanical and neural factors, many of which were modified with 24 weeks of stretching. Specifically, passive resistance to stretch was lower in subjects with greater ROM (gymnasts and dancers), and in the group that stretched for 24 weeks, passive resistance of the plantar flexors was reduced. Maximally tolerated passive torque was increased after 24 weeks of stretching. The role of neural factors in determining ROM was underscored by lower electromyographic amplitudes (EMG) at standardized joint angles in ballet dancers, by reduced EMG at standardized angles after 24 weeks of stretching, and by bilateral changes in ROM, passive resistance, EMG and tissue elongation after 24 weeks of stretching. Ballet dancers had longer gastrocnemius medialis muscle fascicles and longer and more compliant Achilles tendons, but neither of these variables nor the amount of intramuscular collagen were altered by 24 weeks of stretching. Hence, this thesis does not provide direct evidence of morphological adaptations. However, the changes in passive resistance after 24 weeks of stretching cannot fully be explained by the observed changes in neural activation, and 24 weeks of stretching led to bilateral increases in tendon elongation, observed using two different methods. These findings may represent genuine structural adaptations, but further research is needed to confirm this.

The gymnasts presented specialized contractile properties, producing greater work, reaching peak knee flexion torque with the knee more extended and displaying a greater functional ROM, despite similar strength compared to controls. Such differences were not evident in dancers. However, 24 weeks of stretching shifted angle of peak torque to a more dorsiflexed ankle angle.

In conclusion, the present thesis shows that long term stretching training leads to neural adaptations and probably structural adaptations, which together translate into altered mechanical properties and have the potential to modify contractile function. The role of central mechanisms for increases in ROM was confirmed by bilateral responses to 24 weeks of stretching, which had previously not been demonstrated.

Sammendrag

Denne avhandlingen hadde som formål å undersøke effektene av langvarig bevegelighetstrening på muskel-sene-morfologi, -mekanikk og funksjon. Eliteutøvere i rytmisk gymnastikk (studie I) og profesjonelle ballettdansere (studie II), med mangeårig bevegelighetstrening bak seg, ble sammenlignet med kontrollpersoner som ikke hadde drevet bevegelighetstrening. Friske, normalt aktive voksne gjennomførte 24 ukers bevegelighetstrening av ett ben, mens motsatt ben fungerte som kontroll, i en "within-subjects" randomisert, kontrollert studie (studie III).

Samlet viser studiene at leddutslag (ROM) er assosiert med et antall morfologiske, mekaniske og nevrale faktorer. Mange av disse faktorene ble endret ved 24 ukers trening. Spesifikt var passiv motstand mot tøyning lavere hos personer med stort ROM (gymnaster og dansere), og i gruppen som trente bevegelighet i 24 uker ble passiv motstand ved dorsalfleksjon redusert. Maksimalt tolerert passivt dreiemoment var økt etter 24 ukers trening. Betydningen av nevrale faktorer for bevegelighet ble understreket av lavere elektromyografisk amplitude (EMG) ved standardisert leddvinkel hos ballettdansere, av redusert EMG ved standardisert vinkel etter 24 ukers trening, og av bilaterale endringer i ROM, passiv motstand, EMG og forlenging av vev etter 24 ukers trening. Ballettdansere hadde lengre muskelfasikler i gastrocnemius medialis og en lengre og mer ettergivende akillessene, men hverken disse variablene eller intramuskulært kollageninnhold var endret etter 24 ukers trening. Denne avhandlingen kan dermed ikke dokumentere morfologiske tilpasninger til bevegelighetstrening. Endringene i passiv motstand kan imidlertid ikke fullstendig forklares av endringer i nevral muskelaktivering, og 24 ukers trening medførte økt forlenging av senevev bilateralt, målt med to ulike metoder. Disse resultatene representerer muligens reelle strukturelle tilpasninger, men dette må bekreftes av ytterligere forskning.

Gymnastene demonstrerte spesialiserte kontraktile egenskaper, ved at de produserte større kontraktilt arbeid, oppnådde maksimalt dreiemoment i knefleksjon med strakere kne, og benyttet et større funksjonelt ROM, sammenlignet med kontrollpersoner. Tilsvarende forskjeller var ikke åpenbare hos ballettdanserne. Derimot endret 24 ukers trening leddvinkelen ved maksimalt dreiemoment til en mer dorsalflektert posisjon.

Oppsummert viser denne avhandlingen at langvarig bevegelighetstrening medfører nevrale tilpasninger og antagelig strukturelle tilpasninger, som til sammen medfører endrede mekaniske egenskaper og har potensiale til å endre kontraktil funksjon. En betydning av sentralt regulerte mekanismer for økning i bevegelighet ble bekreftet av bilaterale endringer etter 24 ukers bevegelighetstrening, som ikke tidligere er dokumentert.

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Oslo, June 2018

Marie M. Moltubakk

Abbreviations, definitions and glossary

Agonist: Role played by a muscle acting to cause a movement

Antagonist: Role played by a muscle acting against another muscle (against the agonist)

ANOVA: Analysis of variance – a statistical method

Aponeurosis: A layer of connective tissue surrounding a muscle or group of muscles

Concentric: Describing a muscular contraction involving shortening of a muscle

Contractile component: Component of muscle enabling development of tension by stimulated muscle fibres

Eccentric: Describing a muscular contraction involving lengthening of a muscle

EMG: Electromyography – a technique to assess neural activation of muscle

Fasciae: See Aponeurosis

Free Achilles tendon: The distal part of the Achilles tendon, from the most distal insertion of the soleus onto the tendon to the calcaneal insertion

GL: The muscle gastrocnemius lateralis

GM: The muscle gastrocnemius medialis

Golgi tendon organ: A tension-sensitive mechanoreceptor located at the end of afferent nerve fibre, found near collagen bundles near the muscle-tendon junction

Goniometer: A device that measures joint angle

Hamstrings: Muscle group on the back of the thigh, responsible for knee flexion/hip extension, consisting of the muscles biceps femoris, semimembranosus and semitendinosus

Hz: Hertz – the measurement unit of frequency

Fascicle: A bundle of muscle fibres, a sub-component of a muscle

Material properties: Qualitative properties of tissue, independent of tissue dimensions. Variables include stress, strain and Young's modulus.

Mechanical properties: Structural properties derived from mechanical testing of structures, e.g. the relationship between applied force and resulting tissue deformation in active and passive states. Variables include length-tension relationship, stiffness and viscoelastic properties.

Morphological properties: Properties describing dimensions and structure of tissue. Variables include length, cross-sectional area, thickness and muscle architecture.

Moment arm: Shortest (perpendicular) distance between a force's line of action and an axis of rotation

MRI: Magnetic resonance imaging

MTJ: Muscle-tendon junction – the anatomical connection between muscle cells and tendinous tissue

MTU: Muscle-tendon unit – the entire system from bony insertion to bony insertion, including muscle, tendon, intramuscular and fascial connective tissue

Muscle spindle: Sensory receptor that provokes reflex contraction in a stretched muscle and inhibits tension development in antagonist muscles

MVC: Isometric maximal voluntary contraction

Nm: Newton meter – the measurement unit of torque

Parallel elastic element: Passive elastic component of muscle, constituted by intramuscular and extra-muscular connective tissue

Passive joint stiffness: A variable calculated as the slope of a fourth-order polynomial fit of the passive torque-angle relation

Pennation angle: The angle between muscle fascicles and the muscle's line of action

PNF: Proprioceptive neuromuscular facilitation – a group of stretching modalities involving alternating contraction and relaxation of the muscles undergoing stretching

POST: A time-point or measurement after an intervention

PRE: A time-point or measurement before an intervention

Quadriceps: Muscle group on the front of the thigh, responsible for knee extension/hip flexion, consisting of the muscles rectus femoris, vastus lateralis, vastus medialis, vastus lateralis

ROM: Joint range of motion

Sarcomere: The smallest functional (contracting) unit of muscle fibres

Series elastic element: Passive elastic component of the tendons comprising the muscle-tendon unit, lying in series with muscle

SD: Standard deviation – quantification of the amount of variation in a set of measurements

SOL: The muscle soleus

Stiffness: See Passive joint stiffness, Tendon stiffness, Young's Modulus

Stretch reflex: Monosynaptic reflex initiated by stretching of muscle spindles and resulting in immediate development of muscle tension

TA: The muscle tibialis anterior

Tendon stiffness: A variable calculated as the slope of the force-elongation curve from a ramped isometric contraction.

TENS: Transcutaneous electrical nerve stimulation

Triceps surae: Muscle group on the back of the calf, responsible for ankle plantar flexion/knee extension, consisting of the muscles gastrocnemius medialis, gastrocnemius lateralis and soleus

Torque: The rotatory effect of a force about an axis of rotation, measured as the product of the force and the moment arm

Viscoelasticity: Property of tissues having both viscous and elastic characteristics when undergoing deformation

Whole Achilles tendon: The full length of the Achilles tendon, including the proximal part where soleus muscle fibres insert: From the calcaneal insertion to the gastrocnemius medialis muscle-tendon junction

Work (in a mechanical context): Force multiplied by the displacement of the resistance in the direction of the force

Young's modulus: A variable calculated as stress divided by strain – a dimensionless measure of stiffness

List of papers

This dissertation is based on the following original research papers, which are referred to in the text by their Roman numerals:

I. Moltubakk, M. M., Eriksrud, O., Paulsen, G., Seynnes, O. R., & Bojsen-Moller, J. (2016). Hamstrings functional properties in athletes with high musculo-skeletal flexibility. *Scandinavian Journal of Medicine and Science in Sports, 26*, 659-665. doi: 10.1111/sms.12488.

II. Moltubakk, M. M., Magulas, M. M., Villars, F. O., Seynnes, O. R., & Bojsen-Moller, J. (2018). Specialized properties of the triceps surae muscle-tendon unit in professional ballet dancers. *Scandinavian Journal of Medicine and Science in Sports*. 2018 May 3. doi: 10.1111/sms.13207 [Epub ahead of print]

III. Moltubakk, M. M., Villars, F. O., Magulas, M. M., Magnusson S. P., Seynnes, O. R., & Bojsen-Moller, J. Altered triceps surae muscle-tendon properties after 6 months of unilateral stretching. Manuscript.

IV. Moltubakk, M. M., Svensson, R. B., Magnusson S. P., Suetta, C., Raastad, T., Seynnes, O. R., & Bojsen-Moller, J. Effects of 6-month stretching training on collagen content of the human soleus muscle. Manuscript.

1. Introduction

Stretching exercise is frequently applied in a variety of populations to improve joint flexibility (Garber et al., 2011). Additional aims range from performance enhancement through maintenance of daily function and health, relaxation and well-being, to injury prevention, rehabilitation and pain management. Half a century of research has ascertained that stretching exercise repeated systematically over time, stretching training, increases joint range of motion (ROM) (Magnusson, Simonsen, Aagaard, Sorensen, & Kjaer, 1996; Weber & Kraus, 1949). However, many of the potential benefits are supported by tradition and practical experiences, rather than by science.

A growing number of studies indicates morphological, mechanical or neural adaptations to stretching training (e.g. Guissard & Duchateau, 2004; Simpson, Kim, Bourcet, Jones, & Jakobi, 2017; Nakamura et al., 2017; Blazevich et al., 2014), but with little uniformity. A large body of research suggests changes related to pain tolerance as the main mechanism for increased ROM. With this background, science currently does not provide satisfactory answers to questions such as: What are the potential benefits of increasing ROM? How does stretching training influence function and performance? Which populations may benefit from stretching training? Which stretching methods will best facilitate the various desired outcomes? ROM is governed by a large number of morphological, mechanical, neural and external factors. To address questions about benefits of and methods for stretching with appropriate specificity, more specific outcome measures must be applied – "flexibility" must be broken down into specific biomechanical and physiological variables. After identifying mechanisms for increases in ROM, the important questions on practical application of stretching training can be more easily addressed.

The present thesis aims to increase knowledge about the mechanisms at play when ROM is increased over months or years of stretching training in healthy adults. In the following theory section, results from intervention studies, research on acute effects and animal studies are integrated to present an exhaustive overview. The relationships between flexibility and stretching training on one side and performance, daily function, health and injury risk on the other side are not part of the research questions of the present thesis, but they are briefly addressed in the theory section in order to examine the relevance of flexibility and stretching training.

2. Theoretical background

2.1. Joint flexibility and nomenclature

The literature offers different definitions of joint flexibility (hereafter referred to as "flexibility"). Some of the classic definitions of flexibility focus on ROM, which may be defined as "the angle through which a joint moves from anatomical position to the extreme limit of segment motion in a particular direction" (Hall, 2006a). In other words, ROM refers to the range of joint angles between anatomically neutral position and the endpoint. The present thesis refers to the single joint angle at the endpoint of ROM as "maximal joint angle".

Other sources offer wider but less operationalized definitions of flexibility, such as "an intrinsic property of the body tissues that determines the ROM achievable without injury at a joint or group of joints" (Holt, Holt, & Pelhan, 1996) or "the ability to rotate a single joint or series of joints smoothly and easily through an unrestricted pain free ROM" (Kisner, Colby, & Borstad, 2018). These definitions encompass the behaviour through the entire ROM, not only the maximal joint angle. "Rotating easily" relates to the passive resistance offered by the musculo-skeletal system throughout the ROM, which results from a number of different factors. The present thesis uses "flexibility" to describe the wider concept involving passive resistance and further variables, while ROM refers to the range of joint angles.

2.2. What determines flexibility?

In human testing of ROM, the maximal joint angle is determined through a cognitive decision by the individual. The factors involved in this decision are related to the willingness to tolerate pain and to sensory input, which is relayed through the nervous system but is also modulated by the structural properties of the muscle-tendon unit (MTU). An overview of factors contributing to flexibility is presented in Figure 1. The present thesis focuses on the structural properties of the MTU (shown in blue) and on neural factors (shown in red). Variables measured in the present thesis are shown on white, square labels. The many interactions between the factors are not shown in the figure. The present section describes the main structural and neural factors that determine flexibility, regardless of their potential adaptive capacity. Research on adaptations to stretching training is presented in section 2.5.

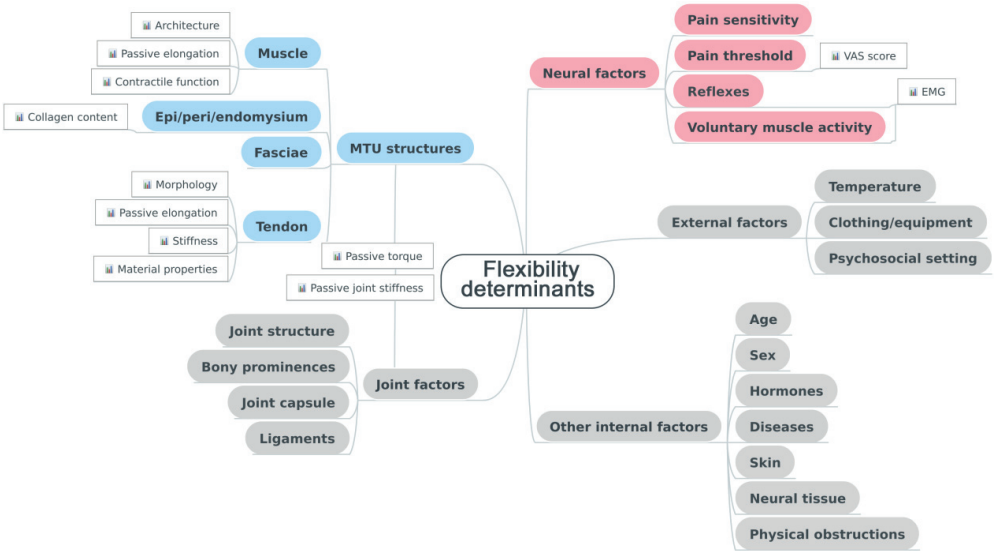


Figure 1. *Overview of factors determining flexibility. Variables investigated by the present thesis are shown on white, square labels. Interactions between factors are not shown. The list of factors is not exhaustive.*

2.2.1. Structural properties of the MTU

The classical Hill model (Hill & Sec, 1938) divides the MTU into a contractile component (i.e. muscle fibres), a series elastic element (i.e. tendon and/or fasciae) and a parallel elastic element. The parallel elastic element is further divided into intramuscular connective tissue (endomysium surrounding muscle fibres and perimysium surrounding muscle fascicles), extra-muscular connective tissue (epimysium surrounding the muscle) and fasciae. The role of fasciae as a parallel or series elastic element varies between muscles. The slack length, the capacity for elongation and the viscoelasticity of these MTU components influence flexibility, as detailed below.

2.2.1.1. Length and compliance

Tendon slack length. When zero tension is applied, the tendon is at its slack length. *In vivo*, true slack length cannot be measured directly, as the tendon interacts with other tissues, but estimations are available (Hug, Lacourpaille, Maisetti, & Nordez, 2013). In the present thesis, *in vivo* approximations of slack length, e.g., MTU passive and joint angle only constrained by the weight of the limb, are referred to as "resting length". Theoretically, an isolated increase in slack length influences flexibility by allowing the joint to take a more extended position before further passive force is applied. Furthermore, an increased slack length (given similar material properties) increases the potential for absolute elongation and thus contribution to MTU elongation.

Tendon compliance/stiffness. Compliance is the inverse parameter of stiffness. When tension is applied, through external forces or muscular contraction, compliance represents the capacity to respond to the applied tension by elongation. Tendon compliance is determined by tendon dimensions (CSA and slack length) and material properties. Tendon material properties are generally described by the Young's modulus (the relation between normalized load and deformation), but may also be represented by magnetic resonance imaging (MRI) pixel signal intensity (Erickson, Prost, & Timins, 1993; Adler & Finzel, 2005).

Muscle slack length. The role of muscle slack length in determining flexibility follows the principles given for tendon slack length. Muscle slack length is governed by the length of myofibrils, muscle fibres or fascicles. The functional unit of muscle is the sarcomere. The classical understanding is that if human muscle could be placed at true slack length, sarcomere length would be relatively constant when averaged across a muscle; hence, the length of the slack myofibril, muscle fibre or fascicle is considered to represent the number of sarcomeres in series (Williams & Goldspink, 1978). However, direct assumptions are dissuaded, given indications that slack sarcomere length is muscle-dependent (Lieber, Roberts, Blemker, Lee, & Herzog, 2017) and further may vary across a myofibril and muscle (Moo, Fortuna, Sibole, Abusara, & Herzog, 2016). Furthermore, in pennate muscles, the portion of the fascicle length that contributes to longitudinal MTU length and elongation is influenced by the pennation angle (Hall, 2006b).

Muscle compliance/stiffness. The isolated sarcomere has large capacity for elongation, being able to produce force at more than 50 % elongation (Gordon, Huxley, & Julian, 1966). Within the sarcomere, the titin filament attaches myosin to the Z-line and is responsible for the elastic tension of isolated muscle fibres (Opitz et al., 2003). *In vivo*, muscle compliance cannot be separated from parallel elastic element compliance.

Parallel elastic element compliance/stiffness. Epimysium, perimysium and endomysium provide structure and transfer forces. This is verified by a 5-fold increase in modulus when comparing muscle fibres *in vitro* with and without their associated extracellular matrix (Meyer & Lieber, 2011). Hence, these tissues govern the compliance of the intact muscle. The compliance of connective tissue is determined by its dimensions and material properties. Material properties are regulated by tissue composition (proteoglycan content, collagen content and types, elastin content) and its structural organization (Nordin, Lorenz, & Campello, 2001). Importantly, perimysium constitutes as a substantial part of the parallel elastic element and is considered a major contributor to passive resistance to stretch (Purslow, 1989). Altogether, the role of the integrated muscle and parallel elastic element in determining flexibility follows the principles given for tendon compliance.

Fasciae force transfer. Though scarcely researched, analyses of muscle deformation suggest that fasciae crossing multiple MTUs or joints may transmit forces between MTUs (for a review, see Maas & Finni, 2018). It is hypothesized that the friction between fasciae and underlying epimysium contributes to the passive resistance to stretch (Krause, Wilke, Niederer, Vogt, & Banzer, 2017); however, these hypotheses do not currently appear to be verified experimentally.

Extensibility of neural tissues. Increased passive resistance to stretch when neural tension was added during passive knee extension has indicated that the passive extensibility of neural tissues may influence flexibility (McHugh, Johnson, & Morrison, 2012).

2.2.1.2. Viscoelastic properties and stress relaxation

Elastic materials strain when stretched but return to slack length when the stress is removed. Viscous materials resist shear flow and strain linearly with time when a stress is applied. The series and parallel elastic elements of the MTU have viscoelastic properties (Hall, 2006b). The viscous properties confer that MTU stiffness is influenced by rate of loading; if forces are applied at a sufficient rate, stiffness increases. On the other hand, when tissue remains elongated, such as with static stretching, compliance increases. When stretching with a constant force, with time, creep increases the elongation. When stretching to a constant length or joint angle, with time, stress relaxation reduces the force required to maintain the elongation. This effect has been demonstrated *in vivo* for stretching in the presence and absence of neural activation (McHugh, Magnusson, Gleim, & Nicholas, 1992).

2.2.2. Neural factors

Flexibility is not determined solely by the structural properties discussed above. Factors related to pain play a role in the subjective determination of ROM. Furthermore, reflexes may activate muscles, increasing active muscle stiffness and hence resistance to stretch.

2.2.2.1. Pain and stretch tolerance

A stimulus that causes or is near causing tissue damage usually elicits protective reactions, e.g. a sensation of pain (Widmaier, Raff, & Strang, 2004). Nociceptors sense potentially damaging stimuli and relay information to levels higher up in the nervous system. Reflex loops in the spinal cord may cause immediate protective reactions, while pain is experienced once the signal reaches the brain (Widmaier et al., 2004). Human stretching guidelines normally specify that the stretching intensity should be to the point of discomfort or the point of pain, but whether typical stretching exercise evokes a nociceptive response does not appear to be documented.

Stretching training studies often indicate improved "tolerance to pain" or "stretch tolerance" (hereafter referred to as "tolerance") as a main mechanism for increases in ROM (Magnusson et al., 1996). Many of the studies do not examine underlying mechanisms of tolerance (Ben & Harvey, 2010; Bjorklund, Hamberg, & Crenshaw, 2001; Halbertsma & Goeken, 1994; Harvey, Batty, Crosbie, Poulter, & Herbert, 2000; Harvey et al., 2003), but speculations include inhibition of pain perception (Laessoe & Voigt, 2004; Mahieu, Cools, De, Boon, & Witvrouw, 2009; Folpp, Deall, Harvey, & Gwinn, 2006), effects related to nociceptive nerve endings, mechanoreceptors or proprioceptors (Magnusson et al., 1996; Law et al., 2009) and psychological factors (Folpp et al., 2006; Law et al., 2009). Psychological factors include how much pain the individual is willing to withstand (hereafter referred to as "pain threshold"). Neural factors that may differ among individuals and may change upon stimulus include the threshold above which nociceptors fire, and the perceived intensity of the nociceptive response (hereafter referred to as "pain sensitivity"). The gate control theory suggests that pain perception during stretching exercise may be reduced by non-painful afferent input from e.g. mechanoreceptors, mitigating nociceptive signals (Melzack & Wall, 1965).

In flexibility research the self-perceived intensity of pain during stretching is sometimes recorded on a visual analogue scale (VAS) (Carlsson, 1983). If pain sensitivity is reduced, the maximally tolerated joint angle should increase while the VAS score remains constant, while if pain threshold is increased, the VAS score should also theoretically increase – although the determination of level of pain is highly subjective.

2.2.2.2. Neural activation due to reflexes

A stretch is often considered passive if neural activation of muscle, measured as electromyographic (EMG) amplitude, is less than 5 % of MVC (Gajdosik, Vander Linden, McNair, Williams, & Riggin, 2005). The passive torque-angle relation is generally associated with the length and compliance of the passive MTU, however, increased EMG amplitudes have been observed at submaximal joint angles (Blazevich et al., 2014), increasing the active stiffness of muscle and hence the passive torque output (Moore & Hutton, 1980). In passive stretching, subjects are instructed to relax their muscles. Hence, voluntary muscle activity is not expected. Nonetheless, EMG amplitudes as high as 17 % of MVC have been reported with passive stretching modalities (Blazevich et al., 2014). The neural activation arising during passive stretching is normally caused by the stretch reflex. The stretch reflex is activated by the muscle spindles, which are intrafusal fibres lying in parallel with the contractile muscle fibres. When the

muscle spindles are stretched with sufficient magnitude or velocity, nerve impulses are relayed to the spinal cord, resulting in reflexive activation of muscle (Kisner et al., 2018).

Reflexive activation may be reduced through autogenic or reciprocal inhibition, resulting in lower passive torque and possibly allowing a greater ROM. The theory behind proprioceptive neuromuscular facilitation (PNF) stretching modalities aiming at autogenic inhibition is based on Golgi tendon organ activation, though this effect is likely minimal (Sharman, Cresswell, & Riek, 2006). The Golgi tendon organs are located at the muscle-tendon junctions (MTJs). In order to avoid critical stresses to the tendon, they respond to increased tension by inhibiting muscle contraction. Reduced activation through reciprocal inhibition may be achieved by voluntary contraction of the antagonist muscles, causing excitatory output to Ia-inhibitory motoneurons of the agonist muscles (Sharman et al., 2006).

2.2.3. Which factors contribute most to flexibility?

All of the aforementioned structural and neural factors potentially play a role for flexibility, but their relative contribution is scarcely researched. For example, pain threshold may limit ROM to a joint angle where the MTU structures are not close to any risk of damage, or reflexive activation may prevent the parallel elastic element from contributing its available elongation. An exploratory factor analysis on flexibility of the hamstrings MTU found that variables grouped as sensory factors explained 37 % of the total variance in ROM and variables grouped as mechanical factors explained 53 % (Chagas et al., 2016). However, neurophysiological variables such as stretch reflexes were not included, and the analysis classified passive torque at maximal joint angle as a mechanical variable, even though maximal joint angle is determined by the subject and likely significantly influenced by sensory factors. A study examining the relationship between straight leg raise ROM and variables related to passive mechanical properties and neural activation found that 79 % of the variability in ROM could be explained by the passive mechanical variables (McHugh, Kremenic, Fox, & Gleim, 1998). These studies indicate that both structural properties of the MTU and neural factors contribute to ROM.

A study testing hip flexion flexibility in surgical patients by applying a standardized force to extend the contralateral knee found no change in joint angle when pain was blocked through general surgical anaesthesia, epidural anaesthesia or femoral nerve block, and an 8° increase when spinal reflexes were blocked through spinal anaesthesia (Krabak, Laskowski, Smith, Stuart, & Wong, 2001). This suggests that neural aspects have a limited influence on the passive torque-angle relation.

Early animal studies have suggested a limited capacity for tendon elongation (2-3 % strain) compared to muscle (20 % strain) (Williams & Goldspink, 1978). However, recent human *in vivo* studies have reported tendon strains around 9 % (Waugh, Blazevich, Fath, & Korff, 2012) and muscle strains around 15 % (Blazevich et al., 2012) . The contribution of tendon and muscle elongation to total MTU elongation and flexibility also depends on slack lengths. A long tendon undergoing 9 % strain may elongate more than a short muscle undergoing 15 % strain. Studies of the gastrocnemius medialis (GM) MTU report that when dorsiflexing the ankle to 30°, muscle contributed 72 % of the total elongation (Abellaneda, Guissard, & Duchateau, 2009), and when dorsiflexing to maximal joint angle, fascicles saw approximately 50 % of the total elongation (Morse, Degens, Seynnes, Maganaris, & Jones, 2008). When rotating between maximally shortened and maximally lengthened position, GM fascicles saw 27 % while tibialis anterior (TA) fascicles saw 55 % of the total elongation (Herbert, Moseley, Butler, & Gandevia, 2002). GM MTU elongation to submaximal joint angles (70 ± 13 % of maximal MTU elongation) indicated that tendon strain contributed most to MTU elongation, but decreasingly with increasing joint angle, fascicle elongation contributed less, but increasingly (27 % at the greatest joint angle), while pennation angle contributed little (6 %) (Herbert et al., 2011). Ultrasonography-based three-dimensional reconstruction of GM MTU elongation to a submaximal joint angle (60 %) by the same group showed that half of the MTU elongation came from fascicle elongation, half from tendon elongation, with little contribution from pennation angle and aponeurosis flattening (Herbert et al., 2015). In summary, both muscle and tendon elongate, but the contributions vary largely with the chosen MTU, joint angles and methods of analysis.

2.2.4. Genetic versus acquired flexibility

Our initial flexibility is determined by genetics. Some individuals have large natural flexibility in specific joints and some are generally hypermobile (Kirk, Ansell, & Bywaters, 1967). ROM in naturally flexible individuals may be manifested through different factors compared to individuals who have acquired large ROM through stretching training: Not all factors may be modified through stretching training. In example, the orientation and depth of the acetabulum, the acetabular labrum and the inclination and declination angles of the neck of the femur influence the ease of performing hip flexion, abduction and extension (Alter, 1996). Joint hypermobility syndrome is a genetic disorder affecting connective tissue matrix proteins (Baeza-Velasco, Pailhez, Bulbena, & Baghdadli, 2015). With regard to factors such as joint structure and connective tissue properties, there may be a continuum from individuals with specific syndromes, through those with benign symptoms, to individuals that simply have restricted natural flexibility.

Populations requiring great flexibility, such as high-level gymnasts and dancers, may have undergone natural selection. It is hard to distinguish which of their specific properties are genetic, and which are acquired through years of stretching and sport specific training. Ultimately, controlled intervention studies are required to document adaptations to stretching training.

2.3. What is stretching training?

The present thesis focuses on the effects of stretching training, which is stretching exercise repeated systematically over time. The aim of stretching training is to increase or maintain flexibility. Exercises similar to those of stretching training are often part of warm-up or cool-down regimes, but if volume and frequency is low, long-term effects on flexibility cannot be expected. The warm-up/cool-down regimes normally aim at acutely increasing flexibility or performance, relaxation and/or well-being. Acute effects are outside the scope of this thesis.

The volume and frequency that is required in order to increase or maintain flexibility is not defined with exact precision, and likely vary with joint/MTU, population, stretching modality, etc. General recommendations regarding frequency include minimum 2-3 times a week (Garber et al., 2011), 5 times a week (Thomas, Bianco, Paoli, & Palma, 2018), and greater gains with daily exercise (Garber et al., 2011). Regarding volume per MTU per session, recommendation include 1 minute (Thomas et al., 2018; Garber et al., 2011) and 3 minutes (Matsuo et al., 2013). Given appropriate methods, altered ROM may be measurable after 3-4 weeks (Garber et al., 2011). On the other hand, a systematic review found trivial effects of 3-8 weeks of stretching on passive resistance, muscle architecture and muscle/tendon stiffness, and speculated that structural adaptations may require greater intensity, greater volume or more than 8 weeks of stretching before becoming measurable (Freitas et al., 2017).

Stretching training may be conducted using a number of different modalities, such as static passive stretching, static active stretching, dynamic stretching or PNF stretching, all of which are effective at increasing ROM (Sainz De & Ayala, 2010). Several studies suggest that no modality is consistently superior (Decoster, Cleland, Altieri, & Russell, 2005; Thacker, Gilchrist, Stroup, & Kimsey, Jr., 2004; Wanderley et al., 2018; Lucas & Koslow, 1984), while a recent systematic review indicates increased gains with static stretching compared to ballistic or PNF (Thomas et al., 2018). Importantly, the various modalities may stimulate different mechanisms for increases in ROM. In example, static stretching training reduced passive torque but did not change tendon stiffness, while ballistic stretching training decreased tendon stiffness but did not change passive torque (Mahieu et al., 2007).

With stretching exercise, intensity refers to the applied load. General advice is to stretch to the point of tightness or discomfort (Garber et al., 2011) or to the point of pain (Blazevich et al., 2014). Some studies indicate that low intensity may give similar (Wyon, Smith, & Koutedakis, 2013; Muanjai et al., 2017) or greater (Wyon, Felton, & Galloway, 2009) gains in ROM than high-intensity stretching, while a review concludes that further research is needed on stretching intensity (Apostolopoulos, Metsios, Flouris, Koutedakis, & Wyon, 2015).

The present thesis does not compare different modalities, volumes, frequencies or intensities. However, the applied combinations must be adept at increasing ROM, in order to address mechanisms behind increases in ROM.

2.4. The relevance of flexibility and stretching training

Flexibility is considered an important component of physical fitness and good health (Corbin & Noble, 1980; Garber et al., 2011). This section briefly reviews whether limited flexibility may be detrimental to health, function and performance, and importantly, whether increasing flexibility through stretching training has been shown to affect health, function, performance or injury risk. The specific adaptations underpinning these relationships are detailed in section 2.5.

2.4.1. Relationship between flexibility and daily function and health

Daily tasks across the life-span require the ability to move comfortably through a large ROM. Inability to handle tasks such as reaching, kneeling, gardening and physical exercise due to restricted ROM may be perceived as reduced life quality (Weiss et al., 2002). Restricted ROM is also associated with low back pain (Sadler, Spink, Ho, De Jonge, & Chuter, 2017).

There are indications but insufficient evidence that stretching training may improve function in elderly (Stathokostas, Little, Vandervoort, & Paterson, 2012) and reduce chronic pain (Geneen et al., 2017). Stretching training has reduced low back pain (Gordon & Bloxham, 2016), improved function in paretic patients (Pradines et al., 2018), improved cardiovascular function (Kruse & Scheuermann, 2017) and reduced injury rehabilitation time (Fournier-Farley, Lamontagne, Gendron, & Gagnon, 2016).

2.4.2. Relationship between flexibility and performance

Sufficient flexibility is of great importance in sports and athletic activities requiring large ROMs, typically in aesthetic sports such as gymnastics, dance, figure skating and diving, but also in specific techniques in martial arts, swimming, athletics, weight lifting, etc. A number of studies

have shown a relationship between sport-specific flexibility and sports performance (for a review, see McNeal & Sands, 2006), but cause-and-effect is not granted.

A large body of literature suggests that static stretching of 60 seconds or more immediately prior to performance may be detrimental, reducing force production (for reviews, see Rubini, Costa, & Gomes, 2007; McHugh & Cosgrave, 2010), power or explosive performance (for reviews, see Shrier, 2004; Rubini et al., 2007). Dynamic stretching has led to minor acute power, sprint or jump performance improvements (Opplert & Babault, 2018; Peck, Chomko, Gaz, & Farrell, 2014; Behm, Blazevich, Kay, & McHugh, 2016). However, effects appear to diminish when stretching is incorporated in a full warm-up, both for dynamic (Blazevich et al., 2018) and static stretching, unless maintained for 120 seconds (Reid et al., 2018).

The aforementioned studies are relevant for planning of training sessions, but do not foresee effects of increasing flexibility long term. Stretching training is suggested to increase force, power and performance in explosive tasks (for a review, see Shrier, 2004).while intervention studies have increased hip and/or ankle ROM without affecting running economy (Godges, MacRae, & Engelke, 1993; Nelson, Kokkonen, Eldredge, Cornwell, & Glickman-Weiss, 2001) or kinematics (Mettler, Shapiro, & Pohl, 2018).

2.4.3. Relationship between flexibility and injury risk

Limited flexibility is indicated as a risk factor for MTU injury, e.g. limited hip abduction ROM as a risk factor for groin strain (Arnason et al., 2004), low compliance of quadriceps or hamstrings MTUs as a risk factor for developing patellar tendinopathy (Witvrouw, Bellemans, Lysens, Danneels, & Cambier, 2001) or lesions (Witvrouw, Danneels, Asselman, D'Have, & Cambier, 2003), and greater muscle damage following eccentric exercise in subjects with greater passive joint stiffness (McHugh et al., 1999).

No clear consensus exists on the ability of stretching to reduce injury risk, neither when applied immediately before exercise (for reviews, see Shrier, 1999; McHugh & Cosgrave, 2010; Behm et al., 2016), nor with stretching training (for reviews, see Thacker et al., 2004; Weldon & Hill, 2003). Stretching during warm-up did not reduce the risk of exercise-related injuries in army recruits (Pope, Herbert, Kirwan, & Graham, 2000), but reduced rates of muscle injury and low back pain in another sample of army recruits (Amako, Oda, Masuoka, Yokoi, & Campisi, 2003). In these types of analyses, results may vary with the included types of injuries. When limiting the scope to MTU injuries, a positive effect of pre-activity stretching on injury risk is claimed (Small,

Mc Naughton, & Matthews, 2008), but the major body of research suggests that general injury prevention is not a valid incentive for conducting stretching exercise.

2.4.4. How much flexibility is ideal?

Analyses of how flexibility or stretching training influence function, health, performance or injury risk may also be confounded by the non-linear relationships between these factors. Too much or too little flexibility (Jones & Knapik, 1999) and general hypermobility (Grahame, 1971; Grahame & Jenkins, 1972) may be risk factors for injury. Large flexibility may cause excessive energy expenditure through increased stabilisation (Gleim, Stachenfeld, & Nicholas, 1990) or coordinative challenges, e.g. through impaired joint proprioception (Mallik, Ferrell, McDonald, & Sturrock, 1994). Hence, extreme flexibility may be an asset only in specific populations such as ballet dancers (Grahame & Jenkins, 1972). For any given task, there is probably an optimal level of flexibility, which should be observed when choosing study populations and interpreting results.

2.5. Adaptations to stretching training

2.5.1. Range of motion

There is consensus that stretching training increases ROM in various MTUs (Harvey, Herbert, & Crosbie, 2002), including the hamstrings (for reviews, see Decoster et al., 2005; Medeiros, Cini, Sbruzzi, & Lima, 2016) and triceps surae (for reviews, see Young, Nix, Wholohan, Bradhurst, & Reed, 2013; Medeiros & Martini, 2017). However, ROM is not always increased after stretching training in healthy adults (Worrell, Smith, & Winegardner, 1994) or in clinical populations (Harvey et al., 2003), possibly resulting from too low volume (durations of 3 and 4 weeks) and/or large inter-individual variability in the response to stretching training (Worrell et al., 1994). Stretching training interventions typically last 3-10 weeks. Known exceptions applied stretching for 15 (Donti et al., 2018), 16 (Simao et al., 2011) and 31 weeks (Santonja Medina, Sainz De Baranda, Rodriguez Garcia, Lopez Minarro, & Canteras, 2007).

2.5.2. Structural properties of the MTU

The section below addresses variables that describe the MTU as a whole. Subsequent sections discuss tendon and muscle separately. *In vivo*, it is difficult to separate the properties of intra- and

extra-muscular connective tissue from those of the contractile components. Hence, these variables are discussed together.

2.5.2.1. Whole MTU behaviour

Joint flexibility is often assessed by the passive resistance to stretch, represented by the passive torque-angle relation. The recorded torque represents resistance from all parts of the neuro-musculo-skeletal complex, including e.g. active muscle stiffness, joint capsules, ligaments and synergist MTUs – not merely the MTU(s) responsible for the main joint action (Johns & Wright, 1962). From the passive torque-angle relation, a number of mechanical variables describing flexibility or the response to stretching training may be extracted, as detailed below.

Passive torque. Passive torque gives different information depending on the joint angle at which it is reported. At maximal joint angle, which typically increases with stretching training, passive torque may also be increased, because the greater joint angle requires greater MTU elongation and, unless slack lengths are changed, greater strains of the MTU components. Hence, torque at an increased maximal joint angle should not be used to evaluate passive resistance, but may indicate neural adaptations such as reduced pain sensitivity. Contrary, reduced passive torque at a standardized joint angle, i.e. same angle before and after an intervention, indicates that the same MTU elongation is obtained with less tension applied – less passive resistance to stretch.

In human cross-sectional comparisons, flexible subjects have consistently displayed lower passive torque across joint angles (Magnusson et al., 1997) or lower passive torque at standardized joint angles (Blazevich et al., 2012; Abellaneda et al., 2009) compared to less flexible subjects. Stretching intervention studies have demonstrated right-shifted passive torque-angle curves, meaning that when torque and joint angle are plotted on (x,y) axes, torque is reduced across a range of angles (Kubo, Kanehisa, & Fukunaga, 2002; Toft, Espersen, Kalund, Sinkjaer, & Hornemann, 1989; Guissard & Duchateau, 2004) and reduced passive torque at standardized joint angles (Nakamura, Ikezoe, Takeno, & Ichihashi, 2012; Chan, Hong, & Robinson, 2001; Mahieu et al., 2007) in passive ankle dorsiflexion and in passive hip flexion. Contrasting, a number of studies have increased ROM without any change in passive torque (Ben & Harvey, 2010; Law et al., 2009; Mahieu et al., 2009; Folpp et al., 2006; Magnusson et al., 1996; Konrad & Tilp, 2014a; Konrad & Tilp, 2014b).

A few studies have observed increased passive torque at maximal joint angle after stretching training (Halbertsma & Goeken, 1994; Nakamura et al., 2017; Reid & McNair, 2011; Blazevich et al., 2014). Some studies have followed the time course of passive torque changes, revealing increased ROM and increased passive torque at maximal joint angle after 2-4 weeks, and further

increased ROM while passive torque at maximal joint angle was reduced back to (Chan et al., 2001) or below (Guissard & Duchateau, 2004) initial level after 4-8 weeks. In these studies, early increases in ROM may have been achieved primarily through sensory adaptations, in which case increased maximal passive torque would be a consequence of increased strain of the MTU components and eventually reflexive activation. The subsequent reduction in passive torque despite continued increase in maximal joint angle could indicate that the early sensory adaptations allowed a greater stimulus on the force-bearing structures of the MTU, laying ground for structural adaptations and/or reduced reflexes, in turn reducing passive torque. It has been suggested that interventions up to 8 weeks may increase ROM without reducing passive torque due to initial adaptations in non-muscular structures, which contribute marginally to the passive torque but may be richly innervated with receptors affecting the sensation of pain (Nordez et al., 2017). Differences between studies with regard to maximally tolerated passive torque may relate to total duration, weekly volume, intensity, the investigated MTU or differences in the magnitude of sensory adaptations.

A systematic review found a significant increase but small effect size for maximal passive torque, but no difference in torque at standardized joint angles or joint angle at standardized passive torque (Freitas et al., 2017), but large heterogeneity suggests that further research is needed to understand the impact of stretching training on the passive torque-angle relation.

Passive joint stiffness. Passive joint stiffness measured *in vivo* is defined as the slope of the linear portion of the passive torque-angle relation. "The linear portion" is defined differently in former studies, applying either a standardized range of joint angles (Kubo et al., 2002; Marshall, Cashman, & Cheema, 2011), the last X degrees of the ROM (Kay et al., 2016), the last X % of the ROM (Reid & McNair, 2011), or the entire ROM (Konrad, Gad, & Tilp, 2015). Furthermore, different mathematical models applied to same data set gave different outcomes (Nordez, Cornu, & McNair, 2006). Results of previous stretching interventions range from reduced passive joint stiffness (Guissard & Duchateau, 2004; Kubo et al., 2002; Marshall et al., 2011) through unchanged passive stiffness (Konrad & Tilp, 2014b; Konrad & Tilp, 2014a; Kay et al., 2016; Konrad et al., 2015) to increased passive stiffness (Reid & McNair, 2011). Heterogeneity between mathematical models and study designs mean that there is no consensus on the effect of stretching training on passive joint stiffness (Freitas et al., 2017).

Viscoelastic stress relaxation. An intervention study found less stress relaxation after 6 weeks of stretching, speculating that the training had led to rearrangement of collagen fibres, relaxation of collagen fibres or altered unfolding of titin (Peixoto et al., 2015), however, further studies are needed to determine the mechanisms behind finding.

2.5.2.2. Tendon

It has been suggested that tendon must be strained to a threshold in order to induce adaptations such as increased tendon stiffness or region-specific hypertrophy (Arampatzis, Karamanidis, & Albracht, 2007). However, there are indications that tendinous tissue is metabolically responsive to low-intensity tensile loading (Bojsen-Moller, Kalliokoski, Seppanen, Kjaer, & Magnusson, 2006). Tendinous adaptations to stretching have been investigated as detailed below.

Tendon dimensions. Static stretching for 6 weeks did not alter Achilles tendon length or thickness (Simpson et al., 2017). Other human studies investigating tendon dimension adaptations do not appear to be available (Freitas et al., 2017).

Tendon compliance/stiffness. Most human studies applying static stretching training have observed no effect on Achilles tendon stiffness measured during ramp contractions (Mahieu et al., 2007; Mahieu et al., 2009; Kubo et al., 2002; Konrad & Tilp, 2014b) or during passive trials (Blazevich et al., 2014). However, one study found reduced tendon stiffness after 8 weeks of static stretching (Kubo, Kanehisa, Kawakami, & Fukunaga, 2001). Other stretching modalities have produced contrasting outcomes, from reduced tendon stiffness after PNF (Konrad et al., 2015) or ballistic (Mahieu et al., 2007) stretching training, through unchanged tendon stiffness after ballistic stretching training (Konrad & Tilp, 2014a) to increased tendon stiffness after dynamic stretching training (Kay et al., 2016). Possibly, stretching modalities involving muscular contractions near maximal joint angle are more potent at placing strain on the tendon, increasing the stimulus for tendinous adaptations. Acute dynamic stretching exercise caused a proximal displacement of the GM MTJ in resting position, suggesting that this type of stretching reduces tendon stiffness more than muscle stiffness (Samukawa, Hattori, Sugama, & Takeda, 2011).

Tendon elongation/strain during passive stretching. Altered tendon elongation during passive stretching suggests altered tendon dimensions and/or altered relative compliance of the tendon versus other MTU components. A stretching intervention that increased ROM found reduced tendon strain at maximal joint angle, along with increased muscle strain (Blazevich et al., 2014), suggesting greater muscular adaptations compared to tendon.

2.5.2.3. Muscle and connective tissue

Repeated loading alters human muscle architecture and muscle size, e.g. in the form of strength training (Widmaier et al., 2004). Part of the stimulus for production of actin and myosin filaments in series and parallel relates to mechanotransduction (Goldspink, 1999). If stretching loads are sufficient to activate this system, adaptations on muscle level may occur even with stretching.

Muscle size. In animal studies, denervation or external weight application have been used to apply long-term overload, leading to increased muscle weight, size or fascicle CSA (Barnett, Holly, & Ashmore, 1980; Holly, Barnett, Ashmore, Taylor, & Mole, 1980; Sola, Christensen, & Martin, 1973; Heinemeier et al., 2007). These are extreme interventions, however, stretching of rat soleus (SOL)for 40 minutes every third day for 3 weeks, mimicking human stretching, also increased muscle fibre area (Coutinho, Gomes, Franca, Oishi, & Salvini, 2004). The analysis and stretching methods used in animal studies are not applicable in human populations, but there are some indications that stretching training has the capacity to alter muscle size. In large-volume passive stretching of osteoarthritic subjects for 4 weeks, CSA of muscle fibres of hip adductors increased (Leivseth, Torstensson, & Reikeras, 1989). Static stretching for 6-8 weeks increased ankle dorsiflexion ROM and gastrocnemius thickness (Mizuno, 2017). In contrast, 4 weeks of static ankle dorsiflexion stretching did not modify gastrocnemius thickness (Nakamura et al., 2012), two studies of 8 weeks of static hip flexion stretching did not alter biceps femoris thickness (e Lima, Carneiro, de S Alves, Peixinho, & de Oliveira, 2015; Freitas & Mil-Homens, 2015) and 8 weeks of static knee flexion stretching did not modify vastus lateralis thickness (e Lima et al., 2015)

Pennation angle. There are a few indications of altered pennation angle with stretching training. PNF stretching for 6 weeks increased GM pennation angle at anatomical joint angle but not at maximal joint angle (Konrad et al., 2014). High-intensity static stretching led to a near-significant reduction in biceps femoris pennation angle (Freitas & Mil-Homens, 2015). In contrast, static or ballistic stretching did not change pennation angle in GM (Nakamura et al., 2012; Konrad & Tilp, 2014b; Konrad & Tilp, 2014a) or in vastus lateralis and biceps femoris (e Lima et al., 2015).

Muscle length and sarcomeres in series. Longitudinal muscle growth is accomplished through addition of sarcomeres in series, particularly near the MTJ (Dix & Eisenberg, 1990; Williams & Goldspink, 1971). Sarcomerogenesis is known to occur in post-natal growth (Goldspink, 1968), with surgical bone lengthening (Boakes, Foran, Ward, & Lieber, 2007), with altered operating range (Butterfield, Leonard, & Herzog, 2005) and with unaccustomed eccentric strength training (Lynn & Morgan, 1994). Elevation of cytoplasmic calcium activates signalling pathways for sarcomere number regulation during contractile activity (Herring, Grimm, & Grimm, 1984). It is not clear whether human stretching exercise causes sufficient muscle fibre lengthening to raise cytoplasmic calcium through stretch-activated calcium channels (Snowdowne, 1986), but some indications exist that stretching training has the potential to increase muscle length. In animal studies extreme stretching modalities utilizing immobilization, denervation, external weight application or tendon transfer may increase the number of sarcomeres in series (Ashmore &

Summers, 1981; Cox et al., 2000; Tabary, Tabary, Tardieu, Tardieu, & Goldspink, 1972; Dix & Eisenberg, 1990; Williams & Goldspink, 1971; Takahashi, Ward, Marchuk, Frank, & Lieber, 2010). Among animal studies mimicking human stretching, some (Coutinho et al., 2004) but not all (Peixinho, Martins, de Oliveira, & Machado, 2014) found increased serial sarcomeres. In human studies, increased resting fascicle length is sometimes taken as an indication of increased serial sarcomeres. This may be valid if the overlap of actin and myosin is the same during each measurement. *In vivo*, true fascicle slack length and number of sarcomeres in series cannot be determined. Stretching training has increased fascicle length in the gastrocnemii (Pradines, Masson, Portero, Giroux, & Gracies, 2016; Simpson et al., 2017) and biceps femoris (Freitas & Mil-Homens, 2015), while other studies did not find altered fascicle length in GM (Blazevich et al., 2014; Konrad & Tilp, 2014b; Konrad & Tilp, 2014a; Konrad et al., 2015; Nakamura et al., 2012) or biceps femoris and vastus lateralis (e Lima et al., 2015).

Muscle elongation and stiffness. The adaptability of intramuscular connective tissue is indicated by an *in vitro* study where tension on fibroblasts caused remodelling of the cytoskeleton and reduction of connective tissue stiffness (Langevin et al., 2011). In a human study, 3 weeks of static stretching increased muscle and fascicle strain at standardized and maximal dorsiflexion angles (Blazevich et al., 2014). Passive muscle stiffness may be calculated as change in passive torque over change in muscle length. Static stretching for 4 weeks (Nakamura et al., 2017) or dynamic stretching for 6 weeks (Kay et al., 2016) decreased passive muscle stiffness of the GM MTU. Recently the hardness of individual muscles has been evaluated by supersonic shear wave elastography (e Lima, Costa Junior, Pereira, & Oliveira, 2018). Static stretching for 4-5 weeks has increased ROM and reduced muscle hardness of the gastrocnemii (Akagi & Takahashi, 2014), hamstrings (Ichihashi et al., 2016), infraspinatus and teres major (Yamauchi et al., 2016). This indicates that stretching training causes adaptations in the muscle belly, but does not currently specify what may be the physiological source of reduced hardness, such as passive properties of muscle fibres, neural activation and/or connective tissue properties.

Connective tissue quantity and structure. Animal studies have revealed that stretch may rearrange collagen fibres (de la Tour, Tabary, Tabary, & Tardieu, 1979) and increase connective tissue quantity (Tabary et al., 1972; Williams & Goldspink, 1984). Three weeks of incrementally applied stretch increased intramuscular connective tissue CSA from 15 % to 19 %, but the amount returned to initial values with maintenance stretching (Cox et al., 2000). Increased intramuscular collagen turnover has been observed after both acute loading in human models (Miller et al., 2005; Holm et al., 2010; Crameri et al., 2004) and chronic loading in animal models (Heinemeier et al., 2007). Expression of collagen I, III and IV transcripts has been observed after

acute loading in humans (Hyldahl et al., 2015). These data suggest that strength-training loads may yield changes in muscle collagen content, but the links between newly synthesized collagen and total amount thereof remain unclear. A recent study found increased muscle collagen synthesis after 15 days of intermittent stretching in a rat model, while at the same time, collagen content was reduced, underscoring the complexity of the links between collagen net synthesis balance and deposition into the extracellular matrix (Peviani et al., 2018). While intense stretching models in animals have induced increased collagen content and increased muscle stiffness, studies on human fibre preparations *in vitro* have demonstrated that intramuscular collagen content is only weakly correlated to passive muscle tissue stiffness (Smith, Lee, Ward, Chambers, & Lieber, 2011), leaving the impact of stretching training on connective tissue unclear.

2.5.3. Neural factors

While many studies explain increased ROM with improved tolerance (Folpp et al., 2006; Ben & Harvey, 2010; Bjorklund et al., 2001; Halbertsma & Goeken, 1994; Harvey et al., 2000; Harvey et al., 2003; Magnusson et al., 1996; Law et al., 2009; Mahieu et al., 2009), few studies have specifically investigated the role of neural factors in increasing ROM with stretching training.

2.5.3.1. Pain and tolerance

The effect of stretching training on pain sensitivity was studied using thermal quantitative sensory testing, where subjects scale pain during application of increasing, standardized temperatures to the skin (Bishop & George, 2017). Stretching for 8 weeks did not induce a response different from controls, while ROM after the intervention period was more associated with passive torque and pain sensitivity than whether the subject had been in the stretching or control group, indicating that 8 weeks of stretching did not override the initial properties of the subjects.

In accordance with the gate control theory (Melzack & Wall, 1965), transcutaneous electrical nerve stimulation (TENS) has been applied prior to stretching exercise, to relieve pain. Acutely, this technique has allowed subjects to stretch at a greater joint angle with a greater passive resistance, causing greater increase in ROM and greater reduction in muscle hardness compared to static stretching exercise alone (Karasuno et al., 2016). Another study found greater acute increase in ROM by combining stretching exercise with massage balls than by combining with TENS, proposing that only the first combination engaged sensory fibres contributing to pain tolerance (Capobianco, Almuklass, & Enoka, 2018). Stretching for 8 weeks during application of TENS increased ROM more than stretching alone, in children with short hamstrings MTUs (Piqueras-Rodriguez, Palazon-Bru, & Gil-Guillen, 2016). While these studies indicate

opportunities for greater increases in ROM, it is not obvious that the greater gains are caused by reduced pain sensitivity alone, since muscle contraction and massage also infer mechanical elongation of tissues.

2.5.3.2. Neural activation due to reflexes

Extensive static stretching acutely decreases the afferent output from the muscle spindles, reducing the muscle's reflex excitability (Guissard, Duchateau, & Hainaut, 1988). Studies have investigated whether reciprocal inhibition may be responsible for increases in ROM by artificially inducing the Hoffmann reflex, through peripheral nerve electrical stimulation (Knikou, 2008). Acutely after stretching exercise, the H:M ratio (maximal Hoffman reflex to maximal direct motor response) was reduced, indicating reduced spinal reflex excitability (Behm et al., 2013; Avela, Kyrolainen, & Komi, 1999). Ankle dorsiflexion stretching for 6 weeks increased ROM, reduced passive muscle stiffness at standardized joint angles, and reduced the amplitude of the H:M ratio as well as the tendon reflex (Guissard & Duchateau, 2004). The authors suggest that neural adaptations contribute to the increased ROM and reduced passive torque by reducing reflexive neural activation, but also mention that reduced tendon reflex could be caused by increased compliance. Changes in ROM and passive torque occurred earlier than decreases in reflex activity, suggesting multiple mechanisms. In contrast, 6 weeks of dorsiflexion stretching increased ROM but did not change motoneuron excitability, leading the authors to suggest that ROM increased due to altered mechanical properties or improved tolerance (Hayes et al., 2012).

2.5.3.3. EMG amplitude

Given the limited research on specific neural adaptations, studies measuring EMG amplitude during stretching may add insight. A cross-sectional study observed lower EMG amplitude at standardized joint angles in flexible compared to less flexible subjects (Abellaneda et al., 2009), primarily demonstrating that neural activation increases when approaching maximal joint angle. In other cross-sectional studies, peak EMG amplitude was similar in flexible and less flexible subjects (Blazevich et al., 2012; Magnusson et al., 1997). Intervention studies have found either minimal EMG amplitudes at standardized joint angles, unaffected by training (Magnusson et al., 1996) or large peak EMG amplitudes but unaffected by training (Blazevich et al., 2014), suggesting that stretching training does not modify the reflex response to a magnitude that affects EMG amplitude.

2.5.3.4. Cross-education effects

Cross-education – transfer of training effects from a trained limb to the contralateral limb – has been described for strength tasks and skill learning (for a review, see Ruddy & Carson, 2013), with strength gains in the untrained side of around 35 % of the trained side (Munn, Herbert, & Gandevia, 2004). Similarly, exercising one muscle group appears to fatigue other muscle groups, particularly in the lower limbs (for a review, see Halperin, Chapman, & Behm, 2015).

Acutely, static and dynamic lower body stretching exercise has led to increased upper body ROM and vice versa (Behm et al., 2016; Wilke, Vogt, Niederer, & Banzer, 2017). Similarly, static and dynamic unilateral lower body stretching exercise has acutely increased contralateral ROM (Chaouachi et al., 2017; Killen, Zelizney, & Ye, 2018). A study evoking spinal reflexes by transcutaneous spinal cord stimulation found reduced reflex amplitudes during stretching exercise in both stretched and non-stretched muscles on the ipsilateral side, but not on the contralateral side, and no effect on either side immediately after exercise (Masugi, Obata, Inoue, Kawashima, & Nakazawa, 2017). These findings suggest an acute inhibitory effect of stretching on monosynaptic spinal reflexes, but do not foresee cross-education effects of stretching training.

A number of studies applying unilateral stretching training report no contralateral increases in ROM (Akagi & Takahashi, 2014; Ben & Harvey, 2010; Kubo et al., 2002; Guissard & Duchateau, 2004; Minshull, Eston, Bailey, Rees, & Gleeson, 2014; Nelson et al., 2012), no reduction in passive torque properties (Akagi & Takahashi, 2014; Kubo et al., 2002) and no reduction in muscle hardness (Akagi & Takahashi, 2014) with 3-10 weeks of stretching.

2.5.4. Which factors contribute most to ROM increases?

Research on the relative contribution of neural factors versus structural properties to increases in ROM does not seem available, but the relative contribution of various structural properties is examined, mainly as muscle versus tendon elongation. Acutely after stretching, increased ankle dorsiflexion ROM has been associated with increased compliance of the muscle belly relative to tendon (Morse et al., 2008), or increased tendon elongation but unchanged muscle elongation (Kato, Kanehisa, Fukunaga, & Kawakami, 2010), or similar increases in absolute elongation of muscle and tendon (Theis, Korff, Kairon, & Mohagheghi, 2013). In a 3-week intervention, muscle and fascicle strain increased while tendon strain was unchanged at maximal joint angle, and muscle rather than tendon accounted for the increased MTU elongation (Blazevich et al., 2014). Taken together, these studies suggest that both muscle and tendon contribute to greater MTU elongation occurring with greater ROM, but further studies are needed.

2.6. Implications for contractile function

A major incentive for stretching training is an expectation that increased flexibility improves function or performance. The present thesis focuses on the effects of stretching training on the MTU and its contractile function.

2.6.1. Isometric strength

Studies have found increased isometric force of the plantar flexors (Rees, Murphy, Watsford, McLachlan, & Coutts, 2007) and the knee flexors and extensors (Handel, Horstmann, Dickhuth, & Gulch, 1997) following 8 and 4 weeks of PNF stretching, respectively. A number of other studies conducting 3-8 weeks of passive or PNF stretching found no changes in peak torque or maximal force (Akagi & Takahashi, 2014; Blazevich et al., 2014; Guissard & Duchateau, 2004; Konrad & Tilp, 2014b; Konrad & Tilp, 2014a; Konrad et al., 2015; Kubo et al., 2002; Minshull et al., 2014; e Lima et al., 2015). These findings suggest that the loads applied during stretching are generally insufficient to induce muscle hypertrophy, although stretching modalities involving muscular contractions may be executed in a manner that may influence muscle strength.

2.6.2. Isokinetic torque-angle properties

Isokinetic strength. Concentric peak torque of the knee flexors and/or knee extensors increased following some (Chen et al., 2011; Handel et al., 1997; Worrell et al., 1994; Batista, Vilar, de Almeida Ferreira, Rebelatto, & Salvini, 2009) but not all (Ferreira, Teixeira-Salmela, & Guimaraes, 2007; LaRoche, Lussier, & Roy, 2008; Marshall et al., 2011) studies applying 3-12 weeks of stretching. Eccentric peak torque of ankle plantar flexors (Abdel-Aziem & Mohammad, 2012), knee flexors and/or knee extensors (Handel et al., 1997; Worrell et al., 1994) increased following 3-8 weeks of stretching.

Angle of peak torque. A cross-sectional study found that subjects with low hip flexion ROM exerted maximal knee flexion torque at joint angles corresponding to a more flexed knee compared to controls (Alonso, McHugh, Mullaney, & Tyler, 2009). Stretching interventions have shifted knee flexor angle of peak torque toward extended positions (Ferreira et al., 2007; Chen et al., 2011), while a study investigating both concentric and eccentric angle of peak torque of knee flexors and extensors found a shift only for quick eccentric loading ($120°\cdot s^{-1}$) of the knee extensors (Handel et al., 1997). An intervention study stretching the hip extensors found no change in angle of peak torque or any other variable (LaRoche et al., 2008). Possibly, the total volume of 12 sessions was insufficient to induce changes in flexibility.

Theoretically, angle of peak torque may shift towards an extended position if the conditions for optimal myofilament overlap are altered (Gordon et al., 1966), e.g. through addition of serial sarcomeres or increased tendon elongation, or if the contribution from passive resistance to the torque output is reduced. However, effects of addition of serial sarcomeres may be counteracted by increased tendon compliance, causing the tendon to elongate more for a given contractile force, which in turn would lead to muscle working at a shorter, less optimal length.

Isokinetic work. Stretching training increased concentric work of knee flexors and extensors (Handel et al., 1997), or increased work at some but not all concentric and eccentric velocities of knee flexion and extension (Ferreira et al., 2007). Theoretically, increased work may result from reduced passive resistance or from increased fascicle length. Additional sarcomeres in series would enable the muscle to rotate the limb at the same angular velocity with reduced sarcomere shortening velocity, enabling increased torque production, as well as sarcomeres remaining near optimal length across a wider range of joint angles (for a review, see Lieber & Friden, 2000). Further research is needed in order to understand the interplay between compliance, passive resistance, serial sarcomeres and torque production in relation to stretching training.

2.7. Summary

Stretching exercise is frequently applied in a variety of populations, with a broad range of aims. While flexibility is relevant for function and performance in specific tasks, less is known about physiological responses to stretching training. Stretching training increases ROM, but the mechanisms behind are not sufficiently understood. Both sensory adaptations related to pain and structural adaptations in the MTU are proposed.

On MTU level, stretching has reduced passive torque and/or passive joint stiffness at standardized joint angles, in some but not all studies. Reduced passive torque or stiffness may result from altered morphology, mechanical properties and/or neural activation, but the relevance of or contribution from single factors is not well elucidated.

Studies documenting muscle or tendon morphological adaptations to stretching training do not appear available. Increased fascicle length is shown in a few studies, but while muscle has capacity for longitudinal growth, there is no evidence of muscle growth in response to stretching training. Furthermore, research on the effect of human stretching training on connective tissue composition or structural organization does not seem available

With regard to mechanical properties, stretching training has led to increased, unchanged or decreased tendon stiffness. Studies have shown that the passive elongation and strain of the

tendon, muscle belly and/or muscle fascicles may change with stretching training. Changes in elongation at given force levels or MTU lengths indicate altered compliance of one or more MTU components. During passive stretching, compliance and passive torque may be altered by reflexive neural activation. Reports of neural adaptations to stretching training are scarce, but reduced tonic reflex activity has been indicated. On the other hand, EMG amplitude has not been shown to change with stretching training.

If neural adaptations are important for increases in ROM, cross-education effects could be hypothesized. However, contralateral increases in ROM have only been shown acutely after stretching exercise, not with stretching training.

With regard to contractile function, stretching training has shifted angle of peak torque and increased work in some but not all studies.

Collectively, previous studies provide an inconsistent overview of the roles of structural properties of the MTU and neural factors in increasing ROM, and of the interplay between morphological properties, mechanical properties, material properties, neural activation and pain.

It has been speculated that early adaptations to stretching training occur mostly on a sensory level, while adaptations to the force-bearing tissues of the MTU require greater durations in order. Most studies have applied stretching for 3-10 weeks. The known studies exceeding this duration (Santonja Medina et al., 2007; Donti et al., 2018; Simao et al., 2011) measured only ROM and, in one study, muscle strength (Simao et al., 2011).

Cross-sectional studies comparing groups with varying amounts of flexibility provide relevant information about factors that contribute to ROM and that may have developed in response to years of stretching, but do not document causality. A number of previous studies underline the need for stretching intervention studies with greater total time under tension and/or longer intervention periods in order to examine adaptations to stretching training and mechanisms behind increases in ROM (Ben & Harvey, 2010; Folpp et al., 2006; Magnusson et al., 1996; Freitas et al., 2017; Chan et al., 2001).

3. Research aim and hypotheses

In light of the available research on the field, the aim of the present thesis was to examine the effects of long-term stretching training on hamstrings and triceps surae muscle-tendon morphology, mechanics and function, by comparing populations that had undertaken years of systematic stretching to active control subjects with no history of stretching (studies I-II), and by conducting a 24-week stretching training intervention (study III). The specific hypotheses were:

1. ROM is greater in populations with years of stretching training compared to controls, and ROM is increased with 24 weeks of stretching.
2. Passive resistance to stretch, as one of the determinants of flexibility, is represented by lower passive torque at standardized joint angle in populations with years of stretching training compared to controls, and passive torque at standardized joint angle is reduced with 24 weeks of stretching. In contrast, passive torque at maximal joint angle is greater in flexible populations compared to controls, and is increased with 24 weeks of stretching.
3. Lower active muscle stiffness, as one of the determinants of passive resistance to stretch, and hence flexibility, is indicated by lower EMG amplitudes at standardized joint angle of passive stretching in populations with years of stretching training compared to controls, and EMG amplitudes at standardized joint angle are reduced with 24 weeks of stretching.
4. The greater MTU elongation seen in populations with years of stretching training compared to controls is achieved through greater elongation of both muscle and tendon, with muscle playing a greater role. The maximal elongation of muscle and tendon is increased and the contribution from muscle versus tendon is altered with 24 weeks of stretching.
5. Populations with years of stretching training may display different morphological properties compared to controls. However, tendon and morphological properties and tendon material properties are unchanged, while intramuscular collagen content is decreased, with 24 weeks of stretching.
6. Populations with years of stretching training reach peak isokinetic torque at joint angles corresponding to a more extended position compared to controls, and the angle of peak torque is increased with 24 weeks of stretching.

4. Methods

The present thesis consists of three studies, which are presented in the thesis as four original scientific papers plus additional material. An overview of the studies, study designs, investigated MTUs and scientific papers is given in Figure 2.

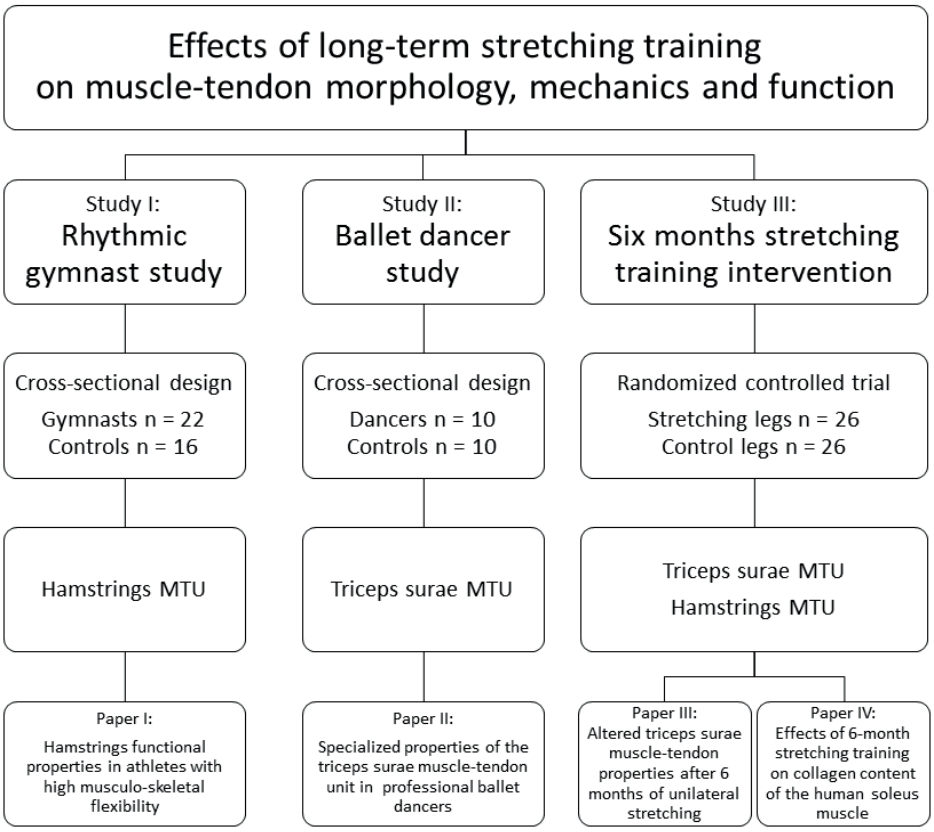


Figure 2. *Overview of the studies, study designs, MTUs (muscle-tendon units) and papers comprising the present thesis.*

4.1. Study designs

4.1.1. Studies I-II: Cross-sectional design

For study I and study II a cross-sectional design was chosen, comparing female subjects from populations that had conducted years of systematic stretching to female control subjects:

In study I, elite rhythmic gymnasts were compared to athletes in sports not involving systematic stretching: Handball, football and cross-country skiing. Groups were matched by age.

In study II, professional ballet dancers were compared to recreationally active controls with no history of systematic stretching. Groups were matched by height and body mass.

The investigators were not blinded to the group affiliation during testing, due to testing of rhythmic gymnasts during Nordic championships and due to the process of recruiting ballet dancers. However, investigators were blinded during all post-intervention analyses.

4.1.2. Study III: Randomized controlled trial

Study III was conducted as a within-subjects randomized controlled trial, where 24 weeks of stretching was applied to one leg, while the contralateral leg served as control. Assignment of stretching leg was made by stratified randomization such that one half of the subjects stretched their dominant leg, while the rest stretched the non-dominant leg. Two subjects had an initial side-to-side ROM difference of >10° and were assigned to stretch their least flexible leg.

The investigators were blinded to the leg assignment during testing and analyses. All measurements were undertaken by the same investigators.

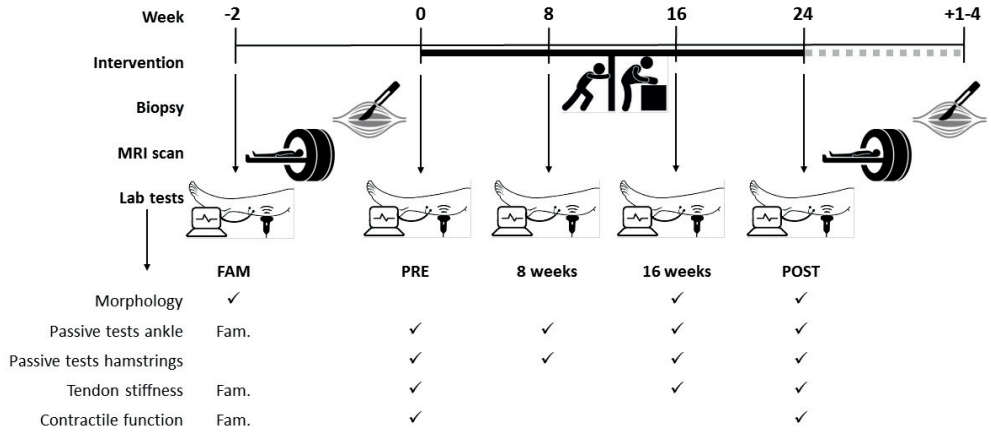
The time course of testing and intervention is shown in Figure 3. Each subject reported to the laboratory five times: A familiarization session where resting measurements of muscle and tendon morphology were also obtained, a pre-intervention test session (PRE) 1-2 weeks later, a subset of tests after 8 and 16 weeks, and a post-intervention test session (POST) 24-48 hours after the last bout of stretching. The subjects were instructed to refrain from training or stretching at least 24 hours prior to each session.

A subgroup of the subjects also underwent MRI scanning and had SOL muscle biopsies taken. Due to scheduling logistics, these subjects continued stretching for 1-4 weeks after their POST test, in order to terminate stretching training 48 hours prior to MRI scanning and muscle biopsy.

In female subjects who did not take constant-dose oral contraceptives, all tests were conducted within 14 days from the last menstruation, to avoid testing during the luteal phase.

The subjects were instructed and reminded to maintain their habitual activity level, refrain from unaccustomed exercise, maintain their normal diet, refrain from anti-inflammatory drugs or nutritional supplements and report any illness or injury during the experimental period.

The long duration of the intervention was considered a risk of substantial dropout. Therefore, taking samples after 8 and 16 weeks was considered important. Since the final dropout rate was low (10 %), most data are analysed and reported only for the PRE and POST time-points.



	FAM	PRE	8 weeks	16 weeks	POST
Morphology	✓			✓	✓
Passive tests ankle	Fam.	✓	✓	✓	✓
Passive tests hamstrings		✓	✓	✓	✓
Tendon stiffness	Fam.	✓		✓	✓
Contractile function	Fam.	✓			✓

Figure 3. *Study III: Time course of familiarization (FAM), tests and stretching intervention. "Fam." indicates trials performed without collecting data. For legibility, the time axis is scaled differently for the pre-intervention (PRE), intervention and post-intervention (POST) phases.*

4.2. Ethics

All studies were conducted according to Good Clinical Practice and the Declaration of Helsinki, using standard procedures that have been used routinely in clinical or research settings at the Norwegian School of Sport Sciences and at Bispebjerg University Hospital. The protocols, written information and consent forms were submitted to the regional committee for medical and health research ethics for approval. The regional committee approved study III, and concluded that studies I-II were outside the mandate of the committee (Appendix I); hence, studies I-II were conducted according to Norwegian law.

In study I, some subjects were between 16 and 18 years of age. The particularly strict ethical demands related to this age group were considered fulfilled, as the study was non-invasive and imposed minimal risk. For all subjects aged 16-17, informed consent forms were signed by the subject and her parent(s).

During data collection and analysis, each subject was represented solely by an identification code. Lists tying codes to individuals were locked in a safe and destructed at the end of data analyses.

Stretching training is relatively risk free. Subjects were given instruction and supervision to ensure correct, safe execution. The endpoint of each flexibility test was determined by the subject through a verbal stop signal. Additionally, the subject could terminate the test immediately by pressing a hand-held stop-button, upon experiencing any excessive discomfort. Stretching of only one leg may cause side-to-side differences, which theoretically may have minor effects on

function or performance. In order to limit side-to-side differences to a minimum, subjects with large pre-intervention (>10°) side-to-side differences stretched their least flexible side.

The muscle biopsy procedure is painful, and as any invasive procedure, involves risks related to bleeding and infections. The procedures were performed and followed up by qualified and experienced personnel. Our laboratory has performed more than 300 biopsies and has experienced very few complications. Biopsies were taken from a subgroup of study III subjects. The subjects were informed that they would be able to participate in the main study regardless of their decision to volunteer for, or subsequently withdraw from, the biopsy procedure.

Altogether, the risks applied to each subject were deemed significantly less than the potential benefits for society; increased scientific understanding of adaptations to long-term stretching, in turn potentially leading to better qualified prescription (or avoidance thereof) of stretching training for health, function, performance and/or rehabilitation.

4.3. Subjects

4.3.1. Power analyses

Study I: A priori sample size calculations on differences in angle of peak torque from a similar cross-sectional study (Alonso et al., 2009), with statistical power of 80 %, indicated that 38 subjects were required. Accordingly, 22 rhythmic gymnasts and 16 controls were recruited.

Study II: Since the population of professional ballet dancers – i.e. dancers who do ballet training and performances as their fulltime job – is very restricted in numbers, inclusion was based on availability for testing in Oslo, rather than on power analyses.

Study III: A priori sample size calculations on PRE-POST changes in passive torque of 3 ± 3 Nm, based on former stretching intervention studies (Kubo et al., 2002; Mahieu et al., 2007), with statistical power of 90 % and an anticipated dropout of 25 % lead to recruiting of 30 subjects (12 men, 18 women) amongst recreationally active university students.

4.3.2. Inclusion and exclusion criteria

In all studies, lower-limb injuries within the past 6 months, musculo-skeletal diseases or any condition preventing stretching of the targeted MTUs (e.g. limited talus posterior glide) were exclusion criteria. Age constraints were 16-22 (study I), 18-38 (study II) and 18-40 (study III).

Study I, rhythmic gymnasts: Subjects needed to compete for their national federation at the 2010 Nordic championships, and to have undertaken systematic stretching for minimum 5 years.

Study II, ballet dancers: Subjects needed to do ballet training and performances as their fulltime job, either at the Norwegian National ballet or in a company with similar standards, and to have undertaken systematic stretching for minimum 10 years.

Studies I-II control subjects, and study III all subjects: Volunteers were excluded if they had a history of systematic stretching (stretching > 10 minutes once a week or more frequently, and/or shorter sessions > 3 times a week).

4.3.3. Recruitment

Study I: Rhythmic gymnasts were recruited among participants at the 2010 Nordic championships, by prior distribution of a written invitation through the participating gymnastics federations. Controls were recruited by distribution of written invitations at trainings of local sports teams. Volunteers were approached one-on-one upon reporting their interest in writing.

Study II: Ballet dancers from the Norwegian National Ballet were recruited by oral information given by the ballet administration. Dancers that reported their interest received further written information. Additional ballet dancers were recruited by sending brief written information directly. Dancers that reported their interest received further written information. Controls were obtained by matching the ballet dancers to the PRE data of female subjects of study III.

Study III: Subjects were recruited among sports biology students at the Norwegian School of Sport Sciences and physiotherapy students at the Oslo Metropolitan University, by use of posters and by presenting the study during classes. Volunteers were approached one-on-one upon reporting their interest in writing.

Paper IV: Among study III subjects, 16 volunteered to contribute muscle samples and have MRI scans taken, of which 14 were drawn by a random number generator to undergo the procedures.

4.3.4. Descriptive data

Paper III: During the intervention, one subject withdrew due to an injury suffered outside of the intervention, three subjects withdrew due to insufficient time or motivation to follow the training program; the final data set for paper III comprised 26 subjects.

Paper IV: One subject drawn for muscle biopsy procedures withdrew from the main study, and three samples were inadequate for analysis; the final data set for paper IV comprised ten subjects.

Descriptive data for the subjects upon which papers I-IV report are given in Table 1. Description of the training background, current training and stretching volume of the subjects upon which papers I-II report is given in Table 2.

Table 1. *Descriptive data for subjects that completed studies I-III. * indicates a difference from controls (P < 0.05).*

Paper	Group	n	Gender	Age (years)	Height (cm)	Body mass (kg)
I	Elite rhythmic gymnasts	22	Female	17 ± 2	166 ± 5	53 ± 6 *
	Controls	16	Female	19 ± 2	166 ± 7	61 ± 5
II	Professional ballet dancers	10	Female	30 ± 4 *	168 ± 6	61 ± 7
	Controls	10	Female	21 ± 1	168 ± 7	60 ± 7
III	Each subject participated with one stretching + one control leg	26	17 females 9 males	22 ± 2	169 ± 7 184 ± 5	61 ± 11 80 ± 12
IV	Each subject participated with one stretching + one control leg	10	5 females 5 males	22 ± 2	171 ± 9 185 ± 6	62 ± 7 83 ± 11

Table 2. *Training status of the subjects that completed studies I-II. * indicates a difference from controls (P < 0.05).*

Paper	Group	Training history (years)	Weekly training (hours)	Weekly stretching (minutes)
I	Elite rhythmic gymnasts	10 ± 3	22 ± 8 *	236 ± 128 *
	Controls	12 ± 3	9 ± 6	9 ± 17
II	Professional ballet dancers	24 ± 5	19 ± 12 *	660 ± 900 *
	Controls	N/A	4 ± 3	0 ± 0

4.4. Stretching training intervention period (Study III)

The stretching training intervention consisted of self-administered static ankle dorsiflexion and hip flexion stretches (Figure 4). A detailed description of the stretches was handed out to the subjects (Appendix II). Self-administered stretches were chosen over e.g. passive stretches in an isokinetic dynamometer, to maximize compliance, minimize dropout rate and to increase the external validity of the findings.

Each stretch was held for 60 seconds and repeated 4 times. This regime was repeated daily for 24 weeks. The subjects were suggested to alternate ankle and hip flexion stretches, for a stretching session lasting 8-10 minutes, but were permitted to distribute the total volume across the day in order to implement the stretching in their daily habits.

Immediately prior to the intervention, subjects received one-on-one instructions and corrections. The stretching technique was controlled at each test session. The subjects were instructed that stretching should feel uncomfortable, but not painful, and to maintain this intensity throughout each stretch by slowly increasing the joint angle as the stretching sensation decreased. After 8 weeks, stretching was progressed such that two of four repetitions were performed with the knee of the stretching leg flexed to approximately 45°, to ensure continuous stretching load on the SOL. Adherence was monitored through written journals and phone follow-ups between the test sessions.

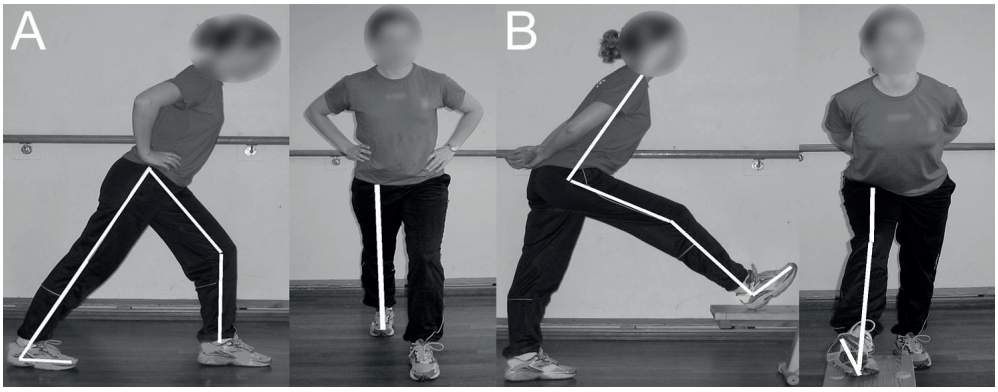


Figure 4. *Static stretching exercises. A) Ankle dorsiflexion. Subjects were instructed to place the stretching leg as far posteriorly as possible, while pushing the heel down to the ground, the forefoot pointing forward. B) Hip flexion. Subjects were instructed to place the stretching leg on an item with a height suitable to induce a solid stretch by leaning the trunk slightly forward, without curving the spine. The knee of the stretching leg should be slightly bent, to prevent hyperextension of the knee. The ankle joint should be in a relaxed position.*

4.5. Equipment, data collection and data processing

4.5.1. Anthropometry

The subjects' body mass and height were measured in all studies. In studies II-III, the standing leg length was measured from the most prominent point of trochanter major to the floor. The calf length was measured from the lateral femoral epicondyle to the posterior point of the calcaneal tuberosity.

4.5.2. Flexibility assessment

In study I, the flexibility of the hamstrings MTU and lower back was assessed using the sit and reach test (American College of Sports Medicine, 2010). The flexibility of the hip joints was

assessed using a gymnastics specific "splits test" (Figure 5). General joint laxity was assessed using the Beighton score (Beighton, Solomon, & Soskolne, 1973).

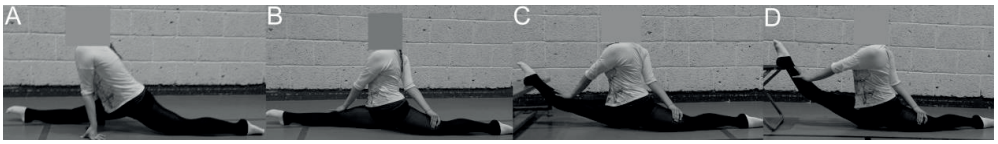


Figure 5. *The flexibility of the hip joints was estimated using a gymnastics specific "splits test". The right and left front splits were graded from 0 points (< 180° split, A) to 3 points (over-split from a 30 cm chair, D).*

In studies II-III, ankle dorsiflexion ROM was determined in an isokinetic dynamometer (HUMAC NORM 770, Computer Sports Medicine Inc., Stoughton, MA, USA). Subjects were seated with 65° hip flexion and the knee of the testing leg extended. Dynamometer settings were individually adjusted and unwanted joint movement was minimized by careful strapping of the limbs (Figure 6A). The foot was manually dorsiflexed to the maximally tolerated dorsiflexion angle (the endpoint of ROM), as signalled verbally by the subject. To prevent visual perception from influencing the determination of ROM, the test was taken with eyes closed.

In study III, flexibility of the hamstrings MTU was assessed by a passive knee extension test (Figure 6B) using the dynamometer and principles described for ankle dorsiflexion ROM. For the knee extension test, lower values signify greater ROM; 0° indicates shank vertically up.

Figure 6. *HUMAC NORM isokinetic dynamometer subject positioning: A) Setup for passive dorsiflexion and contractile function. B) Setup for passive knee extension.*

4.5.3. Pain

In studies II-III, the level of pain experienced at the maximal joint angle of passive dorsiflexion and knee extension was recorded on a 10 cm visual analogue scale (VAS score).

In study III, the level of pain experienced during self-administered stretching exercise during week 1, week 8, week 16 and week 24 was recorded as a VAS score.

4.5.4. Material properties

Intramuscular collagen content and tendon material properties were investigated in 14 of the subjects in study III. Intramuscular collagen content is reported in paper IV, tendon material properties is only reported in the present thesis.

4.5.4.1. Intramuscular collagen content

A SOL muscle biopsy was taken from the control leg at PRE and from the training leg at POST, in order to minimize risks for the subjects. None of the subjects participated in activities that presumably would yield contralateral differences (e.g. jumping, fencing and racket sports).

Muscle biopsies were collected under ultrasound guidance from the medial aspect of the SOL, just distal to the GM MTU. Muscle samples were collected under local anaesthesia with a 6 mm Bergström needle (Pelomi, Albertslund, Denmark), using manual suction, frozen in liquid nitrogen and stored at -80°C.

To quantify muscle collagen content, a collagen to hydroxyproline mass ratio of 7.5 was assumed (Neuman & Logan, 1950). The detailed procedures for specimen treatment are found in Paper III and are as previously reported (Svensson, Smith, Moyer, & Magnusson, 2018). Collagen content is reported as percent of dry weight

4.5.4.2. Tendon material properties

The 14 subjects from which muscle biopsies were taken also underwent magnetic resonance imaging (MRI). All scans were taken as 2D, T2-weighted under a magnetic field strength of 1.5T. Slice thickness was 3 mm with no inter-slice gaps. Sagittal and transversal scans of the lower leg were taken in a supine position with the ankle joint fixed at 0° (Figure 7A), and in right decubitus position with slightly flexed hips and knees and with the ankle joint in a resting position (Figure 7B). For the decubitus scans, an MRI phantom (100g H_2O dist : 1.25g $NiSO_4$ x $6H_2O$ + 5g NaCl, Siemens, 2000 ml) was placed next to the Achilles tendon, for signal intensity normalization.

Regions of interest within the free Achilles tendon were marked (OsiriX v.5.5.1, Pixmeo Sarl, Geneva, Switzerland), from the slice where a proximal part of the bone was visible without retro-calcaneal bursa, to the first slice containing SOL muscular tissue. Signal intensity was normalized to the phantom pixel intensity and averaged for every third of the tendon length (proximal,

middle, distal) after cubic spline interpolation (MATLAB and Statistics Toolbox Release 2015b, The MathWorks, Inc., Natick, Massachusetts, United States).

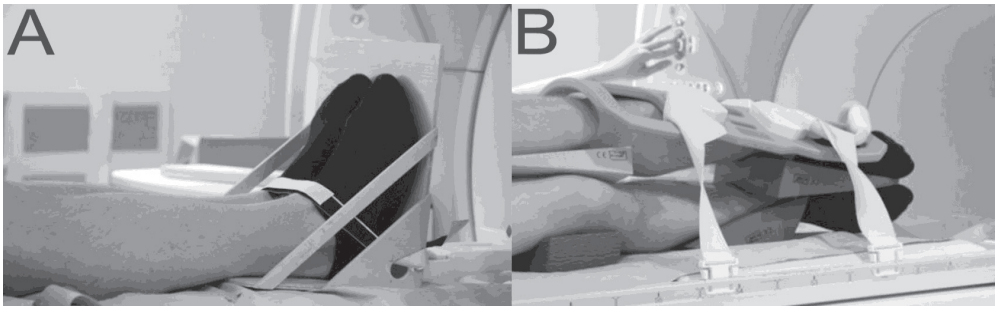


Figure 7. *Subject positioning during magnetic resonance imaging: A) Supine position, ankle joint at 0°. B) Decubitus position, ankle joint in resting position.*

4.5.5. Morphological properties

Resting muscle architecture of SOL and GM was recorded by ultrasonography in studies II-III. Achilles tendon length and CSA were recorded by ultrasonography in studies II-III. Due to data analysis challenges, SOL architecture and tendon CSA are not currently reported for study III. Free Achilles tendon length and CSA were measured by MRI on a subgroup of the study III subjects; these variables are only reported in the present thesis.

4.5.5.1. Muscle architecture by ultrasonography

Muscle architecture was recorded with subjects resting in prone position with the foot hanging freely off the examination bed, using real-time B-mode ultrasonography with a 50-mm linear array transducer (L12-5, Philips, Bothell, WA, USA) and ultrasound system (HD11XE, Philips, Bothell, WA, USA). Fascicle length, pennation angle and muscle thickness were measured from sagittal plane images at mid-length of the muscle belly (Figure 8A).

4.5.5.2. Tendon length by ultrasonography

The locations of the GM and SOL MTJs were identified by ultrasonography and marked on the skin surface. An adhesive scaffold with embedded echogenic wires was placed along the tendon trajectory (Figure 8B). Sagittal plane images were collected along the Achilles tendon from the calcaneal insertion to the GM MTJ. The images were sequentially combined by use of the echogenic markers (Figure 8C) and the lengths of the free and whole Achilles tendon were measured along the tendon path in the sagittal plane using Fiji ImageJ (Schindelin et al., 2012).

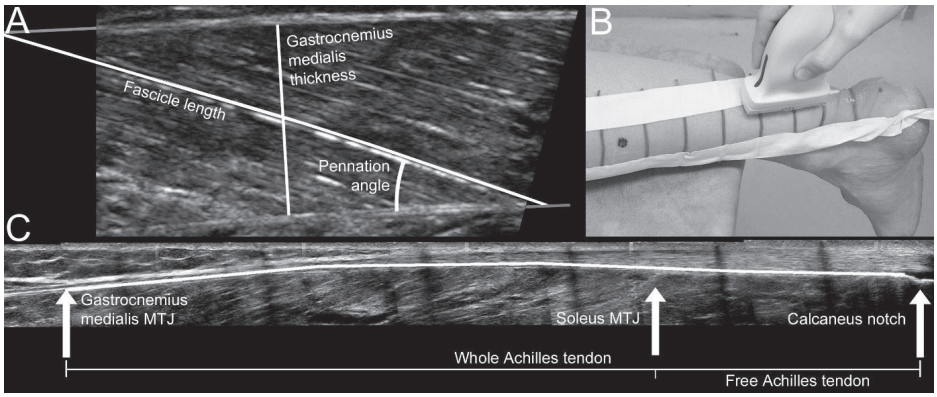


Figure 8. *A) Ultrasound image showing measurement of gastrocnemius medialis fascicle length, pennation angle and muscle thickness. B) Adhesive scaffold with embedded echogenic wires placed along the Achilles tendon, for length measurement. C) Sequentially combined ultrasound images, showing the length of the Achilles tendon from the calcaneal insertion to the soleus (free Achilles tendon) and gastrocnemius medialis (whole Achilles tendon) muscle-tendon junctions (MTJs).*

4.5.5.3. Tendon length and CSA by MRI

The procedures for obtaining MRI scans for calculation of tendon length and CSA are reported in 4.5.4.2. Free Achilles tendon length was calculated from the supine scans, by marking a point at the posterior medial part of the tendon across all slices from the calcaneal insertion to the SOL MTJ, and summing the three-dimensional point-to-point distances. Free Achilles tendon CSA was calculated from the supine scans, and was averaged for every third of the tendon length (proximal, middle, distal) after cubic spline interpolation.

4.5.6. Mechanical properties during passive stretching

Passive torque-angle properties were recorded in all studies: For the triceps surae MTU in studies II-III (papers II-III), for the hamstrings MTU in study I (paper I) and in study III (only reported in the present thesis). Papers II-III additionally report elongation and strain of the triceps surae MTU components based on ultrasonography videos recorded during passive stretching.

4.5.6.1. Position setup

In study I, passive resistance of the left hamstrings MTU was measured during slow, passive knee extension in an isokinetic dynamometer (TechnoGym REV 9000, Cesena, Italy). The subjects were seated with the hip joint 100° flexed, dynamometer settings were individually adjusted and unwanted joint movement was minimized by careful strapping of the limbs (Figure 9). In studies II-III, the position setups described for assessment of flexibility in 4.5.2 were used.

4.5.6.2. Data collection

In study I, the ability of each subject to tolerate full passive knee extension (0°) in the dynamometer was confirmed by slow, stepwise increments of knee extension. The knee was then extended and flexed between 93° and 0°, at 15°·s^{-1}. Subjects were instructed to close their eyes and to fully relax their muscles. Two valid trials were recorded.

Figure 9. *TechnoGym REV 9000 isokinetic dynamometer subject positioning for tests of passive knee extension and contractile function.*

In studies II-III, the ankle joint was dorsiflexed from 10° plantar flexion to maximal ROM and back, at 2°·s^{-1}. Subjects were instructed to close their eyes and to fully relax their muscles. The procedure was repeated to secure two valid trials with sagittal plane ultrasound videos of the distal SOL MTJ, two of the distal GM MTJ and two of GM mid-belly fascicles.

Torque, angular velocity and dynamometer angle were obtained from the isokinetic dynamometer. Ankle joint angle was concurrently obtained with a 2D electro-goniometer (Noraxon Inc., Scottsdale, AZ, USA), secured to the medial part of the 1st metatarsal and distal-medial part of the tibia. EMG amplitudes were recorded from SOL, GM and gastrocnemius lateralis (GL) muscles. A function generator (GwinStec, GFG-8215A, Good Will Instrument Co., Ltd, Tucheng City, Taiwan) and an electric trigger signal were used to initiate sampling and synchronize data by producing a visual marker on the ultrasound videos.

In study III, the knee joint was passively extended from a resting position to maximal ROM and back. The procedures described for ankle dorsiflexion were applied, except that ultrasonography and electro-goniometer data were not collected; hence, only two repetitions were performed.

4.5.6.3. Analysis of passive torque-angle properties

Post-processing was performed off-line (MATLAB and Statistics Toolbox Release 2015b, The MathWorks, Inc., Natick, Massachusetts, United States). Torque, dynamometer angle and goniometer angle were filtered using a bidirectional zero-lag second-order (study I) or fourth-order (studies II-III) Butterworth low-pass filter of 10 Hz (2 Hz for study I passive torque), resampled to the ultrasound video frequency (studies II-III) and interpolated at 0.25° (study I) or 0.05° (studies II-III) intervals using a spline function, before valid trials were averaged.

For study I passive knee extension, torque was gravity-corrected by assuming zero resistance to stretch from 75 to 65°; trigonometric functions were applied to the torque in this range to estimate the weight of the leg across the range of joint excursion. For passive dorsiflexion in studies II-III, gravity correction was not considered necessary due to the relatively low mass of the foot and the minor moment arms when the foot is near vertical. For the passive knee extension in study III, the built-in gravity correction of the dynamometer was applied.

In studies II-III, ankle joint angles during passive dorsiflexion were obtained from the electro-goniometer, rather than from the dynamometer, to avoid error induced by misalignment of the foot and dynamometer near maximal dorsiflexion angle. Goniometer angles hence differ from the dynamometer measurement of ROM.

Passive torque was extracted at the following joint angles:

- Study I: At 5° intervals between 80° and 5° knee joint angle.
- Study II: At anatomically neutral ankle joint angle (0°) ("anatomical joint angle"), at the maximal ankle dorsiflexion angle that was common to all subjects ("common joint angle") and at each subject's maximal dorsiflexion angle ("maximal joint angle").
- Study III: At anatomical joint angle, at the maximal ankle dorsiflexion angle that was common to each leg across time-points ("standardized joint angle") and at maximal joint angle at the given time-point.

Passive joint stiffness was calculated as the slope of a fourth-order polynomial fit of the passive torque-angle relation (Magnusson, Simonsen, Aagaard, & Kjaer, 1996) at the following angles:

- Study I: At 15, 10 and 5° knee joint angle.
- Study II: At common joint angle and at maximal joint angle.
- Study III: At standardized joint angle and at maximal joint angle.

4.5.6.4. Passive elongation of MTU components

MTU length across goniometer joint angles was estimated using equations for normalized GM MTU elongation (Grieve, Pheasant, & Cavanagh, 1978) in study II, and for normalized GM and SOL MTU elongation (Hawkins & Hull, 1990) in study III.

In studies II-III, GM fascicle lengths and pennation angles were measured by automatic tracking using optical flow algorithms (Cronin, Carty, Barrett, & Lichtwark, 2011; Gillett, Barrett, & Lichtwark, 2013). Elongation and strain of fascicles are reported based on fascicle resting length.

In study II, length changes of the GM muscle and the series elastic element were estimated by combining fascicle length and pennation angle from the ultrasound images with MTU length

(Fukunaga et al., 2001). Elongation of muscle and series elastic element are reported based on resting lengths and joint angles. Muscle and series elastic element elongation are reported as absolute values and as percent contribution to total MTU elongation.

In study III, length changes of the SOL and GM muscles and tendon were based on MTJ displacement. Displacement of the MTJs was measured by semi-automated tracking (Tracker 4.11.0, Open Source Physics, Aptos, California, USA). Elongation of any structure proximally to the MTJ is referred to as muscle elongation. Elongation occurring distally to the MTJ is referred to as tendon elongation. Muscle and tendon elongation are reported based on lengths at anatomical joint angle. Muscle and tendon elongation are reported as absolute values and as percent contribution to total MTU elongation.

4.5.7. Tendon mechanical properties

In studies II-III, the tensile stiffness, elongation and strain of the free Achilles tendon and the whole Achilles tendon were examined by measuring tendon elongation during isometric ramp contractions, as previously described (Bojsen-Moller et al., 2004). In brief, the ultrasound probe was placed sagittally over the distal MTJ of SOL and subsequently GM, and fixed to the leg using a custom-made rigid cast. The probe was fixed using elastic straps to maintain consistent positioning while minimizing compression. Echo-absorptive tape was applied to the skin to allow post-processing corrections for potential probe displacement relative to the skin.

4.5.7.1. Position setup

Subjects were seated in a custom-made isometric ankle dynamometer (Gym2000, Geithus, Norway) instrumented with a load cell (U2A 500 Hottinger Baldwin Messtechnik, Darmstadt, Germany), with 90° hip flexion, with the testing knee straight and the testing foot strapped to the dynamometer at anatomically neutral ankle joint angle (0°). Dynamometer settings were individually adjusted and unwanted joint movement was minimized by careful strapping of the limbs (Figure 10).

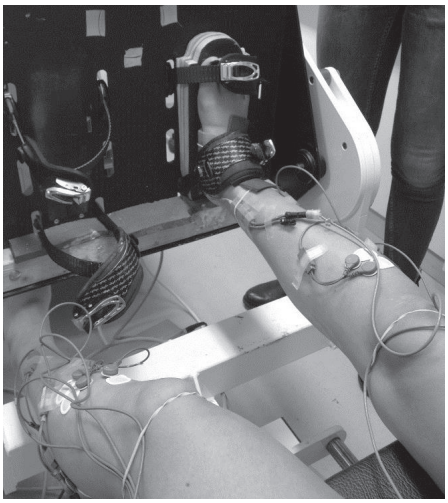


Figure 10. *Isometric dynamometer subject positioning for ankle joint maximal voluntary contractions and ramped isometric contractions.*

4.5.7.2. Data collection

Ramped plantar flexion contractions were carried out at a constant rate of torque development. Target and real-time rate of torque development were displayed, and standardized verbal encouragements were provided. Three valid trials were recorded for each of SOL and GM MTJ.

4.5.7.3. Post-processing

Data were collected and post-processed following the procedures described in 4.5.6. for passive stretching. Plantar flexion torque was calculated by multiplying the load cell force by the perpendicular distance to the axis of joint rotation. Tendon force was calculated by dividing plantar flexion torque by the instantaneous tendon moment arm as derived from ankle joint angle and leg length (Spoor, van Leeuwen, Meskers, Titulaer, & Huson, 1990).

Displacement of the MTJs was interpolated at 50 N intervals using a spline function and the valid trials were averaged. Tendon elongation was defined as proximal displacement of the MTJ, corrected for the influence of ankle joint rotation (Arampatzis, Monte, & Karamanidis, 2008).

Tendon stiffness is reported for the free and the whole Achilles tendon. The individual tendon force and tendon elongation data were cut off at 90 % of maximal force level and fitted with second-order polynomials. Tendon stiffness was determined as the slope of the curve between 80 and 100 % of individual maximal force (studies II-III) and of common force, defined as the greatest force that was achieved by all legs (study II). Tendon elongation and strain are reported at common force and as individual maximum.

4.5.8. EMG amplitudes during passive dorsiflexion

In studies II-III, EMG amplitudes of SOL, GM and GL were recorded during the passive dorsiflexion described in 4.5.6.

4.5.8.1. Preparation and data collection

Standard preparation and placement of EMG electrodes (Ambu, Blue Sensor N, Ballerup, Denmark) on SOL, GM, GL and TA followed SENIAM recommendations (Hermens, Freriks, Disselhorst-Klug, & Rau, 2000). EMG signals were transmitted wirelessly (16-channel TeleMyo 2400 G2 Telemetry System, Noraxon Inc., Scottsdale, AZ, USA) to a receiver (Mini-receiver for TeleMyo G2, Noraxon Inc., Scottsdale, AZ, USA) and synchronized with other data (MyoResearch XP Master Edition 1.08.17, Noraxon Inc., Scottsdale, AZ, USA) as part of the procedures described for passive dorsiflexion in 4.5.6.

4.5.8.2. Post-processing

EMG data were filtered using a bidirectional zero-lag fourth-order Butterworth bandpass filter of 10-500 Hz, rectified and integrated over 500 ms. EMG amplitudes were normalized to amplitudes recorded during (MVC), averaged across trials and extracted at:

- Study II: Anatomical joint angle, common joint angle and peak EMG amplitude.
- Study III: Anatomical, standardized and maximal joint angle.

4.5.9. Contractile function

Isokinetic knee flexion and extension torque-angle properties were investigated in study I. Isometric and isokinetic plantar flexion torque-angle and -velocity properties were investigated in studies II-III. Isokinetic dorsiflexion torque-angle properties were investigated in study III.

4.5.9.1. Isometric plantar flexion

Isometric plantar flexion strength was tested using the same isokinetic dynamometer, position setup, data recording and post-processing as described for passive dorsiflexion in 4.5.6, except that reported angles represent the dynamometer and that data were resampled to 200 Hz.

Isometric maximal plantar flexion torque was determined at 10° plantar flexion, 0°, 5°, 10° and 15° of dorsiflexion. Subjects with ankle dorsiflexion ROM lower than 10 or 15° did not perform the trials at these angles. Standardized verbal encouragements and visual feedback of the instantaneous torque produced were provided during testing.

4.5.9.2. Isokinetic torque-angle properties

In studies II-III, isokinetic plantar flexion torque production was tested and processed following the procedures described for isometric plantar flexion. An overview of the testing protocol is given in Figure 11. Isokinetic concentric plantar flexion peak torque, angle of peak torque and work were determined between 10° of dorsiflexion and 30° of plantar flexion, at each of $30°\cdot s^{-1}$, $45°\cdot s^{-1}$, $60°\cdot s^{-1}$ and $90°\cdot s^{-1}$. In study III, isokinetic concentric dorsiflexion torque, angle of peak torque and work were determined at $30°\cdot s^{-1}$. Standardized verbal encouragements and visual feedback of the instantaneous torque produced were provided during testing.

For statistical analysis of the isokinetic torque-angle relations, torque was interpolated at 2.5° intervals. Positive work was calculated as the area below the torque-angle curve, after interpolating torque at 0.1° intervals.

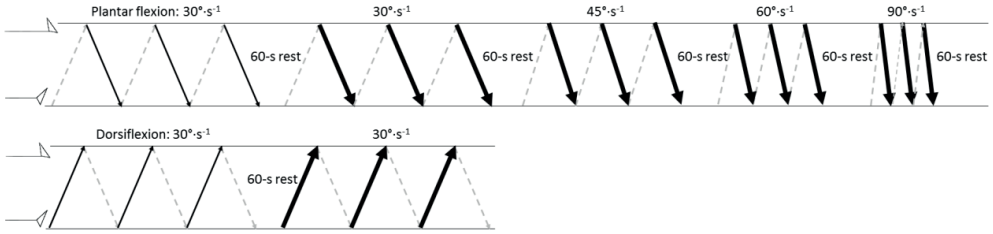


Figure 11. *Protocol for isokinetic concentric torque production tests (studies II-III). Thin black lines represent sub-maximal warm-up repetitions. Thick black lines represent maximal test repetitions. Grey dashed lines represent motion back to starting position. Each repetition/motion was voluntarily initiated. The second line (dorsiflexion) applies only to study III.*

In study I, isokinetic knee flexion and extension torque production was tested using the same isokinetic dynamometer, position setup and post-processing as described for passive knee extension in 4.5.6.

Isokinetic concentric knee extension and knee flexion peak torque and angle of peak torque were determined between 93° and 0°, at 60°·s^{-1}. Standardized verbal encouragements and visual feedback of the instantaneous torque produced were provided during testing.

The torque measured during the knee flexion and extension trials was defined as total torque. The torque obtained by subtracting the passive resistance to stretch was defined as corrected torque. For statistical analysis of the isokinetic torque-angle relations, torque was interpolated at 5° intervals. Work was calculated as the area below the torque-angle curve, between 2.5 and 92.5° of joint excursion. The range of joint excursion (°) during which corrected torque production was above 70 % of peak torque, representing the functional ROM, was determined.

4.6. Statistical analyses

In study I, the inter-group differences in subject morphology, training variables, flexibility variables and peak torque were analysed using unpaired, two-tailed Student's t-tests. Differences in passive and active torque-angle relations were analysed using two-way (group x joint angle) mixed model ANOVA. Post hoc, Bonferroni's multiple comparisons tests were performed. Pearson correlation coefficients were calculated to determine the relations between angle of peak torque and passive torque, stiffness variables, and sit and reach.

In study II, some of the passive torque, strain and tendon stiffness variables did not pass the normality test (D'Agostino & Pearson omnibus). Hence, all inter-group differences relating to passive stretch and tendon stiffness were analysed using Mann-Whitney U tests. The inter-group differences relating to descriptive data, anthropometry, ROM, resting MTU properties and

contractile function were analysed using unpaired, two-tailed Student's t-tests. Differences in passive and active torque-angle relations were analysed using two-way (group x joint angle) mixed model ANOVA. Post hoc, Sidak's multiple comparisons tests were performed, using multiplicity-adjusted *P*-values.

In study III, resting length of the free Achilles tendon, EMG amplitudes and VAS scores for pain during passive stretching tests did not pass the normality test (D'Agostino & Pearson omnibus), and were hence log-transformed to normality. Baseline characteristics and intramuscular collagen content were analysed using paired, two-tailed Student's t-tests. One-way ANOVA for repeated measures with the Geissler-Greenhouse correction was used to identify changes in VAS scores for pain during self-administered stretching exercise. Post hoc, Tukey's multiple comparisons tests were performed, using multiplicity-adjusted *P*-values. Two-way (leg x time) ANOVA for repeated measures was used to identify between-group differences. Two-way (time x joint angle) ANOVA for repeated measures was used to identify differences in isokinetic torque-angle relations. Post hoc, Sidak's multiple comparisons tests were performed, using multiplicity-adjusted *P*-values.

In all studies, the level of significance was set to $\alpha = 0.05$. All data are presented as mean ± SD.

5. Results and discussion

The present section addresses each of the presented hypotheses by underlining the main results from each of the three present studies and discussing them in light of previous studies. The final summary discusses the interplay between the presented variables and their interpretations.

5.1. Range of motion

Studies I-II: All flexibility tests indicated greater hip joint flexibility of the gymnasts compared to controls (study I) and greater ankle dorsiflexion ROM in the ballet dancers compared to controls (study II, Figure 12A).

Study III: Stretching for 24 weeks increased ankle dorsiflexion ROM by 12° (Figure 12B) and knee extension ROM by 10° (Figure 12C) in the stretching leg. Note that for knee extension, lower values signify increased ROM; 0° signifies shank vertically up. The control leg dorsiflexion ROM increased by 4° (Figure 12B), while knee extension ROM was unchanged (Figure 12C). Self-perceived pain during self-administered stretching exercise was reduced for dorsiflexion (Figure 13A) and hamstrings (Figure 13B) stretching exercises.

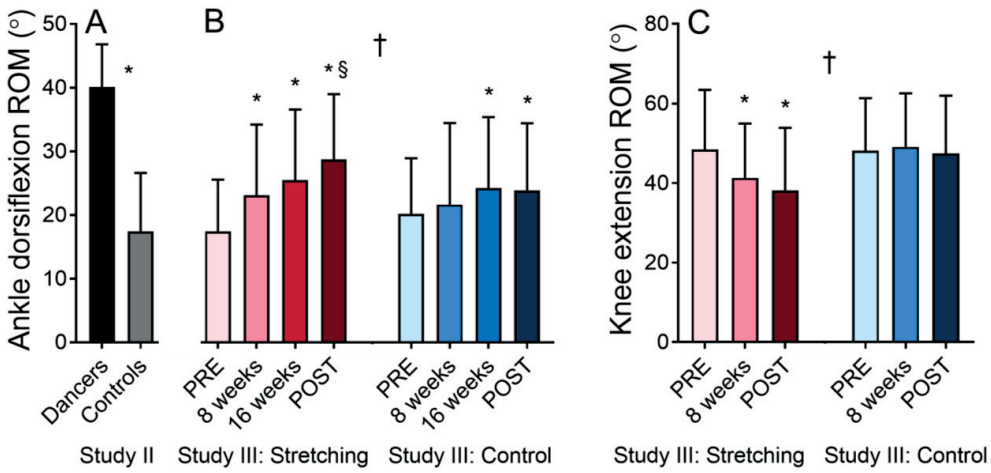


Figure 12. Range of motion (ROM). *A) Ankle dorsiflexion ROM in ballet dancers vs. controls, B) ankle dorsiflexion ROM before (PRE), during and after (POST) 24 weeks of stretching, C) passive knee extension ROM before, during and after 24 weeks of stretching. Note that for knee extension, lower values signify increased ROM; 0° signifies shank vertically up. † represents two-way ANOVA (leg x time) and indicates an interaction effect (P < 0.01), * indicates a difference between dancers and controls in study II (P < 0.01) and a difference from PRE in study III (P < 0.01), § indicates a difference from 8 weeks in study III (P < 0.01).*

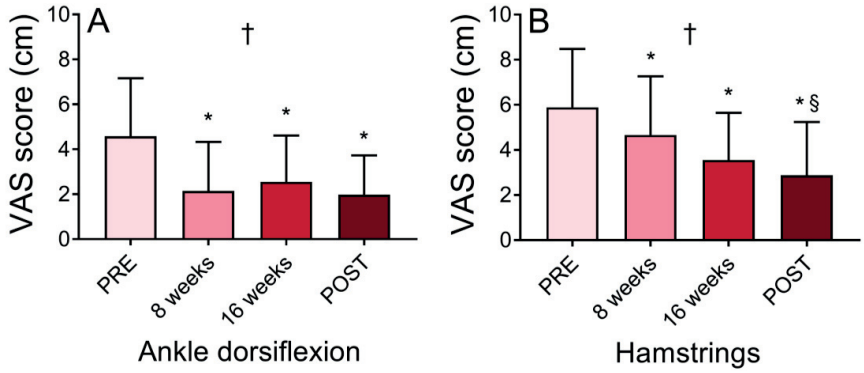


Figure 13. Self-perceived pain during self-administered stretching exercises. *A) Ankle dorsiflexion and B) hamstrings stretching exercises before (PRE), during and after (POST) 24 weeks of stretching. † represents one-way ANOVA and indicates an intervention effect ($P < 0.01$), * indicates a difference from PRE ($P < 0.01$), § indicates a difference from 8 weeks ($P < 0.01$).*

The increases in ROM are similar or greater than reported in previous ankle dorsiflexion intervention studies (Guissard & Duchateau, 2004; Akagi & Takahashi, 2014; Blazevich et al., 2014; Nakamura et al., 2017), and similar to increases reported for hip flexion/knee extension (Bandy, Irion, & Briggler, 1997; Ben & Harvey, 2010; Cipriani, Terry, Haines, Tabibnia, & Lyssanova, 2012; Halbertsma & Goeken, 1994; Marshall et al., 2011).

Stretching for 24 weeks did not increase dorsiflexion ROM to a level comparable to the dancers, indicating that the intervention population as a whole may have further capacity for increasing ROM. Self-perceived pain during stretching was reduced from PRE to 8 weeks, with no change from 8 weeks to POST. The concomitant increase in ROM from 8 weeks to POST suggests that stretching training is effective despite reduced sensation of stretch. From 16 weeks to POST, the lack of significant changes in ROM may be due to a diminished effect of stretching. Possibly, the most flexible subjects approached the limitations imposed by the ankle joint anatomy.

Knee extension ROM did not change between 8 weeks and POST (no measurement taken at 16 weeks), although the hip and knee joint structures did not limit join excursion in this test and the subjects reported greater sensations of stretch than for ankle dorsiflexion. The inability to detect significant changes in ROM after 8 weeks may be related to large variation, possibly due to methodological challenges of standardizing subject positioning for the knee extension test.

The present design cannot ascertain that the measurement of increased ROM in the control leg is due to the intervention, rather than systematic error. In general, changes in the control leg are a threat to the internal validity of the findings. However, for ROM, an interaction effect was found, such that the greater increase in the stretching leg compared to the control is likely due to the

intervention. Furthermore, measurement of ROM offers little opportunity for systematic error, and the contralateral increase in ROM is consistent with a number of other findings. The potential contralateral effects of stretching training are discussed in section 5.7.

ROM was increased using self-administered stretching, and a volume and frequency that is feasible in adult or athletic populations, securing strong external validity of these findings.

In summary, hypothesis 1 was confirmed: ROM was greater in populations with years of stretching training compared to controls, and ankle dorsiflexion and knee extension ROM was increased already after 8 weeks of stretching. Further research should clarify whether the flexibility of the hamstrings MTU may be increased with durations greater than 8 weeks.

5.2. Morphological and material properties

Study II: Ballet dancers displayed longer GM fascicles, shorter SOL fascicles and a longer whole Achilles tendon at rest, compared to controls (Table 3).

Study III: Stretching for 24 weeks did not induce any changes in GM fascicle or tendon length at rest, as recorded by ultrasonography (Table **4**), in free Achilles tendon length, CSA or signal intensity as measured from MRI scans (Table 5), nor in intramuscular collagen content (PRE 1.4 ± 0.5 %, POST 1.1 ± 0.4 %, $P = 0.17$) as measured from muscle samples.

Table 3. *Morphological properties of gastrocnemius medialis (GM), soleus (SOL) and the Achilles tendon (AT) in ballet dancers compared to controls (study II).*

	Dancers	Controls	*P*
SOL fascicle length (mm)	37 ± 4	42 ± 5	< 0.05
GM fascicle length (mm)	55 ± 5	47 ± 6	< 0.01
Free AT length (mm)	65 ± 28	51 ± 19	0.22
Whole AT length (mm)	207 ± 33	167 ± 10	< 0.01

Table 4. *Morphological properties of gastrocnemius medialis (GM) and the Achilles tendon (AT), as recorded by ultrasonography, before (PRE) and after (POST) 24 weeks of stretching (study III).*

		PRE	POST	*P* inter.	*P* time	Post hoc *P*
GM fascicle length (mm)	Stretching	54 ± 6	54 ± 6	0.52	0.48	0.57
	Control	54 ± 8	54 ± 7			1.00
Free AT length (mm)	Stretching	55 ± 20	58 ± 21	0.34	0.47	0.42
	Control	53 ± 19	54 ± 21			0.98
Whole AT length (mm)	Stretching	179 ± 22	184 ± 22	0.19	0.18	0.13
	Control	182 ± 22	182 ± 22			1.00

Table 5. *Morphological properties of the free Achilles tendon (AT), as measured from magnetic resonance imaging scans, before (PRE) and after (POST) 24 weeks of stretching (study III).*

		PRE	POST	*P* inter.	*P* time	Post hoc *P*
Free AT length (mm)	Stretching	59 ± 17	60 ± 17	0.22	0.21	0.17
	Control	62 ± 14	62 ± 14			0.99
Free AT cross-sectional area (mm^2)						
Proximal third	Stretching	0.49 ± 0.10	0.48 ± 0.09	0.98	0.08	0.35
	Control	0.47 ± 0.07	0.46 ± 0.07			0.37
Middle third	Stretching	0.49 ± 0.10	0.49 ± 0.10	0.15	0.25	0.97
	Control	0.50 ± 0.10	0.48 ± 0.09			0.14
Distal third	Stretching	0.63 ± 0.12	0.63 ± 0.12	0.30	0.24	0.99
	Control	0.64 ± 0.13	0.63 ± 0.13			0.25
Free AT signal intensity						
Proximal third	Stretching	0.017 ± 0.005	0.017 ± 0.004	0.33	0.36	1.00
	Control	0.017 ± 0.006	0.015 ± 0.004			0.34
Middle third	Stretching	0.016 ± 0.004	0.018 ± 0.005	0.37	0.47	0.44
	Control	0.015 ± 0.005	0.015 ± 0.005			0.99
Distal third	Stretching	0.010 ± 0.004	0.011 ± 0.006	0.49	0.08	0.68
	Control	0.011 ± 0.006	0.013 ± 0.006			0.17

Tendon material properties or intramuscular collagen content do not appear to be reported in previous cross-sectional or stretching intervention studies. However, stretching for 6 weeks did not change Achilles tendon length or thickness (Simpson et al., 2017), while increased GM fascicle length is demonstrated in some (Pradines et al., 2016; Simpson et al., 2017) but not all (Blazevich et al., 2014; e Lima et al., 2015; Konrad & Tilp, 2014b; Konrad et al., 2015; Nakamura et al., 2012) intervention studies. Potentially, the increased fascicle length may be explained by additional modes of rehabilitation (Pradines et al., 2016), instrumented stretching or protein supplementation (Simpson et al., 2017).

The greater fascicle length and whole Achilles tendon in ballet dancers compared to controls must be handled with caution. The study design does not allow concluding that the dancers' years of stretching have induced adaptations on the sarcomere level or increase tendon length. Firstly, eventual adaptations may have been due to other modes of ballet training, rather than stretching. Secondly, longer fascicles and tendons in ballet dancers may be a consequence of natural selection. The comparison to fascicle lengths in the intervention study, although representing both sexes including taller men, indicates that dancer fascicles are not exceptionally long, however, dancer tendons are longer than observed in the intervention study. However, the lack

of changes in fascicle and tendon length with 24 weeks of stretching suggests that ROM is increased through means other than addition of serial sarcomeres or increased tendon length.

The lack of changes in Achilles tendon signal intensity, which represents tendon material properties, was expected, given that tendon adaptations appear to require tendon strains above a certain threshold (Arampatzis et al., 2007). While intramuscular collagen content is scarcely researched in human intervention studies, reduced amount was expected based on a recent animal study (Peviani et al., 2018), but a reduction was not found.

In summary, hypothesis 5 was partially confirmed: Although GM fascicles were longer in ballet dancers compared to controls, the cross-sectional design limits the interpretations of this finding. Hence, this thesis did not provide evidence of increased number of serial sarcomeres, fascicle length or tendon length, nor altered tendon material properties or intramuscular collagen content, with human stretching training. However, other imaging techniques or variables from invasive procedures may be able to point out morphological changes contributing to increased ROM.

5.3. EMG amplitude during passive stretching

Study II: Ballet dancers displayed lower triceps surae EMG amplitudes compared to controls during passive dorsiflexion, at common joint angle (5°) and at peak EMG (Figure 14A).

Study III: There was a time effect of stretching for 24 weeks on triceps surae EMG amplitudes at standardized joint angle, with reduced amplitude in both legs ($P < 0.01$-0.05), while EMG amplitudes at anatomical joint angle and at maximal joint angle (comparable to peak EMG amplitudes) were unchanged (Figure 14B-C).

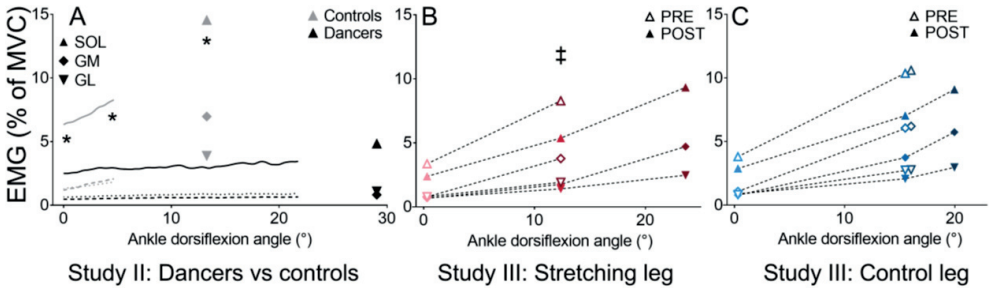


Figure 14. Electromyographic (EMG) amplitudes during passive dorsiflexion. *A) Ballet dancers vs. controls, continuous lines represent group mean up to group common joint angle, symbols represent mean peak EMG, B) stretching leg and C) control leg before (PRE) and after (POST) 24 weeks of stretching, at anatomical, standardized and maximal joint angle. EMG amplitudes are normalized to amplitudes during maximal voluntary contraction (MVC). Error bars are left out for legibility. SOL, soleus; GM, gastrocnemius medialis; GL, gastrocnemius lateralis. * indicates a difference between dancers and controls at anatomical and common joint angle and peak EMG in study II ($P < 0.05$), ‡ represents two-way ANOVA (leg x time) and indicates a time effect at standardized joint angle in study III ($P < 0.01$). Statistical analyses do not differ between SOL, GM and GL.*

The finding of lower EMG amplitudes at common joint angle in ballet dancers compared to controls is partially in line with a study finding lower GM amplitude but similar SOL amplitude in flexible subjects compared to controls at a submaximal dorsiflexion angle of 30° (Abellaneda et al., 2009). The present finding of lower peak EMG in ballet dancers compared to controls contrasts a study showing no differences in peak EMG between flexible and less flexible subjects (Blazevich et al., 2012), despite group differences in ROM being comparable to the present group differences. The present finding of reduced EMG amplitudes at standardized joint angle following 24 weeks of stretching are also in contrast to a study reporting unchanged EMG amplitudes following 3 weeks of stretching (Magnusson et al., 1996) but appear consistent with reduced tonic reflex reported following 6 weeks of stretching (Guissard & Duchateau, 2004).

As with ROM, the changes in EMG amplitude of the control leg may be an indication of systematic error. The lack of an interaction effect for EMG amplitude indicates that either the intervention affected both legs similarly, or systematic error affected both legs similarly. However, further analyses of the EMG data normalised to MVC torque (not shown) do not show a systematic drift of this parameter, and the reduced EMG during passive dorsiflexion is in line with reduced passive torque, increasing the likelihood of an intervention effect.

The present findings indicate that although subjects are requested to relax completely during passive stretches, approaching the endpoint of ROM is associated with neural activation, most likely initiated by reflexes. The mechanisms for differences between dancers and controls and between PRE and POST cannot be explained by the present results. However, it may be speculated that stretching training has led to structural adaptations in the MTU, leading to less tension on the MTU structures and thereby reduced reflex amplitudes, potentially in parallel with neural adaptations involving pain. As this thesis did not identify any changes in morphological properties, reduced strains and reduced reflexes cannot presently be explained by increased length but theoretically, strains and reflexes may be influenced by altered mechanical properties.

The unchanged EMG amplitudes at the increased maximal joint angle with 24 weeks of stretching could indicate a role of EMG amplitude in the subjective determination of ROM. On the other hand, ballet dancers displayed lower peak EMG compared to controls. Most likely, variables other than EMG amplitude lead to the determination of ROM in the ballet dancers. It is possible that ballet dancers were approaching the limitations imposed by the ankle joint structure.

In summary, hypothesis 3 was confirmed: GM, GL and SOL EMG amplitudes at standardized joint angle of passive dorsiflexion were lower in ballet dancers compared to controls, and EMG amplitudes at standardized joint angle were reduced through 24 weeks of stretching.

5.4. Elongation of MTU components during passive stretching

Study II: Maximal GM fascicle elongation during passive dorsiflexion was greater in ballet dancers compared to controls (Figure 15A), with a maximal ballet dancer fascicle strain of 35 ± 8 %. Maximal muscle elongation was greater ($P < 0.01$) in dancers (21 ± 4 mm) compared to controls (14 ± 4 mm), while maximal series elastic element elongation was not significantly greater ($P = 0.09$) in dancers (25 ± 8 mm) compared to controls (19 ± 4 mm). Muscle accounted for maximally 63 % of the MTU elongation in dancers and 53 % in controls but the group difference did not reach significance ($P = 0.09$).

Study III: There was a time effect of stretching for 24 weeks on GM fascicle elongation during passive dorsiflexion, with increased elongation at maximal joint angle in both legs (Figure 15B). There was a time effect of stretching for 24 weeks on maximal fascicle strain ($P < 0.01$), with strain increasing from 15 ± 14 % to 25 ± 14 % in the stretching leg ($P < 0.01$) and from 14 ± 11 % to 25 ± 12 % in the control leg ($P < 0.01$). Increased maximal joint angle necessitates increased total MTU elongation, which was accomplished through increased tendon elongation in both legs and increased muscle elongation in the stretching leg, both for GM MTU (Figure 16A) and for SOL MTU (Figure 16B). The changes in MTU elongation at maximal joint angle constituted an increased contribution from tendon elongation, in both legs for the SOL MTU (Figure 16B), in the stretching leg for the GM MTU (Figure 16A), and a tendency ($P = 0.08$) towards an increased contribution from tendon elongation in the control leg GM MTU.

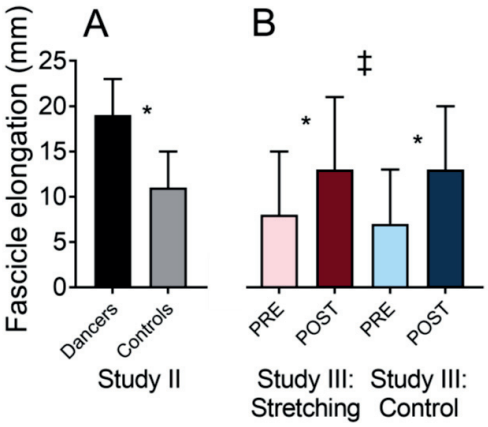


Figure 15. Gastrocnemius medialis fascicle elongation during passive dorsiflexion.
*A) Ballet dancers vs. controls, B) stretching and control leg before (PRE) and after (POST) 24 weeks of stretching. ‡ represents two-way ANOVA (leg x time) and indicates a time effect ($P < 0.01$), * indicates a difference between dancers and controls in study II ($P < 0.01$) and a difference between PRE and POST in study III ($P < 0.01$).*

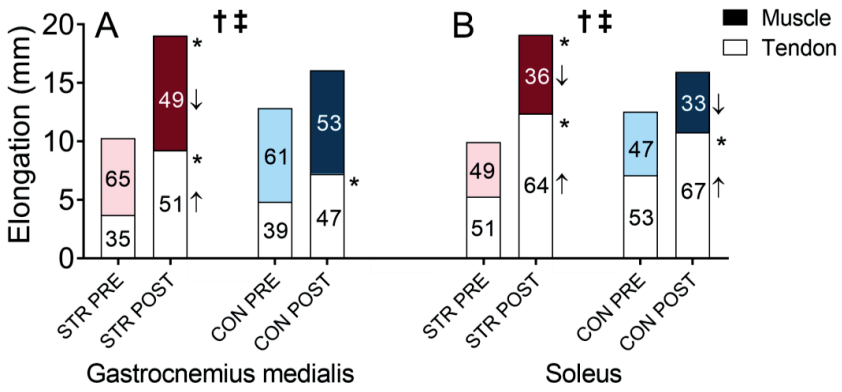


Figure 16. Maximal muscle and tendon elongation during passive dorsiflexion. *A) Gastrocnemius medialis and the whole Achilles tendon, B) soleus and the free Achilles tendon, in the stretching leg (STR) and control leg (CON), before (PRE) and after (POST) 24 weeks of stretching. Numbers on the bars represent percent contribution to total muscle-tendon unit elongation. † represents two-way ANOVA (leg × time) and indicates an interaction effect for elongation (P < 0.01), ‡ represents two-way ANOVA (leg × time) and indicates a time effect for elongation and contribution (P < 0.01-0.05), * indicates significant post hoc tests for elongation (P < 0.01), arrows indicate significant post hoc tests for contribution (P < 0.01).*

The greater elongation and strain of GM fascicles and greater elongation of muscle in ballet dancers compared to controls is in line with previous cross-sectional studies (Blazevich et al., 2012; Abellaneda et al., 2009). In a 3-week stretching intervention, muscle and fascicle strain increased while tendon strain was unchanged at maximal joint angle, and muscle rather than tendon accounted for the increased MTU elongation (Blazevich et al., 2014). The increased muscle and fascicle strain matches the present findings. However, the present study also found increased tendon elongation and increased contribution from tendon.

The 3-week study (Blazevich et al., 2014) increased ROM to a less extent than the present intervention and did not observe changes in passive torque at comparable joint angles, which may lay ground for differences in elongation of the MTU components. In study III, the smaller increase in control leg ROM compared to stretching leg ROM constitutes a less increase in total MTU elongation, potentially explaining why control leg muscle elongation did not increase significantly. Furthermore, with the present methods, muscle elongation may be slightly underestimated and tendon elongation overestimated due to slight stretching of the skin when approaching large joint angles. However, upon these results one may speculate that while unilateral stretching training may induce neural adaptations modulating ROM bilaterally, stretching training may also induce structural changes localized to the stretched muscle belly, possibly related to reduced connective tissue stiffness seen in stretched fibroblasts (Langevin et al., 2011).

The similar changes in both legs in study III may, again, be related to systematic error. However, the increased tendon elongation during passive dorsiflexion is matched by increased tendon elongation during ramped isometric contractions, both at common force level and at maximal elongation (Paper III). Structural adaptations increasing the toe strain limit of the Achilles tendon may be hypothesized to explain these findings.

In summary, hypothesis 4 was partially confirmed: Stretching for 24 weeks increased maximal elongation of muscle and tendon. Ballet dancers displayed greater elongation of muscle but not series elastic element compared to controls, however, the contribution from muscle elongation to MTU elongation was not clearly different. In contrast, 24 weeks of stretching increased the contribution from tendon elongation to GM and SOL MTU elongation.

5.5. Passive resistance to stretch

Study I: Torque during passive knee extension was lower in gymnasts compared to controls, from 30° to maximal joint angle (Figure 17A). Passive joint stiffness at 5° was lower ($P < 0.01$) in gymnasts (0.28 ± 0.12 $Nm \cdot {}^{o-1}$) compared to controls (0.74 ± 0.24 $Nm \cdot {}^{o-1}$).

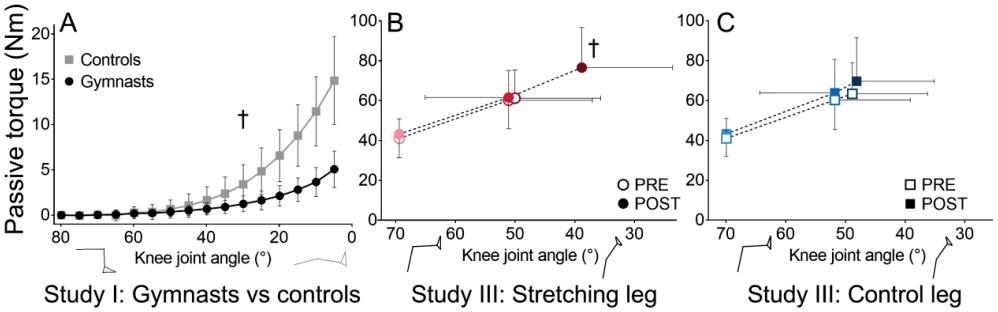


Figure 17. Passive torque during passive knee extension. *A) Gymnasts vs. controls, from onset to 5°, B) stretching leg and C) control leg before (PRE) and after (POST) 24 weeks of stretching, at 70°, standardized and maximal joint angle. The different testing positions of study I vs. study III are illustrated on the X axes. † represents two-way ANOVA and indicates an interaction effect (group x joint angle) in study II ($P < 0.01$) and an interaction effect (leg x time) at maximal joint angle in study III ($P < 0.05$).*

Study II: Torque during passive dorsiflexion was lower in ballet dancers compared to controls at anatomical and common joint angle but similar at maximal joint angle (Figure 18A). Passive joint stiffness was lower at common joint angle ($P < 0.01$) in dancers (0.6 ± 0.5 $Nm \cdot {}^{o-1}$) compared to controls (1.3 ± 0.5 $Nm \cdot {}^{o-1}$) but similar ($P = 0.54$) at maximal joint angle (dancers 2.9 ± 1.3 $Nm \cdot {}^{o-1}$, controls 2.5 ± 1.3 $Nm \cdot {}^{o-1}$).

Study III: There was a time effect of stretching for 24 weeks on ankle dorsiflexion passive torque at anatomical joint angle, with reduced torque in the stretching leg ($P < 0.01$), and at standardized joint angle, with reduced torque in both legs ($P < 0.01$), while passive torque at maximal joint angle increased in the stretching leg ($P < 0.01$, Figure 18B-C). There was also a time effect on passive joint stiffness at standardized joint angle (interaction $P = 0.63$, time $P < 0.01$), with reduced stiffness in both legs (stretching PRE 2.6 ± 1.3 Nm·°$^{-1}$, POST 1.7 ± 0.9 Nm·°$^{-1}$, $P < 0.01$, control PRE 2.7 ± 1.4 Nm·°$^{-1}$, POST 2.0 ± 1.2 Nm·°$^{-1}$, $P < 0.01$). On the other hand, stiffness at maximal joint angle was unchanged (interaction $P = 0.13$, time $P = 0.34$, stretching PRE 2.6 ± 1.3 Nm·°$^{-1}$, POST 3.1 ± 1.7 Nm·°$^{-1}$, control PRE 2.8 ± 1.5 Nm·°$^{-1}$, POST 2.6 ± 1.3 Nm·°$^{-1}$). Stretching for 24 weeks did not change knee extension passive torque at anatomical or standardized joint angle, while passive torque at maximal joint angle increased in both legs ($P < 0.01$-0.05, Figure 17B-C).

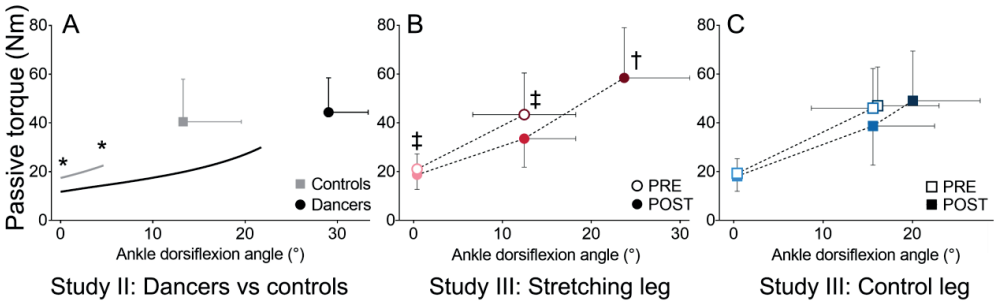


Figure 18. Passive torque during passive dorsiflexion. *A) Ballet dancers vs. controls, continuous lines represent group mean up to group common joint angle, symbols represent mean at maximal joint angle, B) stretching leg and C) control leg before (PRE) and after (POST) 24 weeks of stretching, at anatomical, standardized and maximal joint angle. * indicates a difference between dancers and controls at anatomical and common joint angle in study II ($P < 0.05$), † represents two-way ANOVA (leg × time) and indicates an interaction effect at maximal joint angle in study III ($P < 0.01$), ‡ represents two-way ANOVA (leg × time) and indicates a time effect at anatomical and standardized joint angle in study III ($P < 0.01$).*

Previous cross-sectional studies have demonstrated lower passive torque across joint angles (Magnusson et al., 1997) or lower passive torque at standardized joint angle (Blazevich et al., 2012; Abellaneda et al., 2009) in flexible compared to less flexible subjects, which is consistent with the lower passive torque seen in gymnasts and dancers compared to controls.

Studies of the effect of stretching training on passive torque are less consistent. A number of studies have increased ankle dorsiflexion, knee extension or hip flexion ROM without changing passive torque (Ben & Harvey, 2010; Law et al., 2009; Mahieu et al., 2009; Folpp et al., 2006; Magnusson et al., 1996; Konrad & Tilp, 2014a; Konrad & Tilp, 2014b; Blazevich et al., 2014). Other stretching interventions have right-shifted passive torque-angle curves (Kubo et al., 2002;

Toft et al., 1989; Guissard & Duchateau, 2004), reduced passive torque at standardized joint angles (Nakamura et al., 2012; Chan et al., 2001; Mahieu et al., 2007) or reduced passive joint stiffness (Guissard & Duchateau, 2004; Kubo et al., 2002; Marshall et al., 2011).

The present intervention study reduced passive torque at anatomical and standardized joint angle of passive dorsiflexion but did not of passive knee extension. This shows that the same intervention, applied to the same population, may yield different results in different joints, requiring caution when comparing studies. There are also large between-study differences in duration and volume, and lack of changes in passive torque at standardized joint angle may have been caused by too brief interventions.

In the present intervention study, knee extension ROM was not significantly increased after 8 weeks, which may explain the lack of reduction in passive torque. On the other hand, subjects subjectively reported greater stretch and greater improvements for knee extension compared to ankle dorsiflexion, so methodological challenges of standardizing subject positioning for the knee extension test compared to the dorsiflexion test might be responsible for the inability to detect further increases in ROM and/or reductions in passive torque.

The present intervention study revealed increased passive torque at maximal joint angle after 24 weeks of stretching, both for ankle dorsiflexion and for knee extension, matching findings of a few other stretching intervention studies (Halbertsma & Goeken, 1994; Nakamura et al., 2017; Reid & McNair, 2011; Blazevich et al., 2014). Other studies have followed the time course of passive torque changes, revealing increased ROM and increased passive torque at maximal joint angle after 2-4 weeks, and further increased ROM while passive torque at maximal joint angle was reduced back to (Chan et al., 2001) or below (Guissard & Duchateau, 2004) initial levels after 4-8 weeks. Increases in passive torque at maximal joint angle are relatively similar in the present study (dorsiflexion 35 %, knee extension 24 %) and in interventions lasting a few weeks (e.g. 28 % after 3 weeks (Blazevich et al., 2014)), regardless of the broad differences in ROM gains. This suggests that a decrease in pain sensitivity or an increase in pain threshold may occur in the first weeks of stretching, allowing subjects to tolerate a greater maximal joint angle without corresponding adaptations on MTU level but it is not clear why the maximally tolerated torque remained increased after 24 weeks in the present study but not in shorter, former interventions.

Passive stiffness followed the same trend as passive torque in all studies, with the exception that passive stiffness at maximal joint angle did not change with 24 weeks of stretching. The present data cannot explain this finding but it may be hypothesized that the rate of change in passive torque, rather than passive torque itself, influences the subjective determination of ROM.

The present studies did not aim to identify mechanisms for right-shifted torque-angle relationships or increases in maximal passive torque but some speculations may be offered. Theoretically, reduced passive resistance at comparable joint angles may be caused by altered passive properties such as increased tissue length or compliance. This could be the case in dancers, who displayed longer GM fascicles and a longer and more compliant Achilles tendon compared to controls. However, lengths were not altered in the intervention study. On the other hand, 24 weeks of stretching reduced EMG amplitudes at standardized joint angles, likely contributing to reduced active muscle stiffness and hence reduced passive torque, although the magnitude of this contribution cannot be estimated. EMG amplitude is not likely to regulate passive torque alone, as indicated by reduced torque and unchanged EMG amplitude at anatomical joint angle, or by unchanged EMG amplitude but increased torque at increased maximal joint angle. Tissue compliance likely plays a role, and although tendon stiffness at high force levels did not change with the present intervention, increases in toe strain limit are hypothesized (Paper III), potentially right-shifting the passive torque-angle relation.

In summary, hypothesis 2 was partially confirmed: Passive torque at standardized joint angles was lower in flexible subjects and was reduced with 24 weeks of ankle dorsiflexion stretching but not with hamstrings stretching. Torque at maximal joint angle was similar between dancers and controls but increased with 24 weeks of ankle dorsiflexion and hamstrings stretching. Further research should clarify whether hamstrings stretching training also may reduce passive torque.

5.6. Contractile function

Study I: The isokinetic knee flexion torque-angle relationship differed between gymnasts and controls, with gymnasts producing greater torque from 5° to 30° when correcting for passive resistance (Figure 19A). Peak isokinetic knee flexion torque was reached at joint angles corresponding to a more extended knee ($P < 0.05$) in gymnasts (41 ± 14°) compared to controls (60 ± 17°). Work production corrected for passive torque was greater ($P < 0.05$) in gymnasts (74 ± 11 J) compared to controls (62 ± 20 J). There were no differences in muscle strength.

Study II: The isokinetic plantar flexion torque-angle relationship differed between ballet dancers and controls at 45°·s^{-1} (Figure 19B) but not at 60°·s^{-1} and 90°·s^{-1}. Peak isokinetic plantar flexion torque was reached at similar joint angles in ballet dancers and controls ($P = 0.12$-0.33 across angular velocities). Analyses of work production did not reach statistical significance but showed a tendency ($P = 0.06$-0.07) towards greater work in dancers compared to controls (dancers

145 ± 21 J, 133 ± 21 J, 115 ± 18 J at 45, 60 and 90°·s^{-1}, respectively, controls 124 ± 29 J, 113 ± 24 J, 98 ± 20 J). There were no differences in muscle strength.

Study III: The isokinetic plantar flexion torque-angle relationship displayed a similar interaction (joint angle x time) across velocities in both legs, with increased torque at the most dorsiflexed joint angles (Figure 19C). There was a time effect of stretching for 24 weeks on angle of peak torque at 30°·s^{-1}, 45°·s^{-1} and 60°·s^{-1} of isokinetic plantar flexion (interaction P = 0.62-0.84, time P < 0.01-0.05), with peak torque shifting towards dorsiflexion in both legs (Figure 19C) but no effect at 90°·s^{-1} (interaction P = 0.56, time P = 0.11). Work at 30°·s^{-1} was unchanged (interaction P = 0.41, time P = 0.31, stretching PRE 156 ± 45 J, stretching POST 160 ± 38 J, control PRE 155 ± 35 J, control POST 156 ± 38 J). There were no changes in plantar flexion muscle strength.

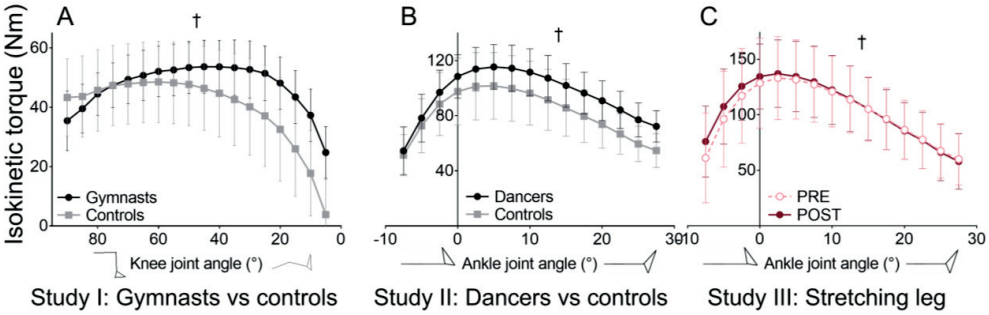


Figure 19. Isokinetic torque-angle relations. *A) Knee flexion torque at 60°·s^{-1} in gymnasts and controls, B) ankle plantar flexion torque at 45°·s^{-1} in ballet dancers and controls, C) ankle plantar flexion torque at 30°·s^{-1} in the stretching leg, before (PRE) and after (POST) 24 weeks of stretching. Study III control leg data are similar to and give the same statistical outcomes as stretching leg data, and are not shown. † represents two-way ANOVA and indicates an interaction effect (group x joint angle) in studies I-II (P < 0.01-0.05) and an interaction effect (time x joint angle) in study III (P < 0.01).*

A cross-sectional study found peak knee flexion torque at joint angles corresponding to a more flexed knee in inflexible subjects compared to controls (Alonso et al., 2009), and studies applying 3-6 weeks of hamstrings stretching demonstrated a shift in the angle of knee flexion peak torque towards extended positions, and/or a gain in knee flexor work (Ferreira et al., 2007; Chen et al., 2011), consistent with the present findings in gymnasts compared to controls. The large flexibility seen in the hamstrings MTUs of the gymnasts was associated with specific functional features of the hamstring muscles, enabling these athletes to exert a greater knee flexion torque towards extended positions and greater knee flexion work compared to controls.

The ballet dancers did not differ from controls in terms of angle of peak torque but showed a tendency (P = 0.06-0.07) towards greater work production. Greater work would fit well with the greater fascicle length seen in dancers compared to controls, whereby more sarcomeres in series

would enable greater work through similar torque production over a wider range of joint angles (for a review, see Lieber & Friden, 2000), indicating a functional advantage of greater ROM.

Speculations of added sarcomeres were, however, not supported by the present intervention study, where stretching for 24 weeks did not increase plantar flexion work. However, angle of peak torque was shifted towards a more dorsiflexed position at the lower angular velocities. Taken together, unchanged torque, work production and muscle architecture do not suggest addition of serial sarcomeres with the present stretching intervention. Hence, the shift in angle of peak torque may be related to increased tendon elongation and/or reduced passive torque, representing a minor effect of stretching training on contractile function. The contrast between the cross-sectional studies and the intervention study in terms of work production may be related to the weaknesses of cross-sectional designs, where it cannot be ascertained whether the group differences are caused by the gymnasts' and dancers' years of stretching, by the other modalities of their training, or by natural selection.

In summary, hypothesis 6 was partially confirmed: Gymnasts but not ballet dancers reached peak torque at joint angles corresponding to a more extended joint angle. Stretching for 24 weeks shifted angle of peak torque towards a more dorsiflexed joint angle.

5.7. Effects of unilateral stretching on the contralateral leg

In study III, 24 weeks of unilateral stretching increased ankle dorsiflexion ROM (Figure 12B), reduced EMG amplitude at standardized joint angle (Figure 14C), altered passive elongation of GM fascicles (Figure **15**B), SOL and GM muscle and tendon (Figure 16), and reduced passive torque at standardized joint angle of passive dorsiflexion (Figure 18C) in the contralateral leg.

This appears to be the first study reporting bilateral increases in ROM after unilateral stretching training, contrasting former interventions with unchanged control leg ROM (Akagi & Takahashi, 2014; Ben & Harvey, 2010; Kubo et al., 2002; Guissard & Duchateau, 2004; Minshull et al., 2014; Nelson et al., 2012) and passive torque (Akagi & Takahashi, 2014; Kubo et al., 2002).

The present study design does not rule out an influence of systematic error on the aforementioned variables. An interaction effect was seen for ROM, maximal elongation of muscle/tendon and maximal passive torque, while the time effect for the remaining variables represents either systematic error, a bilateral response to the intervention, or a combination thereof. The findings across variables are however consistent and may be explained physiologically or biomechanically. The bilateral changes are hence ascribed to a cross-education effect similar to that reported with strength training (Scripture, Smith, & Brown, 1894). This

interpretation seems supported by reports of acute bilateral increases in ROM following single bouts of stretching exercise (Chaouachi et al., 2017; Killen et al., 2018).

The apparent contrast to other studies may be explained by duration, as the former studies were limited to 3-10 weeks of stretching, while in the present study, increases in control leg dorsiflexion ROM reached significance only after 16 and 24 weeks. The contrast to the unilateral increase in knee extension ROM in the present study may be related to methodological challenges with the knee extension test, and/or unchanged knee extension ROM after 8 weeks.

Centrally regulated sensory or neural factors are indicated as mechanisms for increases in ROM (Freitas et al., 2017; Magnusson et al., 1996), laying ground for bilateral effects of stretching training. The present findings cannot pinpoint the mechanisms for bilateral changes but changes in pain threshold, neural adaptations related to pain sensitivity and reflex activity could explain the bilateral changes in torque and EMG amplitudes. However, cross-education effects involving motor learning (for a review, see Lee & Carroll, 2007) or systemic increases in hormone levels affecting collagen metabolism (Hansen & Kjaer, 2016) are also possible explanations which should be further investigated.

While not hypothesized prior to data collection, 24 weeks of stretching appears to involve neural adaptations that have the potential of altering ROM and mechanical properties of joints and MTUs bilaterally. However, further research with a control group not involved in stretching is needed to verify bilateral effects of stretching training.

5.8. Summary

The endpoint of ROM is subjectively determined. Hence, increases in ROM may be achieved through a combination of improved tolerance and reduced sensory input during passive stretching. A role of tolerance, e.g. increased pain threshold or reduced pain sensitivity, possibly affecting sensory input, is indicated by the greater maximally tolerated passive torque in the stretching leg, and may further have influenced active muscle stiffness. Reduced sensory input is further anticipated with reduced passive resistance to stretch, as indicated by reduced passive torque at comparable joint angles, whereby reduced tension on tissues would affect sensory afferences.

Passive resistance to stretch arises from the deformation and behaviour of the MTU components. Reflex activity may contribute to passive torque production via its contribution to active muscle stiffness. This is supported by the present finding of corresponding reductions in EMG amplitude and passive torque at standardized joint angle. However, reduced passive torque

at anatomical joint angle, where almost no EMG amplitude was observed, suggests that neural activation does not explain the entire change in passive torque. While the present thesis did not reveal any morphological adaptations to stretching training, a change in structural and mechanical properties is the most likely co-factor to neural activation in explaining reduced passive torque. The hypothesis of altered structural properties is supported by increased tendon elongation at lower force levels after stretching training.

The cross-sectional designs of the gymnast and ballet dancer studies impose limitations on the interpretation of the results, in that group differences cannot be directly attributed to the athletes' years of stretching but may be influenced by the other modalities of their training, or by selection bias to these elite populations. The findings of greater work production in gymnasts and longer muscle fascicles in ballet dancers thus provide information about the specialized MTU properties in these populations but cannot be taken as evidence of contractile adaptations to stretching training.

The within-subjects design of the intervention study imposes limitations on the interpretations of the changes occurring in both the stretching and the control leg. However, the consistent interplay between these variables supports a role of neural adaptations contributing to bilateral changes in ROM as well as passive torque, EMG amplitude and tissue elongation during stretching and contraction.

6. Conclusions

In conclusion, the present thesis demonstrated:

1. Ankle dorsiflexion ROM and hip flexion ROM were greater in ballet dancers and gymnasts with years of stretching training compared to controls. Ankle dorsiflexion ROM and knee extension ROM were increased after 8 weeks of stretching.
2. Passive torque at standardized joint angles was lower in gymnasts compared to controls for knee extension, and in ballet dancers compared to controls for ankle dorsiflexion. Passive torque at standardized joint angle of ankle dorsiflexion – but not knee extension – was reduced through 24 weeks of stretching. Passive torque at maximal joint angle was similar between dancers and controls but increased in the subjects that underwent 24 weeks of stretching.
3. Triceps surae EMG amplitudes at standardized joint angle of passive dorsiflexion were lower in ballet dancers compared to controls and were reduced in the subjects that underwent 24 weeks of stretching.
4. Ballet dancers displayed greater elongation of GM muscle compared to controls but the relative contribution of muscle versus tendon to total MTU elongation was not different from controls. With 24 weeks of stretching, elongation of both muscle and tendon increased, with an increased relative contribution of the Achilles tendon to total MTU elongation, in both GM and SOL.
5. Resting lengths of the GM muscle fascicles and the whole Achilles tendon were greater in ballet dancers compared to controls, but neither GM muscle architecture, SOL intramuscular collagen content nor Achilles tendon length and CSA changed in the subjects that underwent 24 weeks of stretching.
6. Gymnasts reached peak knee flexion torque at joint angles corresponding to a more extended knee ankle. The same was not found in ballet dancer plantar flexion. However, stretching for 24 weeks shifted angle of peak plantar flexion torque toward a more dorsiflexed ankle angle.
7. Stretching training appears to involve neural adaptations that, over the course of 24 weeks of stretching, contributed to bilateral changes in ankle dorsiflexion ROM, properties of passive dorsiflexion (torque, EMG amplitude, fascicle, muscle and tendon elongation), tendon elongation at standardized force levels and angle of peak torque.

7. Perspectives

Based on the present thesis, the following aspects of adaptation to stretching training are suggested for further investigations, with the ultimate aim of clarifying implications for stretching training in various populations and with various aims:

- The time-course of changes in ROM and passive torque in stretched and contralateral limbs should be investigated.
- The relationship between pain, reflexes and EMG amplitude during passive stretching, and their contribution to passive resistance to stretch, should be investigated.
- More advanced imaging techniques and/or additional invasive protocols should be applied to clarify structural and mechanical adaptations in intramuscular connective tissue, the contractile component and tendon, particularly related to the toe strain limit.

Implications for contractile function may be more easily investigated once specific adaptations of the MTU and the nervous system are pinpointed.

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Hamstrings functional properties in athletes with high musculo-skeletal flexibility

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The purpose of this study was to examine whether athletes with highly flexible hamstring muscle-tendon units display different passive and contractile mechanical properties compared with controls. Flexibility, passive, and active torque–angle properties were assessed in 21 female elite rhythmic gymnasts and 16 female age-matched athletes. Passive resistance to stretch was measured during knee extension with the hip fixed at 100° of flexion. Concentric isokinetic maximal voluntary knee flexion and extension torques were measured at 60°/s in the same position. Tests of flexibility and passive resistance to stretch indicated a greater flexibility in the gymnasts. Despite no differences between groups in knee flexion and extension peak torque, gymnasts reached knee flexion peak torque at more extended positions (longer muscle lengths) and displayed significantly different torque–angle relations. When active torque was corrected for passive resistance to stretch, differences increased, gymnasts producing more work, and maintaining ≥ 70% of peak torque over a larger range of joint excursion. In conclusion, individuals with a higher flexibility of the hamstrings MTU present a different torque–angle profile, favoring the production of flexion torque toward extended knee positions, displaying larger functional range of motion and a higher mechanical work output during knee flexion.

Musculo skeletal flexibility is often considered to relate to sports performance (Shrier, 2004; McNeal & Sands, 2006; McHugh & Cosgrave, 2010; Jenkins & Beazell, 2010) and injury risk (Shrier, 1999; Thacker et al., 2004; Witvrouw et al., 2004; McHugh & Cosgrave, 2010). Several studies applying long-term stretching exercises have convincingly demonstrated increases in range of motion (ROM) (Godges et al., 1993; Bandy & Irion, 1994; Halbertsma & Goeken, 1994; Magnusson et al., 1996; Bandy et al., 1997). While altered tolerance to stretch is suggested as a mechanism for increased ROM (Magnusson et al., 1996), increased flexibility could also be ascribed to the mechanical and morphological plasticity of the musculo-tendinous system. However, despite the pivotal role of the muscle-tendon unit (MTU) properties in joint torque production, the influence of flexibility upon the joint mechanical output remains unclear. The muscle-operating length and torque production are intrinsically bound to the MTU structural and mechanical properties. Fascicular length reflects the number of in series sarcomeres and, de facto, is related to maximal muscle excursion and the muscle length at which myofilaments overlap optimally (Gordon et al., 1966). *In situ*, in series, elastic elements act as a mechanical buffer limiting muscle fascicle strain during elongation of the whole MTU. Moreover, the torque output results from the combined active and passive contributions of the contractile and elastic elements, respectively. Hence, in joints with low flexibility, peak torque is seen at shorter MTU lengths than in joints with greater flexibility. Accordingly, a study comparing subjects with low ROM of the hamstrings MTU to control subjects found that the former exerted maximal knee flexion torque at more flexed knee joint angles (Alonso et al., 2009). The same study showed that ROM affected the shape of the torque–angle curve, so that subjects with low ROM produced greater knee flexion torque than control subjects at more flexed positions, and lower torque at more extended positions. These findings indicate that a low ROM affects the mechanical output of the hamstrings MTU. Such an influence could be ascribed to insufficient length and/or compliance of the hamstrings MTU. In individuals presenting high ROM, mirroring observations might be expected on the torque–angle relation. However, positions combining knee extension with large hip flexion are infrequent in daily activities. Whether ROM exceeding the requirements of daily living will influence the hamstrings MTU's mechanical output under these conditions is not clear.

The purpose of this study was therefore to examine whether highly flexible elite rhythmic gymnasts, having undertaken years of systematic stretching exercise, display different passive and contractile mechanical properties of the hamstrings MTU compared with

physically active controls that do not stretch. It was hypothesized that, in a sitting position, passive resistance to stretch would be lower at longer MTU lengths in gymnasts and that the active torque–angle relation would differ from that of less flexible controls; we expected knee flexion peak torque to occur at longer MTU lengths in gymnasts, owing to the lower passive tension or longer fascicles. Moreover, longer fascicles would confer to the gymnasts a larger functional ROM, defined as the capacity to exert a high torque over a greater range of joint excursion.

Methods

Subjects

A priori sample size calculations (statistical power of 0.80) based on differences in angle of peak torque reported in a similar cross-sectional study (Alonso et al., 2009) indicated that 38 subjects were required. Accordingly, 22 female elite rhythmic gymnasts (STR) from national teams of the Nordic countries were compared with 16 age-matched female athletes (NON) from handball, football, and cross-country skiing. Data from one STR subject were discarded because of incomplete torque measurements. The NON group was selected among athletes in weight-bearing sports that do not involve systematic stretching exercise, as confirmed with self-reporting (Table 1). Rhythmic gymnastics is also mainly performed in weight-bearing positions, but includes substantial amounts of stretching exercise (Table 1). The groups had similar age, height, and years of athletic training, but STR weighed less than NON (Table 1). All subjects were above 16 years of age. In accordance with the Declaration of Helsinki, the regional ethics committee approved the study, and each subject signed an informed consent form.

Table 1. Descriptive data for stretching group (STR) and nonstretching group (NON)

	STR	NON
Age (years)	17.3 ± 1.6	18.5 ± 2.3
Years of sports training (years)	10.1 ± 2.7	11.7 ± 2.7
Height (cm)	165.8 ± 5.0	166.2 ± 6.8
Body weight (kg)	53.0 ± 5.6*	61.0 ± 4.6
Weekly training (h)	21.6 ± 8.3*	9.3 ± 6.4
Weekly stretching (min)	236.3 ± 127.7*	9.4 ± 17.4
Weekly stretching (% of hours training)	18.2 ± 13.6*	1.7 ± 5.1

*Indicates significant difference ($P < 0.05$) between STR and NON.

Clinical flexibility tests

In addition to the instrumented testing described below, three clinical measures of passive musculoskeletal flexibility were obtained: The "Beighton score" (Beighton et al., 1973) assessed general joint laxity. The "sit and reach test" (American College of Sports Medicine, 2010) was used to assess the ROM of hamstrings and lower back. A gymnastics-specific "splits test" was used to assess flexibility of the hip joints (Fig. 1).

Passive resistance to knee extension

Following 5 min of warm-up on an ergometer bike, passive resistance of the hamstrings MTU during knee extension was measured with an isokinetic dynamometer (TechnoGym REV 9000, Cesena, Italy) on the left leg, in a seated position. The hip joint was fixed at 100° of flexion and the dynamometer settings were individually adjusted to align the rotation axis of the knee joint with that of the dynamometer. The leg was secured to the lever arm of the dynamometer and the thigh was secured to the seat to minimize mis-

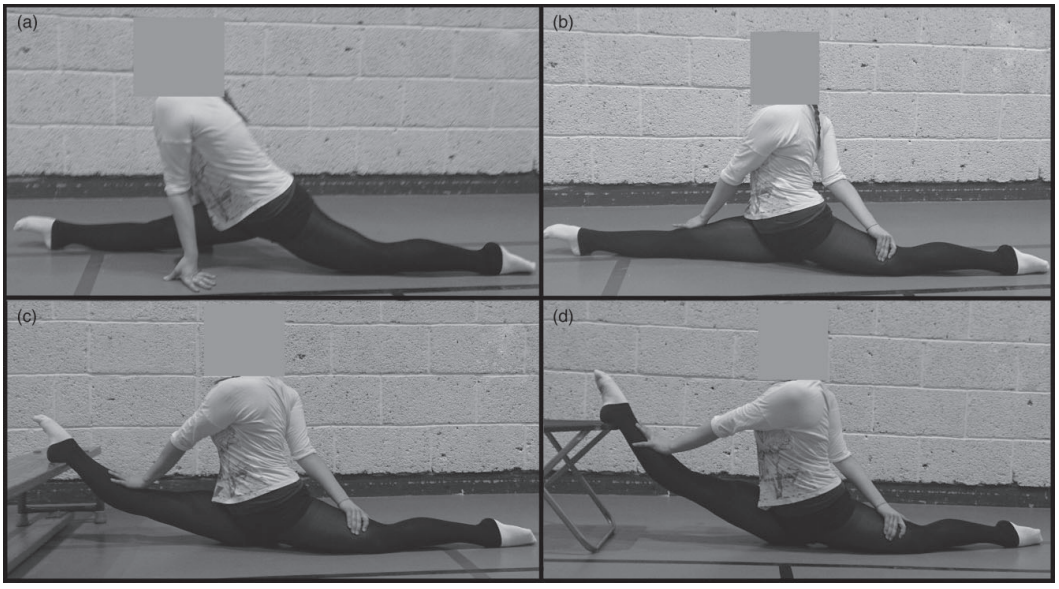

Fig. 1. ROM of the hip joints was estimated using a gymnastics-specific splits test.
The right and left front split was graded from 0 points (< 180° split, a) to 3 points (oversplit from a 30-cm chair, d).

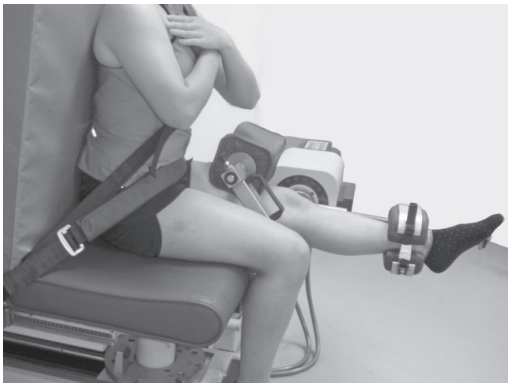

Fig. 2. Subject positioning in the isokinetic dynamometer.

alignment of rotation axes during motion. Belts stabilized the waist and trunk, and arms were crossed. The experimental setup is shown in Fig. 2. Prior to the testing, the ability of each subject to tolerate full passive knee extension (0°) was confirmed on the dynamometer by slow, stepwise increments of knee extension. The knee was then extended and flexed at 15°/s, within a range of motion from 93° to 0°. Subjects were instructed not to offer any active resistance to the movement by avoiding contraction of their hamstring muscles. The procedure was performed twice. Additional trials were performed if hamstrings contraction was detected during movement.

Maximal voluntary torque tests

Isokinetic maximal concentric torque tests were performed during knee flexion and extension, using the same position setup as described above. These tests were preceded by a familiarization phase consisting of four submaximal knee flexor and extensor contractions at 60°/s, within a range of motion from 93° to 0°. After 60 s of rest, subjects performed three maximal knee extensor contractions, followed by a 60-s rest, and three maximal knee flexor contractions. Standardized verbal encouragements and visual feedback of the instantaneous torque produced were provided during testing.

Data processing

Torque and angle signals were recorded at 109 Hz and post-processed in MatLab (The MathWorks, Natick, Massachusetts, USA). Signals were filtered using two-way, zero-lag second-order Butterworth filters with a cutoff frequency of 10 Hz for active tests and passive angle, 2 Hz for passive torque. Torque data were gravity-corrected by assuming zero resistance to stretch during passive knee extension from 75° to 65°. Trigonometric functions were applied to the recorded torque to calculate torque values corresponding to the weight of the leg across the range of joint excursion. Artifactual peaks caused by initial and final accelerations were filtered out.

The passive torque–angle relation representing passive resistance to knee extension was obtained from the passive knee extension trials. Torque data were interpolated using a spline function to extract torque at 0.25° intervals. Statistics were applied to torque values at 5° intervals. Passive musculo-tendinous stiffness was calculated as the slope of a fourth-order polynomial fit of the passive torque–angle relation, at 15°, 10°, and 5° knee joint angle (Nordez et al., 2006). To assess stiffness independently of joint angles, a stiffness index was calculated as two times the leading coefficient from a second-order polynomial fit ($ax^2 + bx + c$) of the passive torque–angle relation (Nordez et al., 2006).

The torque measured during voluntary knee flexor and extensor contractions was defined as total torque. The torque obtained by subtracting the torque due to passive resistance to stretch was defined as corrected torque. At all joint angles, the measured torque during passive extension was subtracted from the corresponding torque during isokinetic trials using MatLab. Active torque data were interpolated using a spline function to extract torque at 0.25° intervals. Statistics were applied to torque values at 5° intervals. For the maximal voluntary torque tests, the trial with the greatest peak torque was selected for subsequent analysis. Peak torque and angle of peak torque were extracted. Work was calculated as the area below the torque–angle curve, between 2.5° and 92.5° of joint excursion. The range of joint excursion (°) during which corrected torque production was above 70% of peak torque, representing the functional ROM, was determined. Torque data were expressed as absolute torques and as normalized to each individual's peak torque.

Statistics

Intergroup differences in subject morphology, training variables, flexibility variables, and peak torque were analysed using unpaired, two-tailed *t*-tests. Differences in passive and active torque–angle relations were analyzed using two-way mixed model analysis of variance, with group and joint angle as factors. Post-hoc, Bonferroni's multiple comparisons tests were performed. Pearson correlation coefficients calculated used to determine the relations between angle of peak torque and passive torque, stiffness variables, and sit and reach. Level of significance was set to $\alpha = 0.05$. All data are presented as means ± SD.

Results

All clinical flexibility tests demonstrated greater flexibility in the group of gymnasts: Beighton score (STR 4.8 ± 1.6 points, NON 1.8 ± 1.4 points, $P < 0.0001$), sit and reach test (STR 25 ± 5 cm, NON 6 ± 8 cm, $P < 0.0001$), and gymnastics-specific splits test (STR scoring 2.4 ± 0.7, while no subjects in the nonstretching group were able to perform 180° splits, $P < 0.0001$). Correspondingly, a lower resistance to stretch was measured in STR than in NON during passive knee extension between 30° and maximal extension [$F(1, 15) = 64.4$, $P < 0.0001$; Fig. 3]. Passive musculo-tendinous stiffness and stiffness index ($P < 0.0001$; Table 2) were also significantly lower in STR.

Peak total torque during the maximal voluntary knee flexion test did not differ between groups (Table 3). However, angle of peak torque was more extended in STR subjects ($P < 0.005$; Table 3). Moreover, the active torque–angle relation displayed higher total torque values in STR subjects in the range of 5° to 15° of knee flexion [$F(1, 17) = 9.70$, $P < 0.0001$; Fig. 4(a)]. When normalizing total torque, torque was higher in STR between 5° and 35°, and lower between 85° and 90° of knee flexion [$F(1, 17) = 11.3$, $P < 0.0001$; Fig. 4(b)].

In respect to corrected torque, no between-groups differences in peak torque were detected (Table 3), but the difference in the angle at which peak torque was

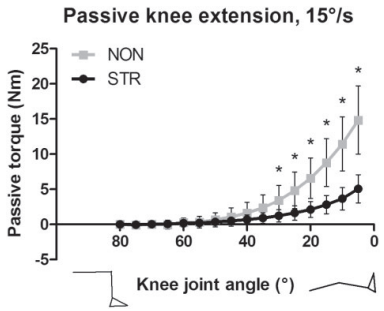

Fig. 3. Passive resistance to knee extension during continuous passive motion from 90° to 0° knee joint angle, at 15°/s. Torque data are interpolated at 0.25° intervals and each 5° interval is reported as mean ± SD. *Indicates significant difference ($P < 0.01$) between STR and NON, starting from 30° and continuing to 5°.

Table 2. Passive musculo-tendinous stiffness at 15°, 10°, and 5° and stiffness index (two times the leading coefficient from a second-order polynomial fit, Nordez et al., 2006), as calculated from polynomial fits of the torque–angle relation from passive motion from 90° to 0° knee joint angle, at 15°/s

	STR	NON
Passive musculo-tendinous stiffness (Nm/°)		
–at 15°	0.16 ± 0.07*	0.49 ± 0.15
–at 10°	0.22 ± 0.09*	0.60 ± 0.19
–at 5°	0.28 ± 0.12*	0.74 ± 0.24
Stiffness index (Nm/°^{2})	–0.003 ± 0.001*	–0.009 ± 0.003

*Indicates significant difference ($P < 0.0001$) between STR and NON.

produced increased ($P < 0.001$; Table 3). The torque–angle relation displayed differences across a greater range of joint excursion, with higher torque in STR subjects between 5° and 30° of knee flexion [Table 3, $F(1, 17) = 17.4$, $P < 0.0001$; Fig. 4(c)], higher normalized torque between 5° and 35°, and lower normalized torque at 90° of knee flexion [$F(1, 17) = 20.5$, $P < 0.0001$; Fig. 4(d)].

Work by the total torque during knee flexion did not differ between groups (Table 3). However, more work was produced in STR than in NON when the contribution of the passive resistance was subtracted ($P < 0.05$; Table 3). Correspondingly, the STR group displayed a larger functional ROM by maintaining a high torque (> 70% of peak corrected torque) over a greater range of joint excursion than the NON group ($P < 0.005$; Table 3).

The joint angle at knee flexion peak torque was negatively related with sit and reach ROM ($r = –0.48$) and stiffness index ($r = –0.60$), and positively related with passive musculo-tendinous stiffness at 15°, 10°, and 5° ($r = 0.53$–0.56).

Table 3. Peak torque and angle of peak torque during maximal voluntary torque tests for knee flexion and extension at 60°/s

	STR	NON
Knee flexion		
Total peak torque (Nm)	56 ± 9	52 ± 14
Total peak torque (Nm/kg$^{2/3}$)	4.0 ± 0.6*	3.4 ± 0.8
Angle of total peak torque (°)	40 ± 13*	57 ± 20
Work by total torque (J)	75 ± 11	66 ± 21
Corrected peak torque (Nm)	56 ± 9	52 ± 13
Corrected peak torque (Nm/kg$^{2/3}$)	3.9 ± 0.6*	3.3 ± 0.7
Angle of corrected peak torque (°)	41 ± 14*	60 ± 17
Work by corrected torque (J)	74 ± 11*	62 ± 20
Range of joint excursion > 70% of peak torque (°)	73 ± 6*	63 ± 13
Knee extension		
Total peak torque (Nm)	140 ± 18	145 ± 21
Total peak torque (Nm/kg$^{2/3}$)	9.9 ± 1.0	9.0 ± 2.3
Angle of total peak torque (°)	64 ± 5	63 ± 6
Work by total torque (J)	154 ± 15	158 ± 21
Corrected peak torque (Nm)	140 ± 18	145 ± 21
Corrected peak torque (Nm/kg$^{2/3}$)	9.9 ± 1.0	9.0 ± 2.3
Angle of corrected peak torque (°)	64 ± 5	63 ± 6
Work by corrected torque (J)	155 ± 16	162 ± 22

*Indicates significant difference ($P < 0.05$) between STR and NON.

Neither total nor corrected peak voluntary knee extension torque differed between groups (Table 3). In addition, no differences were observed in angle of peak torque (Table 3) or in the torque–angle relation of the STR and NON groups [$F(1, 17) = 1.3$, N.S.]. The work produced during voluntary knee extension did not differ between groups (Table 3).

Discussion

The purpose of the present study was to examine the influence of high musculo-skeletal flexibility on muscular function. The clinical flexibility tests and the lower passive resistance to stretch in the hamstrings confirm that the selected group of rhythmic gymnasts was much more flexible than the control athletes. Moreover, our findings show that muscle function differs between these groups, with peak isokinetic knee flexion torque occurring at positions of greater knee extension in the STR group. Furthermore, the work produced by the hamstring muscles during knee flexion was greater in the STR group when passive resistance was accounted for, despite similar peak torque values.

The higher flexibility of the gymnasts is consistent with the higher volume of stretching performed during their training activities (Table 1). Baseline characteristics of the two groups were comparable, with the notable exceptions of a lower body weight, a higher training volume, and a higher flexion torque when normalized to body weight for the STR subjects. However, the fact that the gymnasts' body weight was lower while height was similar indicates that their training activity does not involve any substantial muscular hypertrophy. In

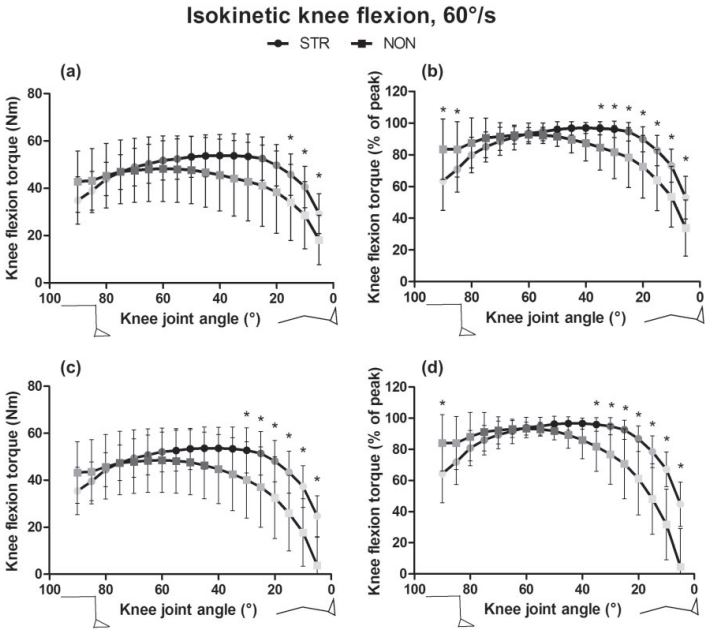

Fig. 4. Active torque–angle relation during isokinetic knee flexion at 60°/s.
Figures a and b show total torque, figures c and d show torque corrected for passive resistance to stretch. Figures a and c show absolute torque, figures b and d show normalized torque. Torque data are interpolated at 0.25° intervals and every 5° interval is reported as mean ± SD. Group by angle interaction $P < 0.0001$ in a–d. *Indicates significant difference ($P < 0.05$) between STR and NON: from 5° to 15° knee flexion for total torque (a), from 5° to 35° as well as from 85° to 90° for normalized total torque (b), from 5° to 30° for corrected torque (c), and from 5° to 35° as well as at 90° for normalized corrected torque (d).

rhythmic gymnastics, hours of weekly training are allocated to the practice of low-intensity technical drills (Jastrjembskaia & Titov, 1998). Despite the higher intrinsic flexion force of the gymnasts, the similar non-normalized torques measured during knee extension and flexion confirm that the groups presented comparable muscular strength around the knee joint. The fact that peak torque production in these athletes was not greater than values reported for nonathletic, young female adults (Lanshammar & Ribom, 2011) is of note. Beside possible differences between ergometers, the unnatural but necessary subject positioning may have influenced the present results.

Despite similar values of knee flexion peak torque, the active torque–angle relation revealed higher torques at more extended positions in the STR group than in the controls. A mirroring trend was observed with a lower torque at more flexed positions in gymnasts, although differences only reached significance when normalized values were considered (Fig. 4(b, d)). These findings are in line with the results of Alonso et al. (2009), who showed that isometric torque–angle curves in inflexible subjects are shifted toward more flexed knee joint angles (Fig. 5) compared with controls. Similarly, intervention studies that applied 3–6 weeks of stretching exercise demonstrated a shift in the angle of knee flexion peak

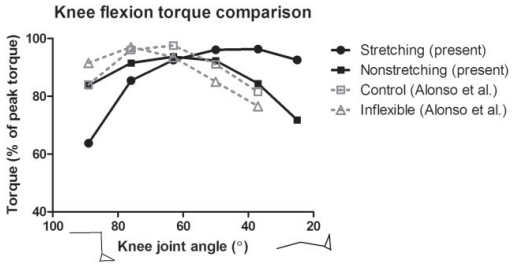

Fig. 5. Knee flexion torque comparison between flexible, control, and inflexible subjects.
Data were obtained from the present results (stretching and nonstretching subjects) and from a previous study with a similar design (inflexible and control subjects, Alonso et al., 2009). Note that hip flexion angles differ, with approximately 140° in the Alonso study and 100° in the present study.

torque toward extended positions, and concurrently, a gain in exerted knee flexor work (Handel et al., 1997; Ferreira et al., 2007). These changes in the torque–angle relation and exerted work match the present results, and suggest that the difference in musculo-skeletal properties between the STR group and the controls may be related to the gymnasts' years of stretching exercise. When accounting for the mechanical contribution of the

passive elements, between-group differences in torque occurred over a greater range of joint excursion. Taken together, the total and corrected torque data indicate that the observed differences in torque–angle relations are attributable to forces exerted by passive elements and to musculo-skeletal architectural features. Since muscular force production is determined by the degree of actin-myosin overlap (Gordon et al., 1966), the joint angle at which optimal overlap is achieved is determined *in vivo* by the combined influences of the compliance of series elastic elements, fascicle length, and absolute MTU length. Albeit speculative, the gymnasts of the present study may present a more compliant and/or longer hamstring MTU than controls, enabling greater muscle shortening during contraction at extended positions, effectively setting the optimum angle of torque production toward this joint range. In addition, hamstrings fascicles may be longer in gymnasts, as suggested by the optimal torque production at positions that are more extended and in particular by the greater range of joint excursion in which a high torque (≤ 70% of peak torque) is maintained. The larger functional ROM and total work measured in the present study are in line with the increased work production reported following stretching interventions (Handel et al., 1997; Ferreira et al., 2007) and suggest that a greater flexibility may confer a functional advantage. These hypotheses are consistent with the correlations observed between the angle of knee flexion peak torque and some of the measurements of flexibility (i.e., sit and reach ROM and passive stiffness).

Interestingly, previous reports have attributed increased ROM to a stretching-induced increase in tolerance to stretch (Halbertsma & Goeken, 1994; Magnusson et al., 1996; Laessoe & Voigt, 2004; Folpp et al., 2006; Law et al., 2009; Ben & Harvey, 2010). For instance, intervention studies where the hamstrings underwent stretching exercise for 3–6 weeks induced an increase in ROM, without altering passive resistance to stretch within the original ROM (Halbertsma & Goeken, 1994; Magnusson et al., 1996; Reid & McNair, 2004). Similarly, three studies report unchanged joint angles at standardized passive torques (Folpp et al., 2006; Law et al., 2009; Ben & Harvey, 2010). In contrast, indications of altered passive resistance to stretch were seen in one study reporting increased ROM without corresponding increases in passive torque (Chan et al., 2001). The contribution of tolerance to stretch in explaining differences in ROM cannot be assessed with the present design. Yet, during knee flexor contraction from extended positions, reflex muscle contractions induced by insufficient tolerance to stretch would not explain the lower total torque measured in less flexible controls. Hence, if tolerance to stretch partly influenced the general flexibility measured in each group, the specific features of their respective torque–angle relations are likely attributable to differences in structural and mechanical properties.

In contrast to the differences in active torque–angle relations during knee flexion, knee extension torque–angle relations were similar in the two groups. This finding may illustrate that the stretching group had conducted years of systematic stretching for the hamstrings MTU, but not for the quadriceps (subjects' self-report). Alternatively, one could ascribe the specific torque–angle curve of gymnasts to the specificity of the mechanical loading associated with rhythmic gymnastics. Biceps femoris fascicle length has been shown to increase with eccentric resistance exercise (Potier et al., 2009). In vastus lateralis, increased fascicle length was seen after resistance exercise with high muscle lengthening velocity (Sharifnezhad et al., 2014), while resistance exercise utilizing large ranges of motion augmented increases in fascicle length (McMahon et al., 2014). Although both groups regularly performed weight-bearing sports involving torque production at similar ranges of joint excursion, musculo-tendinous adaptations stemming from specific operating ranges or contraction modes of the hamstring muscles cannot be ruled out. A detailed comparison of sport-specific biomechanical features is unfortunately not possible here; additional studies with a longitudinal design are required to ascertain that the present findings result primarily from differences in flexibility.

In conclusion, the high knee extension flexibility seen in elite rhythmic gymnasts was associated with specific functional features of the hamstring muscles, enabling these athletes to exert a higher knee flexion torque toward extended positions than less flexible control athletes. In addition, hamstring muscles of gymnasts display a greater functional range of motion and produce more knee flexion work than controls, despite identical peak torque production.

Perspectives

While it is well known that stretching exercise increases joint range of motion, the mechanisms behind this alteration are not well elucidated. This study underlines that the level of musculo-skeletal flexibility affects the functional properties of the MTU. Future research including imaging techniques should support the present observations with direct measurements of structural and mechanical properties of muscle and tendon. For example, ultrasound imaging could be used to determine whether more flexible individuals do indeed have greater fascicle shortening during isometric MVCs at longer muscle lengths. There is also a need for truly long-term stretching exercise interventions, in order to address whether stretching exercise may alter functional properties and thereby affect performance.

Key words: Stretching, range of motion, length-tension, passive resistance, passive stiffness, peak torque.

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Paper II

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ORIGINAL ARTICLE

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Specialized properties of the triceps surae muscle-tendon unit in professional ballet dancers

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This study compared professional ballet dancers (n = 10) to nonstretching controls (n = 10) with the purpose of comparing muscle and tendon morphology, mechanical, neural, and functional properties of the triceps surae and their role for ankle joint flexibility. Torque-angle and torque-velocity data were obtained during passive and active conditions by use of isokinetic dynamometry, while tissue morphology and mechanical properties were evaluated by ultrasonography. Dancers displayed longer gastrocnemius medialis fascicles (55 ± 5 vs 47 ± 6 mm) and a longer (207 ± 33 vs 167 ± 10 mm) and more compliant (230 ± 87 vs 364 ± 106 N/mm) Achilles tendon compared to controls. Greater passive ankle dorsiflexion range of motion (40 ± 7 vs 17 ± 9°) was seen in dancers, resulting from greater fascicle strain and greater elongation of the muscle. Peak electromyographic (EMG) activity recorded during passive stretching was lower in dancers, and at common joint angles, dancers displayed lower EMG amplitude and lower passive joint stiffness. No differences between groups were seen in maximal isometric plantar flexor torque, isokinetic peak torque, angle of peak torque, or work. In conclusion, the greater ankle joint flexibility of professional dancers seems attributed to multiple differences in morphological and mechanical properties of muscle and tendinous tissues, and to factors related to neural activation.

KEYWORDS

fascicle length, length-tension, morphological properties, passive resistance, passive torque, ROM, tendon stiffness, ultrasound

1 | INTRODUCTION

Stretching is frequently applied in sports and exercise and while it is known that habitual stretching increases joint range of motion (ROM),[1,2] the ability for stretching to facilitate functional performance[3,4] or to prevent injuries[4] remains under question. The ROM of a joint is governed by individual tolerance to stretch and by the length and compliance of relevant muscle-tendon structures. Stretching-induced increases in ROM are often attributed to increased tolerance to stretch,[1,5] although the influence of habitual stretching upon musculotendinous variables has not been consistently examined.

Animal studies have demonstrated that extensive stretching induces muscle morphological changes such as an increase in serial sarcomeres and longer muscle fibers.[6,7] In humans, a few stretching intervention studies have observed increases in fascicle length,[8-10] although others have not found any change in this parameter.[11] Tendon length is not expected to increase with habitual stretching,[10,12,13] however, due to large individual differences in resting tendon length,[14] the tendon likely offers varying contribution to total muscle-tendon unit (MTU) elongation during loading regimes. Tendon mechanical properties may theoretically influence ROM through differences in morphological or material properties. Most

human studies applying static stretching have observed no effect of this type of intervention on tendon stiffness,[12,15] although a few studies have reported reduced tendon stiffness after ballistic[15] or PNF[16] stretching training. With respect to tendon and muscle morphological properties, there is to date no evidence of the influence of stretching training on thickness, cross-sectional area or slack length.[5]

From a functional perspective, animal studies have demonstrated that extensive MTU stretching shifts the passive force-elongation relation toward lower force at same MTU length,[6,17] but in human intervention studies where less intensive stretching is applied, only few studies have demonstrated changes in passive torque-angle variables such as reduced passive torque[13,18] or reduced passive joint stiffness.[19] Nonetheless, in human cross-sectional comparisons, flexible subjects have consistently displayed lower passive torque across joint angles[20] or lower passive torque at given joint angles,[12,21,22] compared to less flexible subjects. Although the passive torque-angle relation is associated with mechanical resistance to stretch, increased neural activation is often observed near maximal ROM, contributing to additional torque production.[23] The significance of neural activation during stretching is unclear. One human intervention study found increased maximal ROM and passive torque while electromyographic (EMG) amplitude was not affected by training,[1] whereas cross-sectional studies observed lower EMG amplitude in more flexible subjects during stretching.[21,22]

Taken together, previous studies provide an inconsistent overview of the relative contributions of morphological, mechanical, and neural properties to joint ROM. The duration of previous human intervention studies may have been too short to produce measurable differences.[5] Examining populations with a long history of stretching training may therefore offer better insight into the determinants of flexibility. Previous studies comparing populations with different flexibility[20-22,24] have mainly focused on mechanical or functional properties, and have for the most part not reported whether the involved subjects had undertaken stretching training. Classical ballet requires substantial ankle ROM, from maximal dorsiflexion in grand pliés to maximal plantar flexion in pointé work. Professional dancers have undergone years of systematic and intense stretching, which makes for an interesting study population to examine the mechanisms that govern ROM.

The aim of this study was therefore to investigate whether professional ballet dancers display different morphological, mechanical, neural, and functional properties of the triceps surae MTU, compared to physically active control subjects with no history of stretching training. It was hypothesized that, owing to favorable morphological and mechanical properties (ie lower passive joint stiffness, lower tendon stiffness, and longer muscle fascicles), dancers would have greater maximal ROM and lower passive torque compared to controls. Additionally, dancers were expected to present a greater tolerance to stretch, as indicated by lower EMG amplitude and pain perception during passive stretching.

2 | MATERIALS AND METHODS

2.1 | Subjects

Ten female professional ballet dancers from the Norwegian National Ballet or professional ballet dancers with similar training background were matched by body mass (dancers 61 ± 7 kg, controls 60 ± 7 kg, $P = .94$) and height (dancers 168 ± 6 cm, controls 168 ± 7 cm, $P = .90$) to ten healthy, active female control subjects. The groups had similar leg length (dancers 86 ± 5 cm, controls 85 ± 6 cm, $P = .59$) and calf length (dancers 44 ± 2 cm, controls 45 ± 3 cm, $P = .82$), but the dancers were older than the controls (dancers 30 ± 4 years of age, controls 21 ± 1 years of age, $P < .05$). The training history of the dancers comprised 24 ± 5 years of ballet practice, with a current volume of 19 ± 12 hours per week, of which 11 ± 15 hours were reported as mobility training (ie ballet-specific movements toward maximal ROM and static stretching exercises). The control subjects had no history of systematic stretching and a current training volume of 4 ± 3 hours per week in recreational activities. All subjects attended a familiarization session before returning to the laboratory a week later for data collection. In participants not on constant-dose oral contraceptives, data collection was performed within 14 days from the last menstruation, to avoid testing during the luteal phase. The subjects were instructed to refrain from training or stretching at least 12 hours prior to testing. In accordance with the Declaration of Helsinki, the regional ethics committee approved the study, and each subject signed an informed consent form.

2.2 | Anthropometry

Leg length was measured in standing position as the distance between the most prominent point of trochanter major and the floor. Calf length was measured from the lateral femoral epicondyle to the most posterior point of the calcaneal tuberosity.

2.3 | Resting muscle architecture and morphological properties

Fascicle length, pennation angle and muscle thickness of gastrocnemius medialis (GM) and soleus (SOL), as well as length of the Achilles tendon were measured using real-time B-mode ultrasonography with a 50-mm linear array transducer (L12-5, Philips, Bothell, WA, USA) and ultrasound system (HD11XE, Philips, Bothell, WA, USA).

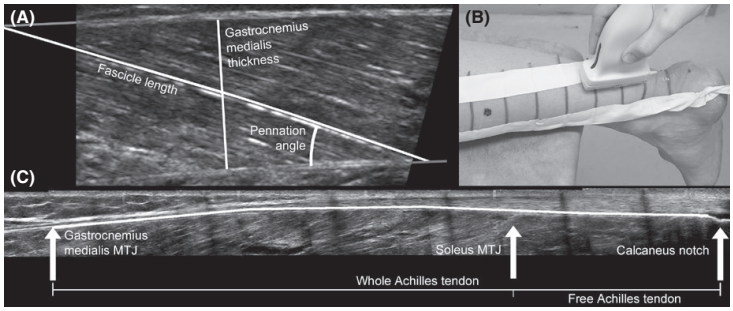

FIGURE 1 Measurement of gastrocnemius medialis architecture and Achilles tendon length. A, Ultrasound image showing the measurement of gastrocnemius medialis fascicle length, pennation angle and muscle thickness. B, An adhesive scaffold with embedded echogenic wires was placed along the Achilles tendon trajectory, for tendon length measurements. C, Sequentially combined ultrasound images, showing the length of the Achilles tendon from the calcaneal insertion to the soleus musculo-tendinous junction (MTJ) (free Achilles tendon) and to the gastrocnemius medialis MTJ (whole Achilles tendon). Length was measured along the tendon path in the sagittal plane

The lower half of the transducer frequency (5-12 MHz) was used with a built-in filter to optimize ultrasound penetration while preserving spatial resolution and contrast. Ultrasound scanning depth was set to 3-5 cm for GM and 4-7 cm for SOL, depending on interindividual differences in muscle thickness.

Subjects were resting in prone position with the foot hanging freely off the examination bed. Resting ankle plantar flexion angle was measured using a manual goniometer.

Fascicle length, pennation angle, and muscle thickness were measured from sagittal plane ultrasound images at midlength of the muscle belly (Figure 1A). To allow measurement of tendon length, the locations of the GM and SOL musculotendinous junctions (MTJ) were identified by ultrasonography and marked on the skin surface. An adhesive scaffold with embedded echogenic wires was placed along the Achilles tendon trajectory (Figure 1B). Sagittal plane ultrasound images were collected along the Achilles tendon from the calcaneal insertion to the GM MTJ. The images were sequentially combined by use of the echogenic markers (Figure 1C) and the lengths of the free Achilles tendon (up to SOL MTJ) and whole Achilles tendon (up to GM MTJ) were measured along the tendon path in the sagittal plane using imaging software (Fiji ImageJ[25]).

Achilles tendon cross-sectional area measures were obtained from transversal plane ultrasound videos sampled at 54 Hz (2-3 cm depth), taken in a rested, seated position as described below, at the proximal, middle, and distal part of the free Achilles tendon, using imaging software (Fiji ImageJ[25]). The proximal region was imaged at the most proximal tendon site without any soleus fibers and marked externally. Images of the distal region were taken at the most distal site before the calcaneal insertion and marked externally. The midlength scan was taken in the middle of the distance between the two marks.

2.4 | Electromyography (EMG)

To ensure optimal skin impedance for recording of EMG signals, standard preparation including shaving, gentle abrasion, and cleaning with isopropanol was performed. EMG electrodes (Ambu, Blue Sensor N, Ballerup, Denmark) were placed on SOL, GM, gastrocnemius lateralis (GL) and tibialis anterior (TA) with an interelectrode distance of 20 mm, as well as a reference electrode on the lateral part of the tibial tuberosity, all in accordance with SENIAM recommendations.[26]

Electromyographic signals were transmitted wirelessly (16-channel TeleMyo 2400 G2 Telemetry System, Noraxon Inc., Scottsdale, AZ, USA) to a receiver (Mini-receiver for TeleMyo G2, Noraxon Inc., Scottsdale, AZ, USA) and synchronized with other data in Noraxon software (MyoResearch XP Master Edition 1.08.17, Noraxon Inc., Scottsdale, AZ, USA).

The raw EMG data were digitized and sampled at 1500 Hz. Postprocessing was performed off-line using a software package (MATLAB and Statistics Toolbox Release 2015b, The MathWorks, Inc., Natick, Massachusetts, United States). Data were filtered using a bidirectional zero-lag fourth-order Butterworth bandpass filter of 10-500 Hz, rectified and integrated over 500 ms.

2.5 | Slow, passive ankle dorsiflexion stretch

Following a minimum of 20 minutes of rest imposed by ultrasound scanning and EMG preparation, passive torque and ultrasound videos were obtained from the left triceps surae MTU during slow, passive ankle dorsiflexion to maximal ROM in an isokinetic dynamometer (HUMAC® NORM TM Model 770, Computer Sports Medicine, Inc. CSMI, Stoughton, MA, USA).

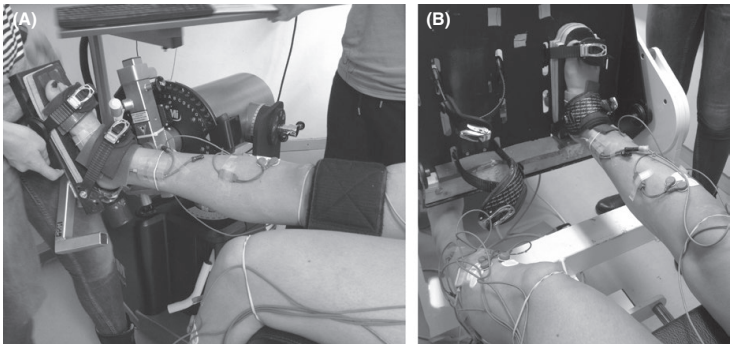

FIGURE 2 Dynamometer position setup. A, Isokinetic dynamometer subject positioning: Setup for slow, passive dorsiflexion stretch and strength tests. B, Isometric dynamometer subject positioning: Setup for maximal voluntary contractions and ramped isometric contractions

Subjects were seated with 65° hip flexion and the left knee extended. The dynamometer settings were individually adjusted to align the mediolateral rotation axis of the ankle joint with that of the dynamometer. Joint movement was minimized by careful strapping of the foot, thigh, waist, and trunk (Figure 2A). Subjects confirmed that they did not sense any stretch in the calf or hamstrings in the starting position.

To determine maximal ankle dorsiflexion ROM, the foot was manually moved at approximately 2°/s, each time by the same test leader, from a resting position into dorsiflexion until the individual limit of tolerance, as signalled verbally by the subject. The corresponding angle was recorded as maximal ROM. To prevent visual perception from influencing the determination of ROM, the test was taken with eyes closed. The level of pain at maximal ROM was recorded on a 10 cm visual analogue scale.

The ankle joint was passively dorsiflexed from 10° plantar flexion to maximal ROM and moved back to 10° plantar flexion, at 2°/s. Subjects were instructed to close their eyes and not to offer any active resistance to the movement. The procedure was repeated six times. The first four repetitions were used to record ultrasound data not presented here, while ultrasound videos of GM midbelly fascicles were sampled during the last two repetitions at 16-25 Hz (3-5 cm depth), depending on ultrasonography settings optimized for each subject. Ultrasound videos of SOL architecture were included in the protocol but were discarded because of the inadequate number of data sets of sufficient quality ($n = 4$ per group). Echo-absorptive tape was applied to the skin to enable correction for eventual probe displacement relative to the skin. Additional trials were performed if muscle contraction was detected during the trial or if the ultrasound video quality was insufficient. Each trial was separated by 120-second rest.

Torque, velocity, and dynamometer angle were obtained from the isokinetic dynamometer. Concurrently, anatomical ankle joint angle was obtained by a 2D electro-goniometer (Noraxon Inc., Scottsdale, AZ, USA), secured to the medial part of the 1st metatarsal and distal-medial part of tibia. EMG activity of SOL, GM, GL and TA was recorded, and all data were sampled using Noraxon equipment (Noraxon DTS TeleMyo System, Noraxon Inc., Scottsdale, AZ, USA). A function generator (GwinStec, GFG-8215A, Good Will Instrument Co., Ltd, Tucheng City, Taiwan) and an electric trigger signal were used to initiate sampling and synchronize data by producing a visual marker on the ultrasound videos.

Torque, velocity, dynamometer angle, goniometer angle, and EMG signals were digitized and sampled at 1500 Hz. Postprocessing was performed off-line using a software package (MATLAB and Statistics Toolbox Release 2015b, The MathWorks, Inc., Natick, Massachusetts, United States). Torque, dynamometer angle, and goniometer angle were filtered using a bidirectional zero-lag fourth-order Butterworth low-pass filter of 10 Hz. EMG amplitude was normalized to activity recorded during isometric maximal voluntary contraction (MVC) (see below).

Ankle joint angles during slow, passive stretch were obtained from the electro-goniometer, rather than from the isokinetic dynamometer, to avoid the error induced by the inconsistent heel attachment near maximal ROM, and hence differ from the dynamometer measurement of individual maximal ROM. Goniometer angle data were fitted to a fourth-order polynomial equation. All data were interpolated at 0.05° intervals using spline functions and the valid trials were averaged (two trials for ultrasonography variables, six trials for torque and EMG amplitude). Data are reported at neutral ankle joint angle (0°), at a common joint angle corresponding to the maximal goniometer ROM of the least flexible subject (5°), and as individual maximal values.

Passive musculo-tendinous joint stiffness was calculated as the slope of a fourth-order polynomial fit of the averaged passive torque-angle relation, at common joint angle and at individual maximal ankle dorsiflexion ROM.[27] Passive joint stiffness independently of joint angle was assessed by calculating the stiffness index defined by Nordez et al[27]—two times the leading coefficient from a second-order polynomial fit of the averaged passive torque-angle relation.

Gastrocnemius medialis fascicle lengths and pennation angles were measured by automatic tracking using optical flow

algorithms in specialized software.[28,29] GM MTU length was estimated from the average goniometer joint angle data using equations derived by Grieve et al.[30] Length change of the GM muscle and the series elastic element was estimated by combining fascicle length and pennation angle from the ultrasound images with MTU length, as described by Fukunaga et al.[31] Elongation of muscle and series elastic element, as well as elongation and strain of fascicles, are reported based on initial lengths and joint angles as measured with subjects resting in prone position. Muscle and series elastic element elongation are given as absolute values and normalized to overall MTU elongation. GM fascicle length and elongation, muscle elongation and series elastic element elongation normalized to resting MTU length follow the same trends as absolute values, and are not reported.

2.6 | Tendon stiffness

Following a 5-minute warm-up on a bike ergometer (Monarch, 828E, Varburg, Sweden), the structural stiffness of the free Achilles tendon and the whole Achilles tendon was examined by measuring tendon elongation during isometric ramp contractions, as previously described.[32] In brief, the ultrasound probe was placed sagittally over the distal MTJ of SOL and subsequently GM, and fixed to the leg using a custom-made rigid cast. The probe was fixed using elastic straps to maintain consistent positioning while minimizing compression. Ultrasound videos were sampled at 38-54 Hz (2-4 cm depth). Echo-absorptive tape was applied to the skin to enable correction for eventual probe displacement relative to the skin.

Participants were seated in a custom-made isometric ankle dynamometer (Gym2000, Geithus, Norway) instrumented with a load cell (U2A 500 Hottinger Baldwin Messtechnik, Darmstadt, Germany), with 90° hip flexion, with the left knee straight and the left foot strapped to the dynamometer at neutral ankle joint angle. The dynamometer settings were individually adjusted to align the axis of the ankle joint with that of the dynamometer. Joint movement was minimized by careful strapping of the foot, waist, and trunk (Figure 2B).

For the purposes of EMG normalization, the participants performed two trials of isometric plantar flexion MVC and two trials of isometric dorsiflexion MVC. Each trial was separated by 60-second rest.

Subsequently, participants performed ramped isometric plantar flexion contractions, following a rate of torque development template fixed to the computer monitor, for a duration of 4-6 second from onset to maximal contraction. Target (45 Nm/s) and produced torque development were displayed real-time, and standardized verbal encouragements were provided. Each trial was preceded by three quick, submaximal plantar flexion contractions serving to precondition the tendon.[32] Six trials were performed to record the displacements of the SOL and GM MTJs. Additional trials were performed if the ramp or ultrasound quality was insufficient. Torque was monitored during experiments to gauge fatigue. If maximal torque was reduced by more than 10% relative to the best trial, an additional trial was performed. Each trial was separated by 120-second rest, during which the foot was freed from the dynamometer.

Plantar flexion torque during the ramped contractions was calculated by multiplying the load cell force by the perpendicular distance to the axis of joint rotation. As ankle rotation was minimal ($4.0 \pm 2.6°$ across groups), the recorded torques were not corrected for TA coactivation.[33] Tendon force was calculated by dividing plantar flexion torque by the instantaneous tendon moment arm as derived from ankle joint angle and leg length using equations by Spoor et al.[34]

Displacement of the MTJs was measured relative to the skin marker by semiautomated tracking of the ultrasound videos in video-tracking software (Tracker Video Analysis and Modeling Tool V.4.62, Open Source Physics, Aptos, CA, USA). At each scan location, displacement was interpolated at 50 N intervals using a spline function and the valid trials were averaged. Tendon elongation was defined as displacement of the MTJ minus correction for unintentional ankle joint rotation. Correction for ankle joint rotation during isometric contraction[35] was performed by applying a linear fit of displacement-angle data from slow, passive ankle rotation from 0° to 3° of plantar flexion, to the instantaneous ankle joint angles as recorded during each ramped contraction.

Tendon stiffness is reported separately for the free Achilles tendon and the whole Achilles tendon. The individual tendon force and tendon elongation data were cut off at 90% of individual maximal force level and fitted with second-order polynomials ($R^2 = 0.95$-0.99). Tendon stiffness was defined as the slope of the force-elongation curve in its linear region: Tendon stiffness at common force levels was calculated from the individual polynomials, at force levels corresponding to 80%-100% of the maximal force of the weakest subject (1589-1986 N). Maximal tendon stiffness was calculated between 80% and 100% of individual maximal force after cutoff.

2.7 | Isometric and isokinetic muscle strength

Isometric and isokinetic muscle strength was tested using the same isokinetic dynamometer and position setup as described for slow, passive stretch. Isometric maximal plantar flexion torque was determined over a 5-second contraction, using the highest peak torque among two trials at each of 10° plantar flexion, 0°, 5°, 10° and 15° of dorsiflexion (dynamometer angles), performed in this order and separated by 60-second rest. Due to low dorsiflexion ROM in some subjects, control $n = 8$ at 10° dorsiflexion and control $n = 6$ at 15° dorsiflexion. Isokinetic concentric plantar flexion torque was

determined as the peak torque over motion from 10° of dorsiflexion to 30° of plantar flexion, using the trial with the highest peak torque among three trials, at each of 45°/s, 60°/s, 90°/s, performed in this order and separated by 60-second rest. These trials were preceded by a familiarization phase consisting of three submaximal plantar flexion contractions at 45°/s. Standardized verbal encouragements and visual feedback of the instantaneous torque produced were provided during testing.

For statistical analysis of the isokinetic torque-angle relations, torque values were interpolated at 5° intervals using a spline function. Work was calculated as the area below the isokinetic torque-angle curve, after interpolating torque at 0.1° intervals in the range of joint angles during which each subject was able to maintain positive torque production.

2.8 | Statistics

Some of the passive torque, strain, and tendon stiffness variables did not pass the normality test (D'Agostino & Pearson omnibus); hence, all intergroup differences relating to slow, passive stretch, and tendon stiffness were analyzed using Mann-Whitney *U* tests. Intergroup differences relating to descriptive data, anthropometry, ROM, resting MTU properties, and muscle strength were analyzed using unpaired, two-tailed Student's *t*-tests. Differences in passive and active torque-angle relations were analyzed using two-way mixed model ANOVA, with group and joint angle as factors. Post hoc, Sidak's multiple comparisons tests were performed. Level of significance was set to $\alpha = 0.05$. All data are presented as mean ± SD.

3 | RESULTS

Maximal ankle dorsiflexion ROM was greater in dancers compared to controls (dancers 40.1 ± 6.7°, controls 17.4 ± 9.2°, $P < .00001$). Experienced pain at maximal ROM was less in dancers compared to controls (dancers 1.9 ± 2.2 cm, controls 5.0 ± 2.0 cm, $P < .005$).

3.1 | Morphological properties of the MTU

The groups had similar resting plantar flexion angles (dancers 26.0 ± 4.6°, controls 24.0 ± 2.3°, $P = .24$). Dancer GM displayed greater thickness and longer fascicles compared to controls (Table 1), while SOL fascicles were shorter in dancers compared to controls (Table 1). Pennation angles were similar between groups both in GM and SOL (Table 1). The free Achilles tendon had similar length between groups, while the whole Achilles tendon was longer in dancers compared to controls (Table 1). Achilles tendon proximal, midtendon, and distal cross-sectional areas were similar between groups (dancers 62 ± 14 mm^2, 59 ± 12 mm^2, 75 ± 15 mm^2 respectively, controls 62 ± 25 mm^2, 53 ± 8 mm^2, 73 ± 20 mm^2, $P = .14$-$.99$).

3.2 | Mechanical properties during slow, passive ankle dorsiflexion stretch

Dancers tolerated greater ankle dorsiflexion (Table 2), but at the maximal ROM, passive torque and passive joint stiffness were similar between groups (Table 2). At common joint angle (5°), dancers had lower passive torque and passive joint stiffness compared to controls (Table 2). The passive torque-angle

TABLE 1 Morphological properties of GM, SOL and the Achilles tendon

	Dancers	Controls	*P*
Gastrocnemius medialis			
Thickness (mm)	22 ± 2	18 ± 2	<.001
Pennation angle (°)	24 ± 2	25 ± 2	.14
Fascicle length (mm)	55 ± 5	47 ± 6	<.005
Fascicle length (% of resting MTU length)	12 ± 1	11 ± 1	<.0005
Soleus			
Thickness (mm)	15 ± 2	17 ± 4	.35
Pennation angle (°)	22 ± 5	24 ± 5	.29
Fascicle length (mm)	37 ± 4	42 ± 5	<.05
Fascicle length (% of resting MTU length)	8 ± 1	9 ± 1	<.05
Whole Achilles tendon			
Length (mm)	207 ± 33	167 ± 10	<.005
Length (% of resting MTU length)	47 ± 7	37 ± 2	<.001
Free Achilles tendon			
Length (mm)	65 ± 28	51 ± 19	.22
Length (% of resting MTU length)	15 ± 6	12 ± 4	.23

TABLE 2 Joint angle, passive torque, passive joint stiffness, and stiffness index during slow, passive stretch

	Dancers	Controls	*P*
Ankle dorsiflexion angle (°)			
Individual maximal values	29.0 ± 4.1	13.3 ± 6.0	<.000005
At common maximal torque (13 Nm)	6.6 ± 5.4	0.2 ± 2.0	<.005
Passive torque (Nm)			
At neutral joint angle (0°)	12 ± 3	17 ± 7	<.05
At common joint angle (5°)	14 ± 5	23 ± 8	<.05
Individual maximal values	44 ± 13	40 ± 17	.58
Passive joint stiffness (Nm/°)			
At common joint angle (5°)	0.6 ± 0.5	1.3 ± 0.5	<.01
Individual maximal values	2.9 ± 1.3	2.5 ± 1.3	.54
Stiffness index (Nm/°^{2})	1.4 ± 0.6	2.3 ± 1.2	<.05

relation displayed lower torque values in dancers compared to controls across the range up to common joint angle ($F = 5.555$, $P < .0001$, Figure 3A). At common passive torque (13 Nm), the dancers displayed greater ankle dorsiflexion angles compared to controls (Table 2). The angle independent stiffness index was lower in dancers compared to controls (Table 2).

Gastrocnemius medialis fascicle length was greater in dancers compared to controls both at common joint angle and at individual maximal length (Table 3), while pennation angles were similar between groups (Table 3). At common joint angle, elongation of the GM muscle as well as elongation and strain of the GM fascicles was similar between groups (Table 3). At common joint angle, GM muscle accounted for 58% of the overall MTU elongation in dancers and 48% in controls (Table 3). Looking at individual maximal values, dancers displayed greater elongation of the GM muscle and GM fascicles (Table 3, Figure 3C) and had greater individual maximal fascicle strain (Table 3). GM muscle accounted for maximally 63% of the MTU elongation in dancers and 53% in controls (Table 3). The EMG amplitudes of SOL, GM, and GL (Figure 3B) were similar between groups at neutral ankle joint angle (dancers 2.4 ± 1.6%, 0.5 ± 0.3%, 0.7 ± 0.6%, respectively, controls 5.8 ± 4.0%, 0.9 ± 0.6%, 1.2 ± 0.8%, $P = .11$-.19), but significantly lower in dancers compared to controls in all three muscles at common joint angle (dancers 2.9 ± 2.0%, 0.5 ± 0.3%, 0.7 ± 0.6%, controls 8.2 ± 4.5%, 2.0 ± 2.4%, 1.8 ± 1.1%, $P < .05$) and at peak EMG, which occurred slightly before maximal ROM in some subjects (dancers 6.2 ± 4.3%, 1.1 ± 0.9%, 1.4 ± 1.5%, controls 14.7 ± 7.5%, 7.1 ± 6.8%, 3.9 ± 2.8%, $P < .05$).

3.3 | Tendon mechanical properties

One dancer was eliminated from free Achilles tendon stiffness analyses due to insufficient ultrasound video quality. Another dancer was eliminated from whole Achilles tendon stiffness analyses because data synchronization signals were lost during data acquisition. Whole Achilles tendon stiffness (Figure 4) was lower in dancers compared to controls, both at common force (1987 N) (dancers 230 ± 87 N/mm, controls 364 ± 106 N/mm, $P < .005$) and at individual maximal force (dancers 261 ± 110 N/mm, controls 401 ± 116 N/

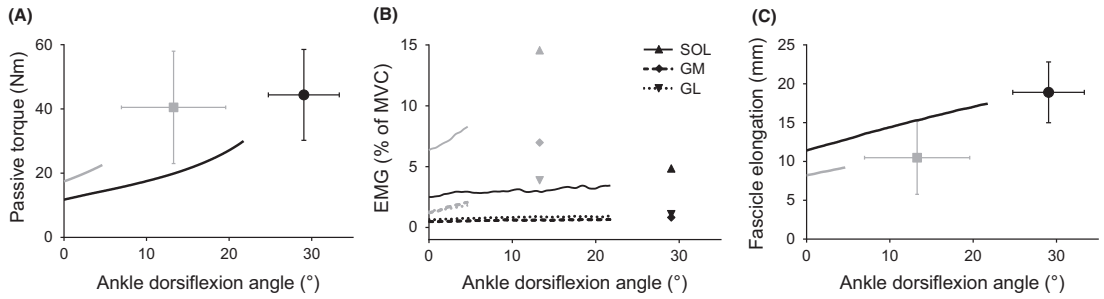

FIGURE 3 Torque, EMG and fascicle elongation during passive ankle dorsiflexion. Group mean passive torque (A), electromyographic (EMG) amplitude normalized to activity recorded during isometric maximal voluntary contraction (MVC) (B) and gastrocnemius medialis fascicle elongation (C) during slow, passive dorsiflexion stretch in dancers (black) and controls (gray). Symbols indicate mean of individual maximal values ± standard deviations in dancers (●) and controls (■). For EMG, error bars are omitted for legibility—we refer the reader to the text for standard deviations

	Dancers	Controls	*P*
GM pennation angle (°)			
At neutral position (0°)	13 ± 2	13 ± 2	.58
At common joint angle (5°)	13 ± 2	13 ± 3	.63
Individual maximal values	14 ± 2	14 ± 2	.85
GM fascicle length (mm)			
At neutral position (0°)	67 ± 6	55 ± 7	<.005
At common joint angle (5°)	68 ± 6	56 ± 7	<.005
Individual maximal values	74 ± 6	58 ± 6	<.0001
GM fascicle elongation (mm)			
At common joint angle (5°)	13 ± 3	10 ± 3	.06
Individual maximal values	19 ± 4	11 ± 4	<.005
GM fascicle strain (%)			
At common joint angle (5°)	23 ± 5	21 ± 9	.14
Individual maximal values	35 ± 8	24 ± 11	<.05
GM muscle elongation (mm)			
At common joint angle (5°)	16 ± 3	13 ± 3	.07
Individual maximal values	21 ± 4	14 ± 4	<.005
GM muscle elongation (% of MTU elongation)			
At common joint angle (5°)	58 ± 15	48 ± 12	.09
Individual maximal values	63 ± 16	53 ± 13	.09
Series elastic element elongation (mm)			
At common joint angle (5°)	12 ± 6	13 ± 3	.30
Individual maximal values	25 ± 8	19 ± 4	.09
Series elastic element elongation (% of MTU elongation)			
At common joint angle (5°)	42 ± 15	52 ± 12	.09
Individual maximal values	53 ± 10	58 ± 10	.22

TABLE 3 Architecture, elongation and strain of GM and the series elastic element during slow, passive stretch

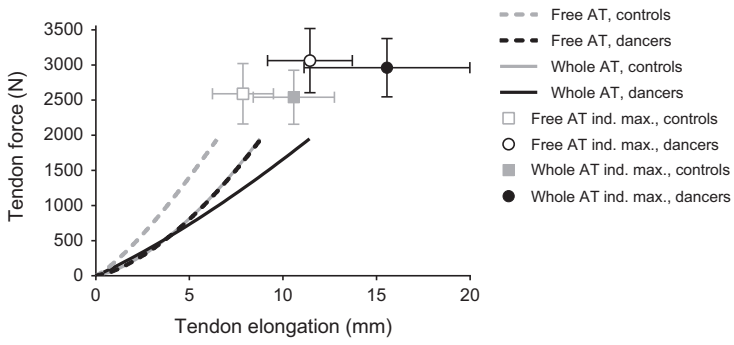

FIGURE 4 Tendon stiffness: Force-elongation. Group mean force-elongation relationship of the free Achilles tendon (dashed lines, open symbols) and the whole Achilles tendon (solid lines, filled symbols) of controls (gray) and dancers (black) during ramped isometric plantar flexion contractions. Symbols indicate mean of individual maximal force and elongation ± standard deviations in dancers (●) and controls (■)

mm, $P < .005$). The individual maximal force was greater in dancers compared to controls (dancers 3090 ± 441 N, controls 2645 ± 423 N, $P < .05$). Stiffness of the free Achilles tendon (Figure 4) was not different between groups at common force (dancers 349 ± 67 N/mm, controls 423 ± 173 N/mm, $P = .55$) nor at individual maximal force (dancers 425 ± 100 N/mm, controls 482 ± 218 N/mm, $P = .84$).

3.4 | Isometric and isokinetic muscle strength

During isometric ankle plantar flexion at joint angles from +10° to −15° (5° increments), dancers and controls produced similar maximal torques (Figure 5B, $P = .71$-$.98$). In isokinetic plantar flexion efforts (45, 60 and 90°/s), the peak

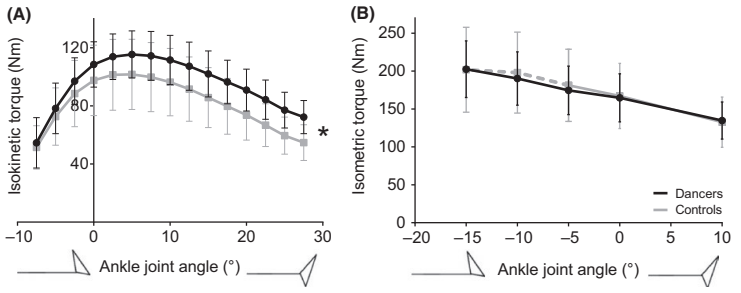

FIGURE 5 Isokinetic and isometric ankle plantar flexion contractions. Torque-angle relations for maximal voluntary concentric isokinetic plantar flexion contractions at 45°/s (A) and isometric plantar flexion contractions (B) in dancers (●) and controls (■). *indicates a significant interaction between group and joint angle ($P < .05$)

torque (dancers 116 ± 127 Nm, 105 ± 16 Nm, 87 ± 15 Nm, respectively, controls 102 ± 24 Nm, 92 ± 20 Nm, 77 ± 16 Nm, $P = .13$-$.15$), angle of peak torque (dancers 5 ± 2°, 7 ± 2°, 8 ± 2° respectively, controls 4 ± 1°, 5 ± 1°, 7 ± 1°, $P = .12$-$.33$), and total work (dancers 145 ± 21 J, 133 ± 21 J, 115 ± 18 J, respectively, controls 124 ± 29 J, 113 ± 24 J, 98 ± 20 J, $P = .06$-$.07$) were similar between groups. At 45°/s only, the isokinetic torque-angle relation displayed a significant interaction between group and joint angle ($F = 1.74$, $P < .05$, Figure 5A). At 60°/s, interaction $P = .58$ and group $P = .058$. At 90°/s, interaction $P = .28$ and group $P = .066$.

4 | DISCUSSION

The aim of this study was to investigate whether highly flexible ballet dancers display different morphological, mechanical, and functional properties of the triceps surae MTU, compared to control subjects with no history of stretching training. The main findings were that dancers presented longer GM fascicles, a longer and more compliant Achilles tendon, as well as greater maximal strain of the GM fascicles and greater maximal elongation of the GM muscle, which likely facilitated the greater maximal ankle dorsiflexion ROM of the dancers. Furthermore, lower levels of normalized muscle activity and pain perception in the dancers support the role of neural factors and suggest that joint flexibility is gained through adaptations at multiple levels.

4.1 | ROM and torque-angle properties

Maximal ankle dorsiflexion ROM was considerably greater and the corresponding perception of pain was much lower in dancers compared to controls, despite similar maximal passive torque and passive joint stiffness at maximal ROM. This is in contrast to the study by Blazevich et al,[21] where the flexible group tolerated torques approximately 50% greater than the less flexible group. The maximal passive torques of the present study are more in line with those reported by Abellaneda et al[22] at 30° dorsiflexion. Between-studies differences in maximal passive torque and ROM may relate to tolerance to stretch, different experimental protocols and individual variations in joint anatomy. Further research is needed to understand the potential influence of passive torque and passive joint stiffness on the maximal ROM as defined by the endpoint of tolerance to stretch.

4.2 | Resting ankle joint angle

The resting ankle joint angle in the prone position was similar between groups. Resting joint angle is determined by the sum of all torques acting around the joint. Albeit speculative, it is possible that the increased operating range of the dancers does not imply different slack length of tissues surrounding the joint but rather depends on their material properties. In the present study, passive torque was already lower in dancers than controls at neutral ankle joint angle. These findings underline that the so-called neutral ankle joint angle (foot perpendicular to the shank) is not necessarily a valid starting point for flexibility measures such as elongation of the different components of the MTU.

4.3 | Muscle activity

When passively stretched to a common joint angle, the dancers displayed lower triceps surae activation compared to controls. This is partially in line with Abellaneda et al,[22] who at a submaximal dorsiflexion angle of 30° found lower GM activation but similar SOL activation in their flexible group compared to controls. The dancers of the present study also displayed lower peak EMG during passive dorsiflexion compared to controls, whereas Blazevich et al[21] saw no group differences in peak EMG, although group differences in maximal ROM were comparable to those of the present study. The lower activation levels in the dancers suggest proprioceptive adaptation at a sensory or neural level, which may be a response to the specific training history of the dancers.

4.4 | Tendon morphological properties

As expected, there were no differences in length or cross-sectional area of the free Achilles tendon. However, the whole Achilles tendon was longer and less stiff in dancers compared to controls. For a given Young's modulus, a longer tendon undergoes a greater total elongation. Therefore, the present group differences may affect the working conditions for the contractile tissues in series. In the present study, the lower stiffness of the whole Achilles tendon suggests that its proximal portion (ie excluding the free Achilles tendon) undergoes greater deformation under tension in dancers. Previous stretching intervention studies which revealed reduced tendon stiffness included training protocols with active muscular contractions near maximal ROM in the form of PNF[16] or ballistic[15] stretching. With these types of stretching, the muscle shortens while the rest of the MTU is being stretched, yielding higher tendon strain and, possibly, inducing greater adaptive response in the tendinous tissue than with passive stretching methods. Ballet dancer Achilles tendons are subjected to large amounts of tendon loading, through repetitive submaximal and maximal jumps, yet do not have greater tendon stiffness compared to controls. In general, tendon stiffness is seen to increase with habitual loading such as strength training.[36] The present finding may be related to the extensive amounts of passive and dynamic stretching in ballet, or to the ballet-specific loading conditions with a large operating range of the MTU.

4.5 | Muscle morphological properties

The small changes in pennation angle during passive stretch and the lack of group differences suggest a limited role for pennation angle with respect to modifying ROM. On the other hand, longer GM fascicles in dancers compared to controls suggests a greater number of sarcomeres in series, which has implications for contractile function through an altered force-length relationship.[37] In line with these results, human intervention studies have measured increased GM fascicle length following stretching training.[8-10] In vivo, true fascicle resting length and number of sarcomeres in series cannot be assessed. The present study obtained morphological measurements in resting prone position, which seems a better approximation to resting length than neutral ankle joint angle, but fascicle length remains a crude approximation of sarcomere number and care must be taken when interpreting these results. Nonetheless, the present findings suggest that human stretching training may constitute a load sufficient to induce adaptations on the sarcomere level. In contrast to the present results, cross-sectional comparisons[21,22] and a stretching intervention study[11] found no differences in GM fascicle length. Further research is needed to understand the relationship between fascicle length and musculoskeletal flexibility.

4.6 | Passive MTU elongation

At or near individual maximal ROM, we found that dancers had greater elongation and strain of GM fascicles and greater elongation of muscle, which is in line with previous studies.[21,22] These results suggest that properties of both muscle and series elastic element contribute to the greater MTU elongation obtained by dancers. The numerical values may indicate that muscle contributes to a greater extent in dancers (63% of maximal MTU elongation) compared to controls (53%); however, these differences did not reach statistical significance. Greater contribution from muscle would be in agreement with Abellaneda et al,[22] where fascicles contributed 72% and tendon 28% at 30° dorsiflexion, and an intervention study which found increased muscle and fascicle strain and decreased tendon strain at a constant joint angle following stretching training.[38] Taken together, these findings suggest that the capacity for muscle elongation plays an important role for ROM.

4.7 | Factors limiting ROM

The mechanisms limiting MTU elongation are not merely anatomical. For instance, tendon strains above 9% have been measured in active conditions,[39] implying that tendon can elongate more than seen during passive stretch in previous studies.[21,22] Under experimental conditions, the maximal passive ROM is driven by maximal tolerance to stretch, which arguably occurs before maximal strain of the MTU. Possibly, there is a two-layer system where neural factors limiting the tolerance to stretch must be modified before morphological adaptations such as the specialized properties seen in the ballet dancer group of the present study may occur.

4.8 | Function

In the present study, the impact of morphological and mechanical properties on function was assessed through maximal isometric and isokinetic contractions. Dancers and controls had comparable maximal strength, and angle of peak torque remained similar, despite longer GM fascicles in the dancers. The torque-angle relation showed a significant interaction between group and joint angle at only one of three isokinetic velocities. These findings contradict former studies showing group differences in the knee flexion torque-angle relation, with flexible subjects displaying angle of peak torque at more extended positions, lower torque at shorter muscle lengths and greater torque at greater muscle length, compared to less flexible subjects.[20,24] The longer GM fascicles found in dancers, if representing more sarcomeres in

series, should theoretically cause an optimal myofilament overlap at greater muscle length[37] and thus shift peak torque to a more dorsiflexed angle. This effect is likely counteracted by the lower tendon stiffness seen in dancers, which may cause the tendon to elongate more for a given contractile force, which in turn could mean that muscles work at a shorter, less optimal length. The seemingly greater plantar flexion work seen in dancers did not reach significance in the present study. However, a type II error may have occurred ($P = .06$-$.07$ across contraction velocities) for this variable. This hypothesis would be in line with previous studies showing greater knee flexor work in flexible compared to less flexible subjects[20] and following stretching interventions.[40] Greater work may be attributed to more sarcomeres in series, enabling similar torque production over a wider range of joint angles, suggesting a functional advantage of greater ROM.

4.9 | Limitations

Ballet dancers undergo years of intensive training where stretching is one of many loading stimuli. For this reason and because of a possible selection bias, the cross-sectional design of the present study provides a valid but limited insight into adaptations to habitual stretching. Longitudinal studies are needed to separate stretching-induced adaptations from inherent properties and from adaptations to other parts of training regimes. Nonetheless, the present study fulfilled the purpose of identifying differences in MTU morphological, mechanical, neural, and functional properties that may influence maximal joint ROM.

4.10 | Conclusions

The greater ankle dorsiflexion ROM seen in professional ballet dancers compared to controls is attributable to longer GM fascicles, greater fascicle strain and greater muscle elongation, a longer and less stiff whole Achilles tendon, lower passive torque and lower passive joint stiffness during passive stretch. Concurrently, lower passive normalized muscle activity and pain perception in the dancers suggest that proprioceptive adaptation to habitual stretching also plays a role in determining maximal joint ROM

5 | PERSPECTIVE

The present study underlines that both neural factors and morphological and mechanical properties of the musculotendinous system differ in individuals with varying levels of musculoskeletal flexibility. Long-term (ie >3-month) stretching intervention studies are needed to understand if these features in fact result from stretching training, and importantly whether such adaptations influence function, performance and injury risk.

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Paper III

Title: Altered triceps surae muscle-tendon properties after 6 months of unilateral stretching

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ABSTRACT

This study examined the effects of 24 weeks of stretching of the triceps surae on ankle joint range of motion (ROM), morphological and mechanical properties of the muscle-tendon unit (MTU), neural activation and contractile function. Daily 4x60-sec static dorsiflexion stretches were applied unilaterally (n=26), with the contralateral leg as control. Torque-angle and torque-velocity data were obtained during passive and active conditions by use of isokinetic dynamometry, while muscle and tendon morphology and mechanical properties were evaluated by ultrasonography. Following the intervention, ROM increased (stretching +11±7°, control +4±8°), while passive torque (stretching -10±11 Nm, control -7±10 Nm), passive joint stiffness and normalized triceps surae electromyographic (EMG) amplitude (soleus: stretching -3±6%, control -3±4%) at standardized dorsiflexion angle decreased. Passive muscle and tendon elongation and standardized-force tendon elongation increased (stretching +1.3±1.6 mm, control +1.4±2.1 mm), and angle of peak torque shifted towards dorsiflexion. However, no changes were seen in gastrocnemius medialis fascicle length, tendon length or tendon stiffness. Conformable changes in ROM, passive dorsiflexion variables, tendon elongation and angle of peak torque were observed in the non-stretched leg indicating a bilateral effect of stretching. The present findings indicate that stretching training increases ROM through a combination of neural adaptations and structural adaptations possibly related to tendon material properties, which together translate into altered mechanical properties and have the potential to modify contractile function. The role of central mechanisms for increases in ROM was confirmed by bilateral responses to stretching training.

Keywords:

Flexibility, passive resistance, length-tension, ultrasound, stretch tolerance

INTRODUCTION

Joint flexibility is an important component of physical fitness and health (Corbin & Noble, 1980; Garber et al., 2011). While it is known that habitual stretching increases joint range of motion (ROM) (Magnusson, Simonsen, Aagaard, Sorensen, & Kjaer, 1996; Lucas & Koslow, 1984), the physiological mechanisms behind increased ROM are not well understood (Freitas et al., 2017). An increased understanding will enable more optimal recommendations regarding the ability of stretching training to facilitate functional performance (Shrier, 2004; Medeiros & Lima, 2017), improve function (Stathokostas, Little, Vandervoort, & Paterson, 2012) or to prevent injuries (Thacker, Gilchrist, Stroup, & Kimsey, Jr., 2004; Weldon & Hill, 2003; Witvrouw, Mahieu, Danneels, & McNair, 2004).

Stretching-induced increases in ROM are often attributed to increased tolerance to stretch or pain (Freitas et al., 2017), when they are observed i) without change in passive torque at a pre-defined submaximal joint angle (hereafter referred to as standardized joint angle) (Magnusson et al., 1996; Konrad & Tilp, 2014b), ii) alongside an increased maximal passive torque (Blazevich et al., 2014) and/or iii) with unchanged joint angle at a given passive torque (Ben & Harvey, 2010). While increased tolerance likely contributes to increases in ROM, the specific neural mechanisms and the roles of pain threshold, pain sensitivity, reflex loops and neural activation are not well understood. Studies of neural adaptations to stretching are scarce and report discrepant findings: One study observed reduced tonic reflex activity following 6 weeks of stretching (Guissard & Duchateau, 2004), while 3-6 weeks of stretching did not reduce electromyographic (EMG) amplitude during passive stretching (Magnusson et al., 1996; Blazevich et al., 2014) or change motoneuron excitability (Hayes et al., 2012).

Contrasting the studies attributing increased ROM to tolerance, reduced passive resistance to stretch is indicated by studies where 3-6 weeks of stretching led to right-shifted

passive torque-angle curves (Kubo, Kanehisa, & Fukunaga, 2002; Toft, Espersen, Kalund, Sinkjaer, & Hornemann, 1989; Guissard & Duchateau, 2004), reductions in passive torque at standardized joint angles (Nakamura, Ikezoe, Takeno, & Ichihashi, 2012; Chan, Hong, & Robinson, 2001; Mahieu et al., 2007) or reductions in passive joint stiffness (Guissard & Duchateau, 2004; Kubo et al., 2002; Marshall, Cashman, & Cheema, 2011). Furthermore, flexible subjects have consistently displayed lower passive torque at standardized joint angles compared to less flexible subjects (Blazevich et al., 2012; Abellaneda, Guissard, & Duchateau, 2009; Magnusson et al., 1997; Moltubakk, Eriksrud, Paulsen, Seynnes, & Bojsen-Moller, 2016; Moltubakk, Magulas, Villars, Seynnes, & Bojsen-Moller, 2018). Reduced passive resistance to stretch may stem from reduced neural activation, altered morphological properties and/or altered mechanical properties, but the contribution of each factor is not well understood. Animal studies have demonstrated that extensive stretching may increase the number of serial sarcomeres and muscle fibre length (Tabary, Tabary, Tardieu, Tardieu, & Goldspink, 1972; Takahashi, Ward, Marchuk, Frank, & Lieber, 2010). Human stretching interventions providing indirect measures of muscular adaptations are inconsistent, with reports of increased fascicle length in some (Freitas & Mil-Homens, 2015; Simpson, Kim, Bourcet, Jones, & Jakobi, 2017), but not all studies (Blazevich et al., 2014; Konrad & Tilp, 2014b; Konrad & Tilp, 2014a; Konrad, Gad, & Tilp, 2015; Nakamura et al., 2012; e Lima, Carneiro, de S Alves, Peixinho, & de Oliveira, 2015). To date, there is no evidence of longitudinal growth with human stretching training, and whether reduced passive resistance is caused by morphological, mechanical and/or neural mechanisms is not known.

The contribution of muscle versus tendon to the MTU elongation during passive stretching is investigated in flexible compared to less flexible subjects, where the greater MTU elongation appears to result from greater elongation of both muscle and tendon, with a potential greater role of muscle (Abellaneda et al., 2009; Moltubakk et al., 2018). Following 3

weeks of stretching, increased ROM was accompanied by increased muscle and fascicle strain at standardized joint angle, suggesting increased muscle compliance, and muscle rather than tendon accounted for the increased elongation at the increased maximal joint angle (Blazevich et al., 2014). Similarly, increased muscle elongation was indicated after 4 weeks of stretching (Nakamura et al., 2012), and passive muscle stiffness of the GM MTU was decreased following stretching for 4 weeks (Nakamura et al., 2017) and 6 weeks (Kay et al., 2016), suggesting altered muscle behaviour with stretching training.

The effects of stretching training on tendon properties are more equivocal. Reports from intervention studies vary between unchanged tendon stiffness (Mahieu et al., 2007; Mahieu, Cools, De, Boon, & Witvrouw, 2009; Kubo et al., 2002; Konrad & Tilp, 2014b), reduced tendon stiffness (Kubo, Kanehisa, Kawakami, & Fukunaga, 2001; Konrad et al., 2015; Mahieu et al., 2007) and increased tendon stiffness (Kay et al., 2016), and differences in stretching modalities cannot fully explain the discrepancies. Theoretically, an increased ROM could be associated with altered tendon morphological properties, which is investigated by one study where 6 weeks of stretching did not alter Achilles tendon length or thickness (Simpson et al., 2017).

One of the advantages attributed to stretching training is the influence that increased ROM may have on contractile function. Angle of peak torque may shift towards an extended position if the conditions for optimal myofilament overlap are altered (Gordon, Huxley, & Julian, 1966), e.g. through addition of serial sarcomeres or increased tendon elongation, or if the contribution from passive resistance to torque output is reduced. Addition of serial sarcomeres would additionally enable the muscle to rotate the limb at the same angular velocity with reduced sarcomere shortening velocity, enabling greater torque production, and a more optimal sarcomere length across a wider range of joint angles (Lieber & Friden, 2000), potentially increasing contractile work. Consistent with these theories, 6-8 weeks of stretching

shifted angle of peak torque towards extended positions (Ferreira, Teixeira-Salmela, & Guimaraes, 2007; Chen et al., 2011), while another intervention study did not see a consistent shift in angle of peak torque (Handel, Horstmann, Dickhuth, & Gulch, 1997). Similarly, the implications of stretching training on isokinetic work production are not clear, as concentric work was increased uniformly in some studies (Handel et al., 1997), while in other studies, this effect was seen only at certain velocities (Ferreira et al., 2007). Collectively, the former studies investigating the effect of stretching training on contractile function neither document any rationale for conducting stretching training for improved contractile function, nor provide consistent indications of mechanisms behind increased ROM.

Taken together, previous studies provide an inconsistent overview of the role of morphological, mechanical and neural properties as mechanisms for increased ROM with stretching training. Studies applying more than 12 weeks of stretching are scarce and have not investigated physiological adaptations (Santonja Medina, Sainz De Baranda, Rodriguez Garcia, Lopez Minarro, & Canteras, 2007; Simao et al., 2011; Donti et al., 2018). A few authors have speculated that early adaptations to stretching training occur on a sensory level (Freitas et al., 2017) or are related to non-muscular structures which may be richly innervated but contribute marginally to passive torque (Nordez et al., 2017), while adaptations altering passive resistance through the structural properties of the MTU may occur with greater stretching durations (Freitas et al., 2017). Such a two-phasic pattern of adaptation is supported by intervention studies where maximal passive torque increased after 2-4 weeks, but returned to (Chan et al., 2001) or below (Guissard & Duchateau, 2004) the initial level after 4-8 weeks. Increased maximal passive torque could result from increased pain tolerance or reduced sensory input due to adaptations in non-muscular structures. The subsequent return to initial passive torque levels, despite increased ROM, could indicate that the first phase of adaptations has enabled the stretching stimulus to reach and modify the force-bearing

structures of the MTU. However, stretching interventions of greater duration are required to assess the interplay between ROM and passive resistance to stretch.

The purpose of this study was therefore to examine the effects of 24 weeks of unilateral triceps surae stretching on ROM, on the morphological and mechanical properties of the MTU, on neural activation and on contractile function. It was hypothesized that ROM would increase by 8 weeks, while by 24 weeks, passive torque, passive joint stiffness and EMG amplitude at standardized dorsiflexion angle as well as tendon stiffness would be reduced, while maximal passive torque and passive elongation of muscle and tendon would be increased, morphological properties would be unchanged, and peak isokinetic torque would occur at a more dorsiflexed joint angle.

METHODS

Subjects

A priori sample size calculations were done by estimating PRE-POST changes in passive torque of 3 Nm, with a standard deviation of 3 Nm, based on former stretching intervention studies (Kubo et al., 2002; Mahieu et al., 2007). Statistical power was set to 90 %. In order to accommodate an anticipated dropout of 25 %, 30 subjects (12 men, 18 women) were recruited amongst recreationally active university students. Exclusion criteria were lower limb injury within the past 6 months, musculo-skeletal diseases, other conditions preventing stretching of the targeted MTUs (e.g. limited talus posterior glide), and/or a history of systematic stretching (stretching > 10 minutes once a week or more frequently,, and/or shorter sessions > 3 times a week). In accordance with the Declaration of Helsinki, the regional ethics committee approved the study, and each subject signed an informed consent form.

One subject withdrew from the study due to an injury suffered outside the intervention, three withdrew for personal reasons. The final data set comprised 26 subjects aged 22.0 ± 1.6, of which 17 were women (169 ± 7 cm, 61 ± 11 kg) and 9 were men (184 ± 5 cm, 80 ± 12 kg), reporting a weekly training volume of 2 ± 2 hours of endurance activities and 1 ± 1 hours of strength training during the intervention period.

Experimental design

A within-subjects design was used, where 24 weeks of stretching was applied to one leg, while the contralateral leg served as control. Assignment of stretching leg was made by stratified randomization such that one half the subjects stretched their dominant leg (stance leg while kicking a ball), while the other half stretched their non-dominant leg. Two subjects had an initial side-to-side ROM difference of > 10° and were assigned to stretch their least flexible leg. The testing order of the legs was randomized at the first test session and repeated identically at subsequent sessions. Passive dorsiflexion, tendon stiffness and contractile function tests were completed for one leg before proceeding to the second leg. The investigators were blinded to the leg assignment during testing and post-intervention analyses. All measurements were undertaken by the same investigators.

Each subject reported to the lab five times: A familiarization session where resting measurements of muscle and tendon morphology were also obtained, a pre-intervention test session (PRE), a subset of tests after 8 and 16 weeks, and a post-intervention test session (POST) 24-48 hours after the last bout of stretching. The subjects were instructed to refrain from training or stretching 24 hours prior to each session. In female subjects who did not take constant-dose oral contraceptives, all tests were conducted within 14 days from the last menstruation, to avoid testing during the luteal phase.

The subjects were instructed to maintain their habitual activity level, refrain from unaccustomed exercise, maintain their normal diet, refrain from anti-inflammatory drugs or nutritional supplements and report any illness or injury during the experimental period.

Stretching training

The intervention consisted of four self-administered 60-sec static ankle dorsiflexion stretches (Figure 1), performed daily for 24 weeks. Prior to the intervention, the subjects received written and individualized verbal instructions. Their stretching technique was controlled at each test session. The perception of pain during the preceding week's stretching training was recorded on a 10 cm visual analogue scale (VAS score) at each test session. Adherence to the stretching program was monitored through written journals and phone follow-ups between the test sessions.

(Insert Figure 1 about here)

Anthropometry, resting muscle architecture and morphological properties

Leg length was measured in a standing position as the distance between the trochanter major and the floor. Calf length was measured from the lateral femoral epicondyle to the most posterior point of the calcaneal tuberosity.

Subjects were lying in a prone position with their foot hanging freely off the examination bed. Resting ankle joint angle was measured using a manual goniometer. Fascicle length, pennation angle and muscle thickness of gastrocnemius medialis (GM) and length of the Achilles tendon were measured using real-time B-mode ultrasonography with a 50-mm linear array transducer (L12-5, Philips, Bothell, WA, USA) and ultrasound system (HD11XE, Philips, Bothell, WA, USA). The lower half of the transducer frequency (5-12

MHz) was used with a built-in filter to optimise ultrasound penetration while preserving spatial resolution and contrast.

All ultrasound still images were analysed using imaging software (Fiji ImageJ (Schindelin et al., 2012)). Fascicle length, pennation angle and muscle thickness were measured from sagittal images recorded at mid-length of the muscle belly (Moltubakk et al., 2018). The lengths of the free Achilles tendon (calcaneal insertion to soleus (SOL) MTJ) and of the whole Achilles tendon (calcaneal insertion to GM MTJ) were measured along the tendon path from sequentially combined sagittal images (Moltubakk et al., 2018).

Electromyography

To ensure optimal skin impedance for recording of EMG signals, shaving, gentle skin abrasion and cleaning with isopropanol was performed in accordance with SENIAM recommendations (Hermens, Freriks, Disselhorst-Klug, & Rau, 2000). EMG electrodes (Ambu, Blue Sensor N, Ballerup, Denmark) were placed on SOL, GM and gastrocnemius lateralis (GL) with an inter-electrode distance of 20 mm, and a reference electrode was positioned on the tibial tuberosity. EMG signals were transmitted wirelessly (16-channel TeleMyo 2400 G2 Telemetry System, Noraxon Inc., Scottsdale, AZ, USA) to a receiver (Mini-receiver for TeleMyo G2, Noraxon Inc., Scottsdale, AZ, USA). EMG signals were filtered using a bidirectional zero-lag fourth-order Butterworth bandpass filter of 10-500 Hz, rectified and integrated over 500 ms. EMG amplitudes were normalized to amplitudes recorded during maximal voluntary contractions (MVC).

Data sampling, synchronization and post-processing

For passive dorsiflexion, tendon stiffness and tests of contractile function, EMG, dynamometer and ankle goniometer data were digitized and sampled at 1500 Hz

(MyoResearch XP Master Edition 1.08.17, Noraxon Inc., Scottsdale, AZ, USA). A function generator (GwinStec, GFG-8215A, Good Will Instrument Co., Ltd, Tucheng City, Taiwan) and an electric trigger signal were used to initiate sampling and synchronize data by producing a visual marker on the ultrasound videos.

Post-processing was performed off-line using a software package (MATLAB and Statistics Toolbox Release 2015b, The MathWorks, Inc., Natick, Massachusetts, United States). Torque, dynamometer angle and goniometer angle data were filtered using a bidirectional zero-lag fourth-order Butterworth low-pass filter of 10 Hz. Goniometer angle data were fitted to a fourth-order polynomial equation. All passive dorsiflexion and tendon stiffness data were resampled to the ultrasound video frequency, contractile function data were resampled to 200 Hz.

Slow, passive ankle dorsiflexion stretch

Passive torque, ankle joint angle, EMG (GM, GL and SOL), and ultrasound videos (SOL MTJ, GM MTJ, GM fascicles) were obtained during passive dorsiflexion to maximal joint angle in an isokinetic dynamometer (HUMAC NORM 770, Computer Sports Medicine Inc., Stoughton, MA, USA).

Subjects were seated with 65° hip flexion and extended knee. The mediolateral axis of the ankle joint was aligned with the axis of the dynamometer. Unwanted joint movement was minimized by careful strapping of the limbs (Moltubakk et al., 2018). Subjects confirmed that they did not sense any stretch in the calf or hamstrings in the starting position. To prevent influence of visual perception, the tests were taken with eyes closed.

To determine ankle dorsiflexion ROM, the foot was manually rotated at approximately $2°{\cdot}s^{-1}$ from a resting position to the maximally tolerated dorsiflexion angle, the dynamometer

angle of which was then recorded as the endpoint of ROM. The self-perceived pain intensity was recorded as a VAS score.

To assess passive resistance to stretch, the ankle joint was rotated by the dynamometer from 10° plantar flexion to maximal joint angle and back at $2°\cdot s^{-1}$. Subjects were instructed to fully relax the muscles during the test. The procedure was repeated six times, separated by 120-sec rest, to secure two sagittal ultrasound videos (17-19 Hz) of the distal SOL MTJ, two of the distal GM MTJ and two of GM mid-belly fascicles. Echo-absorptive tape was applied to the skin to allow post-processing corrections for potential probe displacement relative to the skin. Additional trials were performed if torque or EMG signals indicated muscle contraction, or if the video quality was inappropriate.

Ankle joint angle was concurrently obtained with a 2D electro-goniometer (Noraxon Inc., Scottsdale, AZ, USA), secured to the medial part of the 1st metatarsal and distal-medial part of tibia. For analyses of passive dorsiflexion, ankle joint angles were obtained from the electro-goniometer, rather than from the dynamometer, to avoid the error induced by misalignment of the foot and dynamometer near maximal dorsiflexion angle. Data are reported at anatomically neutral ankle joint angle (0°), at standardized joint angle (defined as the maximal dorsiflexion angle that was common to each leg across time-points) and at maximal joint angle (defined as the maximal dorsiflexion angle achieved by each leg at the separate time-points).

GM fascicle lengths and pennation angles were measured by automatic tracking using optical flow algorithms (Cronin, Carty, Barrett, & Lichtwark, 2011; Gillett, Barrett, & Lichtwark, 2013). Displacement of the MTJs was measured by semi-automated tracking (Tracker 4.11.0, Open Source Physics, Aptos, California, USA). Elongation and strain of fascicles are reported relative to resting length. Elongation of the GM and SOL MTUs was estimated from the average goniometer joint angles and calf length (Hawkins & Hull, 1990).

Elongation of any structure proximal to the MTJ, represented by the distal displacement of the MTJ, is hereafter referred to as muscle elongation. Elongation occurring distally to the MTJ, calculated by subtracting distal MTJ displacement from MTU elongation, is hereafter referred to as tendon elongation. Muscle and tendon elongation are reported based on lengths measured at anatomical joint angle, as absolute values and as percent contribution to total MTU elongation. Elongations normalized to resting MTU length give the same statistical outcomes as absolute values and are not reported.

All data were interpolated at 0.05° intervals using spline functions and the valid trials were averaged. Passive musculo-tendinous joint stiffness was calculated as the slope of a fourth-order polynomial fit of the passive torque-angle relation (Nordez, Cornu, & McNair, 2006; Magnusson, Simonsen, Aagaard, & Kjaer, 1996).

Tendon stiffness

Following a 5-minute warm-up on a bike ergometer (Monarch, 828E, Varburg, Sweden), the tensile stiffness of the free Achilles tendon and the whole Achilles tendon was examined through ultrasound videos (38-53 Hz) recorded during isometric ramp contractions, as previously reported (Bojsen-Moller et al., 2004). The ultrasound probe was placed sagittally over the distal MTJ of SOL and subsequently GM, and fixed to the leg using a custom-made rigid cast enabling stable positioning with minimal tissue compression. Echo-absorptive tape was applied to the skin to allow post-processing corrections for potential probe displacement relative to the skin.

Subjects were seated in a custom-made isometric ankle dynamometer (Gym2000, Geithus, Norway) instrumented with a load cell (U2A 500 Hottinger Baldwin Messtechnik, Darmstadt, Germany), with 90° hip flexion, with the knee straight and the foot strapped to the dynamometer at anatomical joint angle. The dynamometer settings were individually adjusted

to align the axis of the ankle joint with that of the dynamometer. Unwanted joint movement was minimized by careful strapping of the limbs (Moltubakk et al., 2018).

For the purpose of EMG normalization, the subjects performed two trials of plantar flexion MVC, separated by 60-sec rest. Subsequently, ramped plantar flexion contractions were carried out at a constant rate of torque development. Target (45 $Nm \cdot s^{-1}$) and real-time rate of torque development were displayed to the subject, and standardized verbal encouragements were provided. Each trial was preceded by three brief sub-maximal contractions serving to pre-condition the tendon (Bojsen-Moller et al., 2004). Three trials were then performed for each of SOL and GM MTJ, separated by 120-sec rest. Additional trials were carried out if the rate of torque development or video quality was inappropriate.

Plantar flexion torque was determined from load cell force and the perpendicular distance to the axis of joint rotation. Since ankle joint rotation during isometric plantar flexion was negligible (2.9 ± 2.3° across groups), the recorded torques were not corrected for tibialis anterior co-activation (Raiteri, Cresswell, & Lichtwark, 2015). Tendon force was calculated from the plantar flexion torque and the instantaneous tendon moment arm as derived from ankle joint angle and leg length (Spoor, van Leeuwen, Meskers, Titulaer, & Huson, 1990).

Displacement of the MTJs was measured by semi-automated tracking (Tracker 4.11.0, Open Source Physics, Aptos, California, USA), interpolated at 50 N intervals, and the valid trials were averaged. Tendon elongation was defined as proximal displacement of the MTJ, corrected for the influence of ankle joint rotation (Arampatzis, Monte, & Karamanidis, 2008) by combining the instantaneous joint angles recorded during each ramped contraction with the linear relationship between MTJ displacement and joint angle data obtained during a slow, passive ankle plantar flexion (0° to 3°).

Tendon force and tendon elongation data were cut off at 90 % of each individual's maximal force and fitted with a second-order polynomial (R^2 = 0.96-0.99). Tendon stiffness

was determined as the slope of the curve between 80 and 100 %. Tendon elongation and strain are reported at common force (defined as the greatest force that was achieved by all legs across time-points) and as individual maximum.

Contractile function

Contractile function during isometric and isokinetic contractions was tested using the same isokinetic dynamometer and position as for passive dorsiflexion. Isometric maximal plantar flexion torque was determined as peak torque over a 5-sec contraction. Two trials were performed at 10° plantar flexion, at 0°, at 5°, 10° and 15° of dorsiflexion, all trials separated by 60-sec rest. Some subjects did not perform all joint angles due to low dorsiflexion ROM. Isokinetic concentric dorsiflexion and plantar flexion torque was determined between 10° of dorsiflexion and 30° of plantar flexion, at $30°\cdot s^{-1}$ (dorsi- and plantar flexion) and at $45°\cdot s^{-1}$, $60°\cdot s^{-1}$ and $90°\cdot s^{-1}$ (plantar flexion). Three trials were performed at each angular velocity, all trials were separated by 60-sec rest. Warm-up consisting of three sub-maximal contractions at $30°\cdot s^{-1}$ preceded the dorsiflexion and plantar flexion tests. Standardized verbal encouragements and visual feedback of the instantaneous torque were provided.

Isokinetic peak torque, angle of peak torque and work were determined from the trial with the greatest peak torque. Positive work was calculated as the area below the torque-angle curve after interpolating torque at 0.1° intervals.

Statistical analysis

Resting length of the free Achilles tendon, EMG amplitudes and VAS scores for pain during passive dorsiflexion tests were not normally distributed (D'Agostino & Pearson normality test), and were hence log-transformed to normality. Baseline characteristics were analysed using paired, two-tailed Student's t-test. One-way ANOVA for repeated measures was used to

identify changes in VAS scores for pain during self-administered stretching exercise. Two-way (leg x time) ANOVA for repeated measures was used to identify between-group differences. Post hoc, Sidak's multiple comparisons tests were performed, using multiplicity adjusted *P* values. Level of significance was set to $\alpha = 0.05$. All data are presented as mean ± standard deviation.

RESULTS

For technical reasons, one subject was excluded from passive dorsiflexion analyses, one from the passive dorsiflexion EMG analyses, and one from the passive dorsiflexion ultrasound analyses. Due to insufficient ultrasound video quality or goniometer defect, five subjects were eliminated from free Achilles tendon stiffness analyses and three from whole Achilles tendon stiffness analyses. Thus, passive dorsiflexion analyses included 25 subjects, passive dorsiflexion EMG and ultrasound analyses included 24, free Achilles tendon stiffness 21 and whole Achilles tendon stiffness 23. For isometric strength, due to restricted ROM in some subjects, n = 19 at 10° dorsiflexion and 8 at 15° dorsiflexion.

Adherence to the training programme was 89 ± 10 %, resulting in 10.9 ± 1.5 hours of static stretching exercise.

ROM and pain during passive stretching

Stretching resulted in a bilateral increase in ankle dorsiflexion ROM (interaction $P < 0.005$, Figure 2). Self-perceived pain intensity during the passive ROM test was unchanged (interaction $P = 0.58$, time $P = 0.06$, stretching PRE 4.2 ± 2.1 cm, POST 4.0 ± 2.9 cm, control PRE 4.2 ± 2.2 cm, POST 4.3 ± 2.7 cm). Self-perceived pain intensity during self-administered stretching exercise was reduced from the start of the intervention to subsequent time-points

(intervention $P < 0.0001$, PRE 4.6 ± 2.6 cm, 8 weeks 2.2 ± 2.2 cm, 16 weeks 2.6 ± 2.0 cm, POST 2.0 ± 1.7 cm).

(Insert Figure 2 about here)

Resting properties of the MTU

Resting ankle joint angle, Achilles tendon morphological properties and GM fascicle length were unchanged, while GM thickness and pennation angle increased with time in both legs (Table 1). Fascicle length and tendon lengths normalized to resting MTU length give the same statistical outcomes as absolute values and are not reported.

(Insert Table 1 about here)

Mechanical properties and EMG amplitude during passive dorsiflexion

Passive torque (Figure 3AB) decreased with time at anatomical joint angle in the stretching leg (interaction $P = 0.29$, time $P < 0.005$, Figure 3E) and at standardized joint angle in both legs (interaction $P = 0.40$, time $P < 0.0001$, Figure 3F), while passive torque at maximal joint angle increased in the stretching leg (interaction $P < 0.01$, Figure 3G). Passive joint stiffness at standardized joint angle decreased with time in both legs (interaction $P = 0.63$, time $P < 0.0001$, stretching PRE 2.6 ± 1.3 $Nm \cdot {}^{\circ -1}$, POST 1.7 ± 0.9 $Nm \cdot {}^{\circ -1}$, $P < 0.0005$, control PRE 2.7 ± 1.4 $Nm \cdot {}^{\circ -1}$, POST 2.0 ± 1.2 $Nm \cdot {}^{\circ -1}$, $P < 0.005$) but was unchanged at maximal joint angle (interaction $P = 0.13$, time $P = 0.34$, stretching PRE 2.6 ± 1.3 $Nm \cdot {}^{\circ -1}$, POST 3.1 ± 1.7 $Nm \cdot {}^{\circ -1}$, control PRE 2.8 ± 1.5 $Nm \cdot {}^{\circ -1}$, POST 2.6 ± 1.3 $Nm \cdot {}^{\circ -1}$).

EMG amplitudes of GM, GL and SOL (Figure 3CD) at standardized joint angle decreased with time in both legs (interaction $P = 0.43$-0.80, time $P < 0.0005$, Figure 3H). GM

pennation angle change during passive dorsiflexion was limited (CON PRE 14.4 ± 3.5° at 0°, 14.9 ± 3.8° at maximal joint angle. STR POST 13.2 ± 3.5° at 0°, 13.4 ± 3.4° at maximal joint angle) and was not altered by the intervention (interaction P = 0.35-0.57, time P = 0.09-0.65).

(Insert Figure 3 about here)

Elongation of the GM fascicles increased with time, in the control leg at standardized joint angle and in both legs at maximal joint angle (Table 2). Elongation of the SOL and GM MTUs at maximal joint angle (Figure 4B, D) was changed in both legs, with increased elongation of tendon in both legs, increased elongation of muscle only in the stretching leg, and with increased contribution from tendon elongation (except control leg GM, where P = 0.08 for contribution at maximal joint angle). At standardized joint angle (Figure 4A, C), the SOL MTU of the control leg displayed decreased elongation of muscle and increased elongation and contribution of tendon, while the GM MTU displayed no changes.

(Insert Table 2 about here)

(Insert Figure 4 about here)

Tendon mechanical properties

Tendon stiffness was unchanged in the free Achilles tendon (interaction P = 0.22, time P = 0.20, stretching PRE 554 ± 218 N·mm^{-1}, POST 552 ± 190 N·mm^{-1}, control PRE 547 ± 184 N·mm^{-1}, POST 488 ± 144 N·mm^{-1}, Figure 5A) and in the whole Achilles tendon (interaction P = 0.54, time P = 0.42, stretching PRE 349 ± 84 N·mm^{-1}, POST 347 ± 100 N·mm^{-1}, control PRE 361 ± 84 N·mm^{-1}, POST 341 ± 76 N·mm^{-1}, Figure 5B). Tendon elongation and strain at common force level and at maximal elongation increased

with time, in both legs for the whole Achilles tendon (elongation interaction P = 0.49-0.88, time P < 0.0005, strain interaction P = 0.63-0.98, time P < 0.0005, Figure 5B), and in the control leg for the free Achilles tendon (elongation interaction P = 0.22-0.30, time P < 0.01, strain interaction P = 0.36-0.47, time P < 0.005, Figure 5A).

(Insert Figure 5 about here)

Contractile function

Maximal isometric plantar flexor torque was unchanged at all joint angles (interaction P = 0.06-0.64, time P = 0.32-0.84, Figure 6A). Concentric plantar flexor peak torque was unchanged across angular velocities (interaction P = 0.32-0.64, time P = 0.14-0.74, Figure 6B). Concentric dorsiflexion peak torque increased with time (interaction P = 0.67, time P < 0.0005, stretching PRE 16.1 ± 5.1 Nm, POST 17.6 ± 5.8 Nm, P = 0.26, control PRE 16.2 ± 5.7 Nm, POST 17.4 ± 5.8 Nm, P < 0.01). Angle of peak torque shifted towards dorsiflexion with time (Figure 6C), at $30°\cdot s^{-1}$, $45°\cdot s^{-1}$ and $60°\cdot s^{-1}$ (interaction P = 0.62-0.84, time P < 0.005-0.05), but not at $90°\cdot s^{-1}$ (interaction P = 0.56, time P = 0.11). Work at $30°\cdot s^{-1}$ was unchanged (interaction P = 0.41, time P = 0.31, stretching PRE 156 ± 45 J, POST 160 ± 38 J, control PRE 155 ± 35 J, POST 156 ± 38 J).

(Insert Figure 6 about here)

DISCUSSION

The purpose of this study was to examine the effects of 24 weeks of unilateral triceps surae stretching on ROM, on the morphological and mechanical properties of the MTU, on neural activation and on contractile function. In line with the hypotheses, ROM increased, while

passive torque, passive joint stiffness and EMG amplitude at standardized dorsiflexion angle decreased. Moreover, passive muscle and tendon elongation as well as tendon elongation at common force increased, and angle of peak torque shifted towards dorsiflexion, with no indications of altered morphological properties. Similar changes were seen in the non-stretching leg.

ROM and cross-education

Ankle dorsiflexion ROM increased by 12° in the stretching leg and by 4° in the control leg. Self-perceived pain during stretching exercise was significantly reduced at 8-24 weeks compared to PRE, while ROM increased further from 8 weeks to POST, indicating that stretching training may have effect despite reduced sensation of pain or stretch. ROM did not change significantly from 16 weeks to POST. Possibly, the most flexible subjects were approaching limitations imposed by the anatomical constraints of the ankle joint.

To the best of our knowledge, a bilateral increase in ROM after unilateral stretching training is not previously reported. Although reports exist of acute bilateral gains in ROM following unilateral stretching exercise (Chaouachi et al., 2017; Killen, Zelizney, & Ye, 2018), previous unilateral stretching training studies found unchanged control leg ROM (Akagi & Takahashi, 2014; Ben & Harvey, 2010; Kubo et al., 2002; Guissard & Duchateau, 2004; Minshull, Eston, Bailey, Rees, & Gleeson, 2014; Nelson et al., 2012) and unchanged passive torque (Akagi & Takahashi, 2014; Kubo et al., 2002). Previous studies were limited to 3-10 weeks of stretching, while in the present study, increases in control leg ROM did not reach significance until at 16 weeks.

The present study design does not rule out an influence of systematic error on the variables with bilateral changes. The findings across variables are however consistent and may be explained physiologically or biomechanically. Considering the proposed role of

tolerance and sensory factors in increasing ROM (Freitas et al., 2017; Magnusson et al., 1996), the present bilateral responses to stretching training may be ascribed to neural adaptations similar to the cross-education effects reported with strength training (Scripture, Smith, & Brown, 1894).

EMG amplitude and pain during passive dorsiflexion

When passively stretched to a standardized joint angle (i.e. same angle PRE and POST), the EMG amplitudes of GM, GL and SOL decreased in both legs. This is in contrast to previous work reporting unchanged EMG amplitude following 3 weeks of stretching (Magnusson et al., 1996), possibly suggesting that such neural adaptations may require longer stretching durations. Decreased EMG amplitude seems consistent with the reduced tonic reflex reported following 6 weeks of stretching (Guissard & Duchateau, 2004). Combined with the unchanged EMG amplitude and pain at the increased maximal joint angle, these findings support the notion that reflex activity and pain are important mechanisms in the subjective determination of ROM. The present findings allow speculations that reflex activity and pain play a role in the regulation of active muscle stiffness produced at a different joint angles of passive stretching.

Torque-angle properties during passive dorsiflexion

Passive torque and passive joint stiffness were reduced at comparable dorsiflexion angles (i.e. anatomical and standardized angle) after the intervention. Previous intervention studies are inconsistent, with some reporting no change (Magnusson et al., 1996; Konrad & Tilp, 2014b; Ben & Harvey, 2010), while others reported reduced torque at standardized joint angles (Nakamura et al., 2012; Chan et al., 2001; Mahieu et al., 2007) or reduced passive joint stiffness (Guissard & Duchateau, 2004; Kubo et al., 2002; Marshall et al., 2011). The

apparent discrepancies may be related to methodological differences, including intervention duration. In the present study, the reduced passive torque at standardized joint angle corresponds with the reduced EMG amplitude, thus supporting an association between neural activation and the active muscle stiffness, and supporting the influence of the latter upon passive resistance to stretch. However, reduced passive torque at anatomical joint angle, where almost no EMG amplitude was recorded, suggests that changes in neural activation do not fully explain the changes in passive torque. Since structural properties such as connective tissue contribute to passive resistance (Meyer & Lieber, 2011), passive torque may be reduced through an interplay between altered structural properties and reduced active muscle stiffness.

The present 35 % increase in passive torque at maximal joint angle in the stretching leg is relatively similar to previous studies with short interventions (e.g. 28 % after 3 weeks (Blazevich et al., 2014)), regardless of the broad differences in ROM gains. This suggests that sensory adaptations enabling the subject to tolerate a greater maximal joint angle and torque may occur in the first weeks of stretching, while ROM is subsequently increased through factors that also reduce passive resistance, such as neural activation or structural adaptations.

In summary, stretching training reduced passive resistance to stretch, which potentially contributed to increased ROM Reduced passive resistance may partly be explained by reduced neural activation related to reduced pain sensitivity or reduced sensory input. Furthermore, stretching training increased the maximally tolerated passive torque in the stretching leg, further contributing to the increased ROM.

Morphological properties

Unexpectedly, a small but consistent increase in GM thickness and resting pennation angle was observed bilaterally after the stretching intervention. No change was seen in muscle strength and any functional relevance is likely negligible. GM fascicle length and Achilles

tendon and length remained unchanged. A few human intervention studies have reported increased fascicle length after stretching (Freitas & Mil-Homens, 2015; Simpson et al., 2017) and longer GM fascicles have been observed in professional ballet dancers compared to non-stretching controls (Moltubakk et al., 2018). In order to standardize tension, the present study measured resting fascicle length with the foot hanging freely. The corresponding ankle angles displayed a non-significant PRE-POST group difference of 2°. Furthermore, fascicle length at 0° was 4-6 mm greater than at resting length, and increased with time. The susceptibility of fascicle length measurements to low levels of tension demonstrated here may explain the discrepancy between the present measurements and previous studies measuring fascicle length at 0°. It is also possible that the current ultrasonography methods have insufficient sensitivity for observing small changes in fascicle length. For more direct measures of the effect of stretching training on muscle adaptations, invasive methods and/or more advanced imaging techniques, e.g. second harmonic generation microendoscopy (Sanchez et al., 2015) could be applied, but with the current methods, no stretching-induced changes in muscle or tendon length were observed.

Tendon mechanical properties

The unchanged tendon stiffness observed in the present studies is in line with previous studies applying static stretching training (Mahieu et al., 2007; Mahieu et al., 2009; Kubo et al., 2002; Konrad & Tilp, 2014b). However, tendon stiffness has been reduced following PNF (Konrad et al., 2015) or ballistic (Mahieu et al., 2007) stretching training, and in a cross-sectional study, ballet dancers had lower whole Achilles tendon stiffness compared to controls (Moltubakk et al., 2018). It is possible that tendon adaptations require a greater loading stimulus (Arampatzis, Karamanidis, & Albracht, 2007) than was achieved by the present

static stretching protocol. Greater amount of tendon stress and/or strain could theoretically be achieved by stretching protocols involving muscular contractions near maximal joint angle.

The increased tendon elongation and strain seen at common and maximal force levels of ramped isometric contractions is compatible with unchanged tendon stiffness, as an increase in toe limit strain would allow greater elongation at low force levels without altering the force-elongation slope near maximal force. However, a potential effect of stretching training on tendon material properties and toe limit strain should be verified by further studies. Increased tendon elongation in the control leg is surprising, however, consistent with observations in the control leg during passive dorsiflexion. A possible mechanism could be systemic changes driving collagen degradation and synthesis affecting collagen fibril ultrastructure.

Passive MTU elongation

At standardized joint angle, elongation of the GM muscle and whole Achilles tendon was unchanged in both legs. At the increased maximal joint angle, the intervention affected the two legs differently; whole Achilles tendon elongation increased in both legs but more in the stretching leg, while GM muscle elongation increased only in the stretching leg. In the stretching leg, the tendon contribution to total MTU elongation was increased at maximal joint angle; the relative contribution of tendon (35 %) versus muscle (65 %) changed to 51 % and 49 % from PRE to POST. In the control leg, the change in contribution from 39 % and 61 % PRE to 47 % and 53 % POST did not reach significance ($P = 0.08$). GM fascicle elongation and strain increased in the control leg at standardized joint angle, and increased similarly in both legs at maximal joint angle. The differences between the methods of calculating muscle and fascicle elongation (MTJ displacement versus mid-belly scans) do not allow for direct comparisons between the two variables. Furthermore, muscle elongation may

be slightly underestimated due to stretching of the skin when approaching large joint angles. These factors may explain the different contribution ratios reported in a cross-sectional study where GM fascicles accounted for 72 % and tendon 28 % at 30° dorsiflexion (Abellaneda et al., 2009).

In the present study, the SOL MTU responded similarly to the GM MTU, but with increased contribution from tendon in both legs at maximal joint angle, and with decreased muscle elongation and increased tendon elongation in the control leg at standardized joint angle. The lack of changes in the stretching leg at standardized joint angle is in contrast to another intervention study with increased muscle and fascicle strain and decreased tendon strain following 3 weeks of stretching (Blazevich et al., 2014). Differences between studies may be related to the brief duration and the unchanged passive torque at standardized joint angle in the study by Blazevich *et al.* (Blazevich et al., 2014). In the present study, the increased elongation of the free Achilles tendon in the control leg is surprising, and may be caused by methodological difficulties in tracking of the SOL MTJ. However, the finding matches the increased free Achilles tendon elongation seen in the control leg at common forces and maximal amplitude of ramped contractions. Furthermore, there is a similar tendency for both legs and both muscles at standardized joint angle of passive dorsiflexion. Increases in tendon toe limit strain, as speculated above, could potentially facilitate the increased tendon elongation and contribution at lower torque levels.

The present study revealed that when maximal MTU elongation increased following stretching training, elongation of SOL and GM muscle bellies contributed only in the stretching leg, suggesting that stretching training may have modified muscle stiffness. However, control leg ROM increased less than stretching leg ROM, constituting a less increase in total MTU elongation, which, owing to the sensitivity of muscle elongation measures, may explain why muscle elongation did not increase significantly in the control leg.

Greater muscle elongation with greater ROM is in line with cross-sectional studies showing greater maximal muscle or fascicle elongation in flexible subjects (Blazevich et al., 2012; Moltubakk et al., 2018; Abellaneda et al., 2009)**.** On the other hand, after the present intervention, tendon contribution to total MTU elongation increased. These findings match our former observation that both muscle and series elastic element contribute to the greater MTU elongation in flexible ballet dancers (Moltubakk et al., 2018). Potentially, at low tension, muscle/fascicle compliance may be large relative to tendon compliance, while at greater tension, muscle/fascicle compliance may be similar or lower than tendon compliance, letting tendon take up an increased portion of the elongation. The relationship between tension, toe strain limit, muscle compliance and tendon compliance requires closer examination.

Contractile function

Despite a small increase in GM thickness, the present intervention did not increase isometric or isokinetic strength, matching most (Akagi & Takahashi, 2014; Marshall et al., 2011; Blazevich et al., 2014; Guissard & Duchateau, 2004; Konrad & Tilp, 2014b; Kubo et al., 2002; Minshull et al., 2014) but not all (Handel et al., 1997; Chen et al., 2011; Abdel-Aziem & Mohammad, 2012) stretching intervention studies. Plantar flexion work was also unchanged, matching again data (Ferreira et al., 2007) but not all studies (Handel et al., 1997). Angle of peak torque shifted towards a more dorsiflexed position at the lower angular velocities, matching some (Ferreira et al., 2007; Chen et al., 2011) but not all (Handel et al., 1997) other studies. Taken together, unchanged torque and work production along with the architecture data suggest no addition of serial sarcomeres with the present stretching intervention. Hence, the shift in angle of peak torque may be an effect of increased tendon

elongation and reduced passive resistance to stretch, representing a minor effect of stretching training on contractile function.

Limitations

Although the present 24 week intervention within-subjects design is a strong model, limitations do exist. External factors such as changes in body mass, dietary and training status, footwear and season may change but would affect both legs similarly. Moreover, potential cross-over effects may influence control-intervention comparisons. Learning effects in e.g. tests of contractile function can potentially bias results, however, tests were separated by 8-16 weeks, so such effects are unlikely, and there were few changes to variables where learning could be an issue. Investigator bias cannot be ruled out in a longitudinal design, but during passive tests, investigators and subjects had no active role, and torque-angle analyses were automated, meaning that changes to passive torque and EMG variables likely reflect the intervention. Furthermore, investigators were blinded during all post-processing.

The calf and ankle joint anatomy is well suited for ultrasonography, however, anatomical constraints other than MTU properties may restrict ROM and thus the most flexible subjects may have experienced an attenuated response to stretching within 24 weeks. On the other hand, the testing position (i.e., hip flexed and knee extended) may have reduced the available ROM in comparison to e.g. prone position, limiting the range of joint angles available for analyses.

Despite these limitations, the study successfully met its purpose of revealing effects of stretching training on ROM, on mechanical properties of the MTU, on neural activation, and on contractile function.

Overview and conclusions

The present unilateral stretching intervention increased ROM, with a contralateral effect documented after 4-6 months. Unearthing the precise mechanisms driving bilateral changes is beyond the scope of the study, but cross-education effects similar to those seen previously with strength training and acute stretching are plausible.

The endpoint of ROM is subjectively determined. Hence, increases in ROM may be achieved through a combination of improved tolerance and reduced sensory input during passive stretching. A role of tolerance, e.g. increased pain threshold or reduced pain sensitivity, possibly affecting sensory input, is indicated by the greater maximally tolerated passive torque in the stretching leg, and may further have influenced active muscle stiffness. Reduced sensory input is further anticipated with reduced passive resistance to stretch, as indicated by reduced passive torque at comparable joint angles, whereby reduced tension on tissues would affect sensory afferences.

Passive resistance to stretch arises from the deformation and behaviour of the MTU components. Reflex activity may contribute to passive torque production via its contribution to active muscle stiffness. This is supported by the present finding of corresponding reductions in EMG amplitude and passive torque at standardized joint angle. However, reduced passive torque at anatomical joint angle, where almost no EMG amplitude was observed, suggests that neural activation does not explain the entire change in passive torque. While the present thesis did not reveal any morphological adaptations to stretching training, a change in structural and mechanical properties is the most likely co-factor to neural activation in explaining reduced passive torque. The hypothesis of altered structural properties is supported by increased tendon elongation at lower force levels after stretching training.

The mechanisms underpinning the bilateral effects cannot be uncovered from the present results and require further studies. Yet taken together, the present findings indicate

that stretching training may induce neural adaptations reducing passive resistance bilaterally, potentially involving reductions in reflex activity. However, cross-education effects involving motor learning (for a review, see Lee & Carroll, 2007) or systemic increases in hormone levels affecting collagen metabolism (Hansen & Kjaer, 2016) are also possible explanations which should be further investigated.

Further research is required to understand the relationship between pain, reflexes, neural activation, resistance of passive structures, passive torque and ROM. Additional insight may be gained by investigating connective tissue composition and hormonal responses to stretching training, or by applying more advanced imaging techniques during passive motion.

In conclusion, 24 weeks of unilateral stretching increased ankle dorsiflexion ROM, while passive dorsiflexion variables (torque, joint stiffness and EMG amplitude at standardized angle) decreased, passive muscle and tendon elongation as well as whole Achilles tendon elongation at common force increased, and angle of peak torque shifted towards dorsiflexion. No changes were seen in GM fascicle length, tendon length or stiffness. After the first 8 weeks, ROM also increased in the control leg, and passive dorsiflexion variables, tendon elongation and angle of peak torque changed similarly to the stretching leg.

These findings indicate that stretching training increases ROM through a combination of neural adaptations and structural adaptations possibly related to tendon material properties, which together translate into altered mechanical properties and have the potential to modify contractile function. The role of central mechanisms for increases in ROM was confirmed by bilateral responses to stretching training.

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CONFLICTS OF INTEREST AND SOURCE OF FUNDING

The authors declare no conflicts of interest and no external funding. The results of the present study do not constitute endorsement by ACSM. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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APPENDICES

Supplemental Digital Content 1.pdf (table)

Supplemental Digital Content 2.pdf (table)

FIGURES

Figure 1. Stretching exercise. Subjects were instructed to place the stretching leg as far posteriorly as possible, while pushing the heel down to the ground, the forefoot pointing forward. After 8 weeks, the stretching was progressed such that two of four repetitions were performed with the knee of the stretching leg flexed to approximately 45°, to ensure continuous stretching load on the soleus.

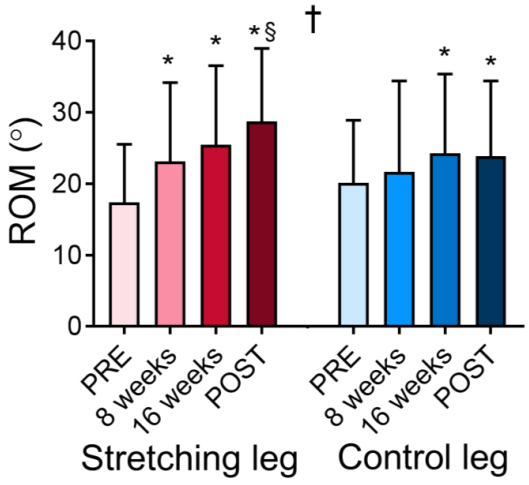

Figure 2. Ankle dorsiflexion range of motion (ROM), as measured in the stretching leg and control leg before (PRE), during and after (POST) 24 weeks of stretching. † indicates an interaction effect ($P < 0.005$), * indicates a difference from PRE ($P < 0.0005$), § indicates a difference from 8 weeks ($P < 0.0005$).

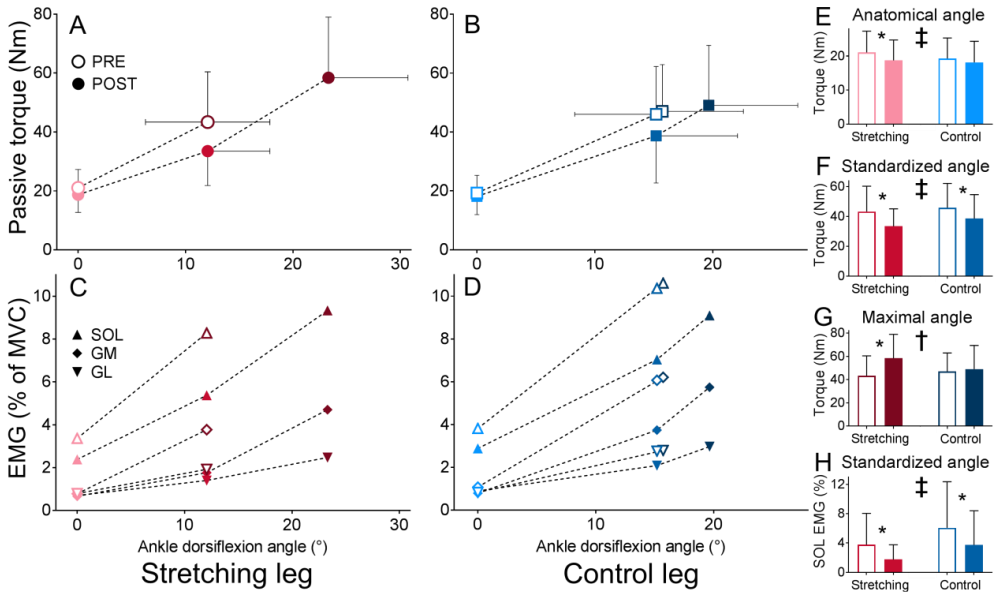

Figure 3. A, B) Passive torque and C, D) EMG amplitudes during passive dorsiflexion. A, C) Stretching leg and B, D) control leg at anatomical, standardized and maximal joint angle, before (PRE) and after (POST) 24 weeks of stretching. In the stretching leg, standardized joint angle = PRE maximal joint angle. EMG amplitudes are normalized to amplitudes during maximal voluntary contraction (MVC). Error bars are left out for legibility. EMG, electromyography; SOL, soleus; GM, gastrocnemius medialis; GL, gastrocnemius lateralis. E-G) Statistical analyses of passive torque and H) SOL EMG amplitude: † indicates an interaction effect ($P < 0.0001$-0.01), ‡ indicates a time effect ($P < 0.0001$-0.005), * indicates significant post hoc tests ($P < 0.0001$-0.05). Statistical analyses of GM and GL EMG are identical to SOL and are not shown. For absolute values and detailed statistics, see Supplemental Digital Content 1 (table).

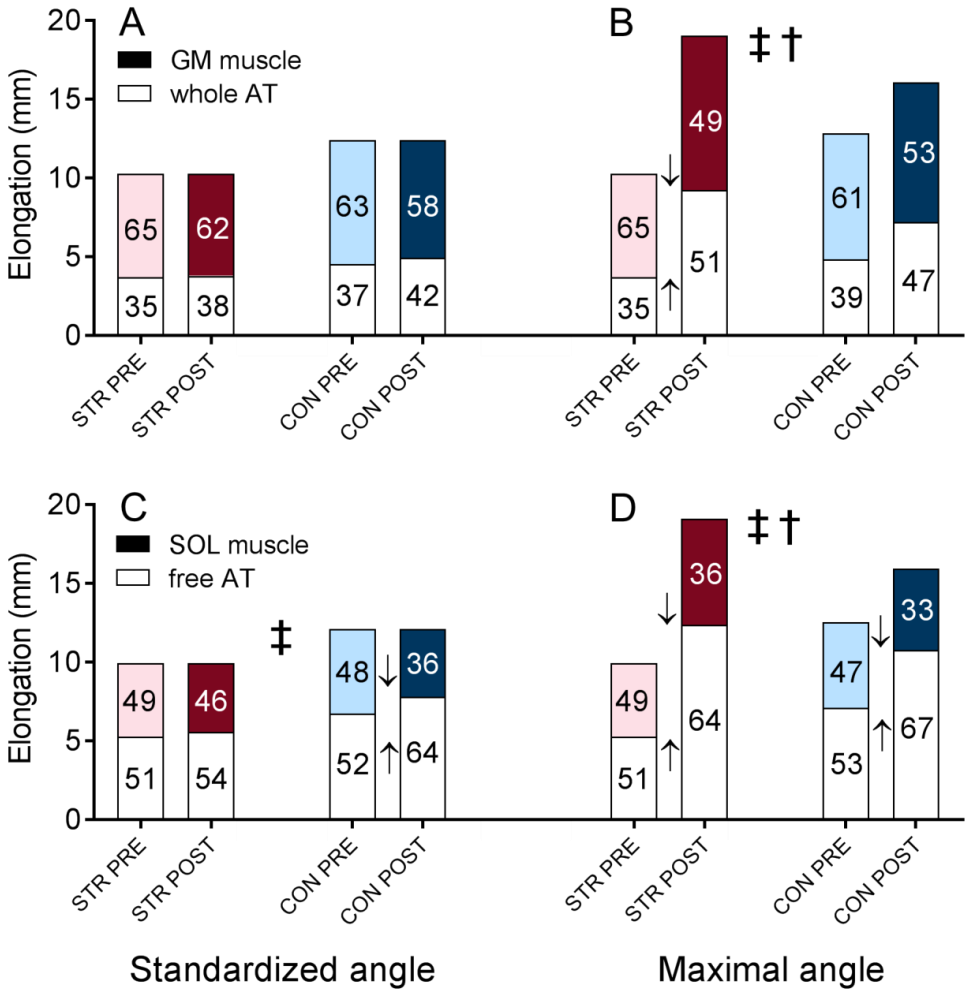

Figure 4. Muscle and tendon elongation during passive dorsiflexion. A-B) Gastrocnemius medialis (GM) and the whole Achilles tendon (AT), C-D) soleus (SOL) and the free Achilles tendon, in the stretching leg (STR) and control leg (CON) at standardized and maximal joint angle, before (PRE) and after (POST) 24 weeks of stretching. Numbers on the bars represent percent contribution to MTU elongation. † indicates an interaction effect for elongation ($P < 0.0001$-0.01), ‡ indicates a time effect for elongation and contribution ($P < 0.0001$-0.05), ↓↑ indicate significant post hoc tests for contribution ($P < 0.001$-0.05). For absolute values and detailed statistics, see Supplemental Digital Content 2 (table).

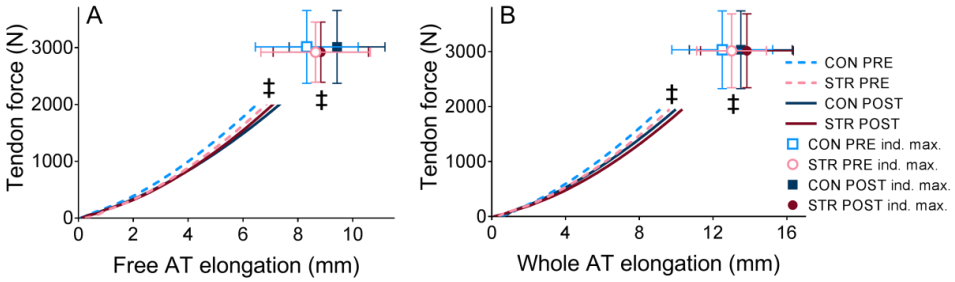

Figure 5. Force-elongation relationship during isometric plantar flexion. A) Free Achilles tendon (AT), B) whole Achilles tendon, in the stretching leg (STR) and control leg (CON) before (PRE) and after (POST) 24 weeks of stretching. Continuous lines represent group mean up to common force level, symbols represent individual maximal force and elongation. ‡ indicates a time effect for tendon elongation ($P < 0.0005$-0.01).

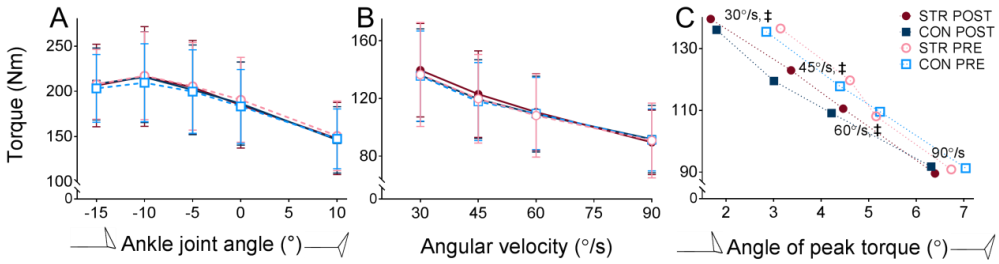

Figure 6. Torque-angle and torque-velocity relations. A) Isometric plantar flexion torque-angle relation, B) isokinetic plantar flexion torque-velocity relation, C) isokinetic torque-angle of peak torque relation, in the stretching leg (STR) and control leg (CON) before (PRE) and after (POST) 24 weeks of stretching. Note that the Y axes differ and are broken. ‡ indicates a time effect ($P < 0.005$-0.05).

TABLES

Table 1. Resting properties of the ankle joint, gastrocnemius medialis and the Achilles tendon.

		PRE	POST	*P* inter.	*P* time	Post hoc *P*
Resting ankle angle (°)	Stretching	22 ± 3	23 ± 4	0.09	0.26	0.09
	Control	23 ± 4	22 ± 4			0.89
Gastrocnemius medialis						
Thickness (mm)	Stretching	20 ± 2	21 ± 2	0.44	< 0.0001	< 0.0001
	Control	20 ± 2	22 ± 3			< 0.0001
Pennation angle (°)	Stretching	22 ± 3	24 ± 3	0.99	< 0.0001	< 0.005
	Control	22 ± 3	24 ± 3			< 0.001
Fascicle length (mm)	Stretching	54 ± 6	54 ± 6	0.52	0.48	0.57
	Control	54 ± 8	54 ± 7			1.00
Achilles tendon						
Free AT length (mm)	Stretching	55 ± 20	58 ± 21	0.34	0.47	0.42
	Control	53 ± 19	54 ± 21			0.98
Whole AT length (mm)	Stretching	179 ± 22	184 ± 22	0.19	0.18	0.13
	Control	182 ± 22	182 ± 22			1.00

Table 2. Length, elongation and strain of gastrocnemius medialis (GM) fascicles during passive dorsiflexion.

GM fascicle length (mm)		**PRE**	**POST**	***P* inter.**	***P* time**	**Post hoc *P***
At anatomical angle	Stretching	58 ± 8	60 ± 9	0.46	< 0.005	0.15
	Control	57 ± 8	59 ± 8			< 0.05
At standardized angle	Stretching	62 ± 9	64 ± 10	0.15	< 0.0001	< 0.05
	Control	60 ± 7	65 ± 9			< 0.0001
At maximal angle	Stretching	62 ± 9	67 ± 9	0.76	< 0.0001	< 0.0001
	Control	60 ± 7	67 ± 10			< 0.0001
GM fascicle elongation (mm)						
At anatomical angle	Stretching	4 ± 7	6 ± 7	0.40	< 0.05	0.48
	Control	4 ± 6	6 ± 5			0.06
At standardized angle	Stretching	8 ± 7	10 ± 8	0.15	< 0.0005	0.13
	Control	7 ± 6	12 ± 6			< 0.001
At maximal angle	Stretching	8 ± 7	13 ± 8	0.56	< 0.0001	< 0.0001
	Control	7 ± 6	13 ± 7			< 0.0001
GM fascicle strain (%)						
At anatomical angle	Stretching	9 ± 14	11 ± 13	0.40	< 0.05	0.55
	Control	7 ± 11	12 ± 10			0.07
At standardized angle	Stretching	15 ± 14	19 ± 15	0.17	< 0.0005	0.16
	Control	13 ± 11	22 ± 11			< 0.005
At maximal angle	Stretching	15 ± 14	25 ± 14	0.62	< 0.0001	< 0.0005
	Control	14 ± 11	25 ± 12			< 0.0001

Note: Elongation and strain of fascicles are reported based on their resting length, as measured with subjects resting in prone position.

SUPPLEMENTARY DIGITAL CONTENT

Supplementary Digital Content 1. Joint angle, passive torque and electromyographic (EMG) amplitude of gastrocnemius medialis (GM), gastrocnemius lateralis (GL) and soleus (SOL) during passive dorsiflexion.

Ankle dorsiflexion angle (°)		**PRE**	**POST**	***P* inter.**	***P* time**	**Post hoc *P***
At maximal angle	Stretching	12 ± 6	23 ± 7	< 0.0001	< 0.0001	< 0.0001
	Control	16 ± 7	20 ± 8			< 0.005
At standardized torque	Stretching	11 ± 6	17 ± 8	0.09	< 0.0001	< 0.0001
	Control	14 ± 7	17 ± 7			< 0.005
Passive torque (Nm)						
At anatomical angle	Stretching	21 ± 6	19 ± 6	0.29	< 0.005	< 0.01
	Control	19 ± 6	18 ± 6			0.29
At standardized angle	Stretching	43 ± 17	34 ± 12	0.40	< 0.0001	< 0.0001
	Control	46 ± 16	39 ± 16			< 0.005
At maximal angle	Stretching	43 ± 17	58 ± 21	< 0.01	< 0.0005	< 0.0001
	Control	47 ± 16	49 ± 20			0.77
EMG SOL (% of MVC)						
At anatomical angle	Stretching	3.4 ± 2.8	2.4 ± 1.9	0.38	0.15	0.89
	Control	3.8 ± 3.7	2.9 ± 3.0			0.20
At standardized angle	Stretching	8.3 ± 6.7	5.4 ± 4.5	0.99	< 0.0001	< 0.005
	Control	10.4 ± 8.6	7.0 ± 5.9			< 0.01

At maximal angle	Stretching	8.3 ± 6.7	9.3 ± 6.8	0.42	0.84
	Control	10.6 ± 8.6	9.1 ± 7.0	0.94	0.78
EMG GM (% of MVC)					
At anatomical angle	Stretching	0.8 ± 0.5	0.7 ± 0.6	0.50	0.59
	Control	1.1 ± 1.4	0.8 ± 0.5	0.53	1.00
At standardized angle	Stretching	3.8 ± 4.3	1.8 ± 2.0	0.43	< 0.0005
	Control	6.1 ± 6.3	3.7 ± 4.7	< 0.0001	< 0.01
At maximal angle	Stretching	3.8 ± 4.3	4.7 ± 5.0	0.18	0.49
	Control	6.2 ± 6.2	5.7 ± 6.4	0.87	0.65
EMG GL (% of MVC)					
At anatomical angle	Stretching	0.8 ± 0.6	0.7 ± 0.6	0.64	0.75
	Control	0.8 ± 0.7	0.9 ± 1.0	0.64	1.00
At standardized angle	Stretching	1.9 ± 1.8	1.4 ± 1.9	0.80	< 0.01
	Control	2.8 ± 2.5	2.1 ± 2.2	< 0.0005	< 0.05
At maximal angle	Stretching	1.9 ± 1.8	2.5 ± 2.7	0.34	0.48
	Control	2.8 ± 2.5	3.0 ± 2.8	0.57	0.96

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Supplementary Digital Content 2. Elongation of the gastrocnemius medialis (GM) and soleus (SOL) muscles and the Achilles tendon, and percent contribution to total muscle-tendon unit (MTU) elongation, during passive dorsiflexion.

GM muscle elongation (mm)		**PRE**	**POST**	***P* inter.**	***P* time**	**Post hoc *P***
At standardized angle	Stretching	6.6 ± 2.4	6.5 ± 2.7	0.57	0.38	0.97
	Control	7.8 ± 3.3	7.5 ± 2.9			0.52
At maximal angle	Stretching	6.6 ± 2.4	9.8 ± 2.6	< 0.001	< 0.0001	< 0.0001
	Control	8.0 ± 3.3	8.9 ± 3.0			0.16
Whole Achilles tendon elongation (mm)						
At standardized angle	Stretching	3.7 ± 3.9	3.8 ± 2.7	0.57	0.38	0.97
	Control	4.6 ± 3.6	4.9 ± 3.0			0.52
At maximal angle	Stretching	3.7 ± 3.9	9.2 ± 4.6	< 0.01	< 0.0001	< 0.0001
	Control	4.8 ± 3.5	7.2 ± 4.1			< 0.01
GM muscle elongation (% contribution to MTU elongation)						
At standardized angle	Stretching	65 ± 21	62 ± 11	0.89	0.12	0.53
	Control	63 ± 24	58 ± 15			0.41
At maximal angle	Stretching	65 ± 21	49 ± 13	0.16	< 0.0001	< 0.0005
	Control	61 ± 24	53 ± 15			0.08
Whole Achilles tendon elongation (% contribution to MTU elongation)						
At standardized angle	Stretching	35 ± 21	38 ± 11	0.89	0.12	0.53
	Control	37 ± 24	42 ± 15			0.41
At maximal angle	Stretching	35 ± 21	51 ± 13	0.16	< 0.0001	< 0.0005
	Control	39 ± 24	47 ± 15			0.08
SOL muscle elongation (mm)						

At standardized angle	Stretching	4.7 ± 2.4	4.4 ± 2.2		0.60
				0.11	< 0.01
	Control	5.4 ± 3.0	4.3 ± 2.8		< 0.005
At maximal angle	Stretching	4.7 ± 2.4	6.7 ± 3.0		< 0.0001
				< 0.001	< 0.05
	Control	5.4 ± 3.0	5.2 ± 3.1		0.79
Free Achilles tendon elongation (mm)					
At standardized angle	Stretching	5.3 ± 3.6	5.6 ± 3.7		0.60
				0.11	< 0.01
	Control	6.7 ± 4.2	7.8 ± 3.7		< 0.005
At maximal angle	Stretching	5.3 ± 3.6	12.4 ± 5.0		< 0.0001
				< 0.01	< 0.0001
	Control	7.1 ± 4.3	10.8 ± 4.8		< 0.0005
SOL muscle elongation (% contribution to MTU elongation)					
At standardized angle	Stretching	49 ± 19	46 ± 15		0.69
				0.13	< 0.05
	Control	48 ± 27	36 ± 17		< 0.05
At maximal angle	Stretching	49 ± 19	36 ± 13		< 0.001
				0.77	< 0.0001
	Control	47 ± 28	33 ± 16		< 0.005
Free Achilles tendon elongation (% contribution to MTU elongation)					
At standardized angle	Stretching	51 ± 19	54 ± 15		0.69
				0.13	< 0.05
	Control	52 ± 27	64 ± 17		< 0.05
At maximal angle	Stretching	51 ± 19	64 ± 13		< 0.001
				0.77	< 0.0001
	Control	53 ± 28	67 ± 16		< 0.005

Note: Muscle and tendon elongation are reported based on lengths measured at anatomical ankle joint angle, as absolute values and as percent contribution to total MTU elongation.

Paper IV

Title: Effects of 6-month stretching training on collagen content of the human soleus muscle

Running head: Stretching training and muscle collagen content

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ABSTRACT

Stretching exercise is frequently applied in sports and rehabilitation settings and is known to increase joint range of motion (ROM). The mechanisms for changes in ROM are not well understood, but neural adaptations described as tolerance to stretch are often presented as the main mechanism. However, it is known that intramuscular connective tissue (IMCT) or collagen content within the extracellular matrix is the main contributor to passive muscle stiffness, potentially affecting passive resistance to stretch. Therefore, the purpose of the present study was to examine the effect of habitual stretching on intramuscular collagen content. Ten participants concluded 24 weeks of daily 4x60-sec unilateral stretching training of the plantar flexors, with the contralateral leg as control. Before and after the intervention, muscle biopsies were obtained from the soleus (SOL) muscle and intramuscular collagen content was estimated based on hydroxyproline, while muscle architecture and passive stretching mechanics were examined by use of ultrasonography and isokinetic dynamometry. Ankle dorsiflexion ROM increased from 16 ± 7° to 25 ± 9° ($P < 0.005$), or by 56 %. Reduced passive resistance to stretch was indicated by time effects ($P < 0.0001$-0.05) on passive torque, with decreased torque at anatomical joint angle in the stretching leg (24 ± 7 Nm to 21 ± 6 Nm), at standardized joint angle in the stretching (46 ± 15 Nm to 35 ± 11 Nm) and the control leg (50 ± 15 Nm to 42 ± 13 Nm), and by decreased passive joint stiffness at standardized joint angle in the stretching leg (2.9 ± 1.5 $Nm \cdot {}^{\circ -1}$ to 1.9 ± 1.0 $Nm \cdot {}^{\circ -1}$). No change was seen in SOL collagen content from PRE to POST (1.4 ± 0.5 % to 1.1 ± 0.4 %, $P = 0.17$) and no changes were seen in muscle architecture of either leg. Despite the known contribution of intramuscular collagen content to passive stiffness of muscle, and although ROM was increased while passive resistance to stretch was reduced following the intervention, no changes were seen in SOL collagen content, suggesting that other factors such as neural adaptations caused the reduction in passive resistance.

Keywords:

Flexibility, length-tension, ultrasound, stretch tolerance

INTRODUCTION

Stretching exercise is often applied in sports and recreational exercise aiming at improving performance or reducing injury risk (Garber et al., 2011), or in rehabilitation (Page, 2012). Previous studies have consistently shown a significant increase in joint range of motion (ROM) following habitual stretching (Magnusson, Simonsen, Aagaard, Sorensen, & Kjaer, 1996; Lucas & Koslow, 1984), however, the influence of stretching training on performance (Shrier, 2004; Medeiros & Lima, 2017) and/or injury risk (Thacker, Gilchrist, Stroup, & Kimsey, Jr., 2004; Weldon & Hill, 2003; Witvrouw, Mahieu, Danneels, & McNair, 2004) is debated. An increased knowledge of the physiological mechanisms that underlie stretching-induced gains in ROM will enable greater understanding of how and if stretching training should be applied in performance, injury prevention and/or rehabilitation settings.

The general consensus from human *in vivo* studies seems to be that the main adaptation to stretching training lies within the nervous system expressed as generic tolerance to stretch (Magnusson et al., 1996), which in turn facilitates increased ROM. Nonetheless, ROM could potentially be modulated reduced passive resistance to stretch, through changes in mechanical properties of tendons or passive muscle stiffness brought about by intra- or extracellular adaptation such as sarcomerogenesis and/or changes in intramuscular connective tissue (IMCT). Animal models applying excessive stretching and/or immobilization volumes have documented length changes in muscle or muscle fascicles manifested by addition of sarcomeres in series (Takahashi, Ward, Marchuk, Frank, & Lieber, 2010). These data demonstrate that stretching does induce adaptation in the contractile tissues, however, in humans, and with use of more feasible stretching volumes, such muscular adaptations have not been observed. With respect to intramuscular force bearing tissues, chronic surgical stretch in a rabbit model increased muscle tissue fibrosis (increases in IMCT), in turn resulting in a leftward shift in the length-tension curve and increased passive muscle stiffness

(Takahashi, Ward, Friden, & Lieber, 2012). Correspondingly, a 5-fold increase in modulus was observed when comparing muscle fibers *in vitro* with and without their associated extracellular matrix (Meyer & Lieber, 2011), which further supports the notion that the IMCT contributes markedly to the passive load-bearing capacity of skeletal muscle.

Increased intramuscular collagen synthesis has been observed after both acute loading in human models (Miller et al., 2005; Holm et al., 2010; Crameri et al., 2004) and chronic loading in animal models (Heinemeier et al., 2007), and expression of collagen I, III and IV transcripts has been observed after acute loading in humans (Hyldahl et al., 2015). Although these data suggest that loading (strength training) may yield changes in muscle collagen content, the links between newly synthesized collagen and total amount thereof remain unclear. A recent study found increased muscle collagen synthesis after 15 days of intermittent stretching in a rat model, while at the same time, intramuscular collagen content was reduced, underscoring the complexity of the links between collagen net synthesis balance and deposition into the extracellular matrix (Peviani et al., 2018). Additionally, while intense stretching models in animals have induced increased collagen content and increased muscle stiffness, studies on human fibre preparations *in vitro* have demonstrated that intramuscular collagen content is only weakly correlated to passive muscle tissue stiffness (Smith, Lee, Ward, Chambers, & Lieber, 2011).

While training and loading in general thus may elicit increases in IMCT, no human *in vivo* studies have examined the influence of stretching training on IMCT. The purpose of the present study was therefore to investigate whether muscle collagen content and passive muscle-tendon mechanical properties change in response to 24 weeks of daily stretching of the triceps surae muscle-tendon unit (MTU). Although relevant information from previous studies is limited and/or contradictory, a reduction in muscle collagen content would in theory contribute to reduced passive stiffness of the muscle and perhaps increased ROM. Thus, it

was hypothesized that muscle collagen content is reduced as a consequence of 24 weeks of stretching in humans *in vivo*.

MATERIALS AND METHODS

The present data are part of a larger study where additional hypotheses are examined. The current methods section describes only procedures relevant for the presently reported parameters, however, the entire protocol may be examined elsewhere (*present PhD thesis, Paper III*). In the overall study, 30 healthy university students participated. Exclusion criteria were lower limb injuries within the past 6 months, musculo-skeletal diseases, other conditions preventing stretching, and/or a history of systematic stretching. The study was conducted in accordance with the Declaration of Helsinki, the regional ethics committee approved the study (ref. 2011/1394 A), and each participant signed an informed consent form.

Participants

From the overall study population, 16 individuals volunteered to also participate in the current invasive protocol. By use of a random number generator, 14 participants were drawn to have muscle biopsies taken before (PRE) and after (POST) the intervention. One participant withdrew from the overall study, while three tissue samples were inadequate for analyses. The present data set thus comprises 10 participants (age 22.0 ± 1.9, 5 women; height 171 ± 9 cm, weight 62 ± 7 kg, and 5 men; 185 ± 6 cm, 83 ± 11 kg). In the course of the study the participants were asked to maintain their habitual activity level, refrain from unaccustomed exercise, maintain their normal diet, refrain from anti-inflammatory drugs, and to report any illness/injury. In addition to stretching, the participants reported a weekly exercise volume of 2 ± 1 hours of endurance activities and 1 ± 1 hours of strength training during the intervention period.

Overall experimental design

The participants completed 24 weeks of unilateral stretching while the contralateral leg served as control. Total stretching time during the study was 686 ± 82 min with an adherence to the training of 90 ± 9 %, as recorded by personal training diaries. Muscle architecture and passive stretching kinetics (isokinetic device) were examined PRE and POST, and soleus (SOL) muscle biopsies were taken prior to and after the intervention.

Stretching training

The assignment of stretching leg was randomized such that 5 participants stretched their dominant leg (stance leg while kicking a ball), while 5 stretched their non-dominant leg. The stretching intervention consisted of daily self-administered 4x60-sec static plantar flexor stretches (Figure 1A), for 24 weeks. Prior to the intervention, the participants received written and individualized verbal instructions. Their stretching technique was controlled at each test session. After 8 weeks, stretching was progressed such that two of four repetitions were performed with the knee flexed to approximately 45°, to ensure continuous stretching load on SOL.

(Insert Figure 1 approximately here)

Muscle architecture

The participants were resting in prone position with the foot hanging freely off the examination bed. Fascicle length, pennation angle and muscle thickness of SOL were measured with ultrasonography (sagittal plane) at mid-length of the muscle belly using real-time B-mode ultrasonography with a 50-mm linear array transducer (L12-5, Philips, Bothell,

WA, USA) and ultrasound system (HD11XE, Philips, Bothell, WA, USA). The lower half of the transducer frequency (5-12 MHz) was used with a built-in filter to optimise ultrasound penetration while preserving spatial resolution and contrast. The ultrasound images were analysed using imaging software (Fiji ImageJ (Schindelin et al., 2012)) as previously reported (Moltubakk, Magulas, Villars, Seynnes, & Bojsen-Moller, 2018).

Passive stretching mechanics

Passive torque and ultrasound videos were obtained during slow, passive ankle dorsiflexion to maximal joint angle in an isokinetic dynamometer (HUMAC NORM 770, Computer Sports Medicine Inc, Stoughton, MA, USA). The participants were seated with 65° hip flexion and extended knee joint, and the ankle joint was aligned with the dynamometer axis of rotation (Figure 1B). Unwanted joint movement was minimized by rigid strapping of all segments. The foot was manually moved at approximately $2°\cdot s^{-1}$ from a resting position (approximately 30° plantar flexion) to the maximally tolerated dorsiflexion angle, as indicated by the participants. The dynamometer angle (relative to anatomically neutral ankle joint angle; 0°) at the endpoint of the rotation was recorded as the endpoint of ROM. Subsequently, passive resistance to stretch was recorded by dorsiflexing the ankle joint from 10° plantar flexion to maximal dorsiflexion angle at $2°\cdot s^{-1}$ while participants remained passive. The procedure was repeated two times (separated by 120 sec) and simultaneous ultrasound video was recorded from the distal SOL musculo-tendinous junction (MTJ) (17 Hz). Echo-absorptive tape was applied to the skin to allow post-hoc correction for unwanted probe displacement. Care was taken to avoid muscle contraction during passive trials, and if contractile activity was observed from the torque or electromyography data (*present PhD thesis, Paper III*), the trial was discarded. To initiate sampling a trigger signal was applied, and to ensure dynamometer data and video synchronization the trigger produced a visual marker on the ultrasound videos.

Torque was obtained from the isokinetic dynamometer. Joint angle was recorded from an external 2D electro-goniometer (Noraxon Inc., Scottsdale, AZ, USA), secured to the medial part of the 1st metatarsal and distal aspect of the lower leg, hence joint angle differs from the dynamometer measurement of ROM. Signals were digitized and sampled at 1500 Hz and filtered using a bidirectional zero-lag fourth-order Butterworth low-pass filter of 10 Hz using processing software (MATLAB and Statistics Toolbox Release 2015b, The MathWorks, Inc., Natick, Massachusetts, United States). Goniometer angle data were fitted to a fourth-order polynomial equation. All data were resampled to the ultrasound video frequency, interpolated at 0.05° intervals using spline functions and trials were averaged. PRE- and POST data are reported at anatomically neutral ankle joint angle (0°), at standardized joint angle (greatest dorsiflexion angle that was common to each leg across time-points), and at maximal joint angle (the greatest dorsiflexion angle achieved by each leg at the separate time-points). Passive joint stiffness was calculated as the slope of a fourth-order polynomial fit of the torque-angle relation, at standardized joint angle and maximal joint angle (Nordez, Cornu, & McNair, 2006; Magnusson, Simonsen, Aagaard, & Kjaer, 1996). Elongation of the SOL MTU was estimated from calf length and joint angle data (Hawkins & Hull, 1990). Displacement of the SOL MTJ was measured by semi-automated tracking (Tracker, Video Analysis and Modeling Tool V.4.62, Open Source Physics, Aptos, California, USA), to enable assessment of muscle and tendon elongation (reported based on length at anatomical joint angle and as contribution to total MTU elongation).

Muscle samples

Muscle biopsies were collected under ultrasound guidance from the medial aspect of the SOL, just distal to the medial gastrocnemius muscle tendon junction. The muscle samples were taken from the control leg at baseline and from the stretching leg following the intervention

(48 hours after final stretching session). Muscle samples were collected under local anaesthesia (10 $mg \cdot ml^{-1}$ xylocaine) with a 6 mm Bergström needle (Pelomi, Albertslund, Denmark), using manual suction. Muscle specimens were washed immediately in 0.9 % saline, and any fat, connective tissue or blood was dissected before the sample was weighed and frozen in liquid N_2. All samples were stored at −80°C until analysis. Hydroxyproline (HYP) was used as a measure of collagen content, assuming a collagen/HYP mass ratio of 7.5 (Neuman & Logan, 1950). Samples of ~30 mg wet weight were freeze dried for 24 hours at room temperature (~5 mg dry weight). Dried samples underwent gas-phase hydrolysis for 24 hours at 110 °C using 37 % HCl containing 0.3 % phenol. Air was removed from the hydrolysis vessel by three consecutive applications of vacuum and nitrogen, ending with vacuum. Hydrolysates were vacuum dried at room temperature overnight and reconstituted in dH_2O at 10 mg/mL. Aliquots of 15 µL were used for analysis of HYP content by an assay based on chloramine-T oxidation and colour reaction with 4-dimethylaminobenzaldehyde (Stegemann & Stalder, 1967). The detailed procedure in our lab has previously been described (Svensson, Smith, Moyer, & Magnusson, 2018). Collagen content is reported as % of dry weight.

Statistical analysis

All data reported were normally distributed (D'Agostino & Pearson normality test). Two-way (leg x time) ANOVA for repeated measures was used to identify between-leg differences in ROM, muscle architecture, passive torque, passive joint stiffness and elongation during passive dorsiflexion. Post hoc, Sidak's multiple comparisons tests were performed, using multiplicity-adjusted *P* values. Collagen content was analysed using paired, two-tailed Student's t-test. Pearson product-moment correlation coefficients were calculated for relevant

changes from PRE to POST. Level of significance was set to $\alpha = 0.05$. All data are presented as mean ± standard deviation.

RESULTS

Stretching for 24 weeks resulted in an increase in ankle dorsiflexion ROM (interaction $P < 0.005$) of 9° in the stretching leg (16 ± 7° to 25 ± 9°, 56 %, $P < 0.0005$), while ROM in the control leg was unchanged (19 ± 9° to 19 ± 7°, $P = 0.99$). Passive torque decreased with time, at anatomical joint angle in the stretching leg (Figure 2C) and at standardized joint angle in both legs (Figure 2D). At maximal joint angle, passive torque increased in the stretching leg (Figure 2E). Passive joint stiffness decreased with time at standardized joint angle in the stretching leg but was unchanged at maximal joint angle (Table 1). No changes were seen in muscle architecture in either leg (Table 2).

(Insert Figure 2 approximately here)
(Insert Table 1 approximately here)
(Insert Table 2 approximately here)

Elongation of the SOL MTU during passive dorsiflexion changed with time at standardized joint angle, with increased tendon elongation and decreased muscle belly elongation in the control leg (Figure 3A). At maximal joint angle, the stretching leg displayed increased tendon elongation and a tendency ($P = 0.08$) for increased muscle elongation, while the control leg did not change (Figure 3B). At maximal joint angle, the contribution to total MTU elongation changed with time, with increased contribution from tendon in the stretching leg (Figure 3B).

(Insert Figure 3 approximately here)

No change was seen in SOL collagen content from PRE to POST (Figure 4). Pearson product-moment correlation coefficients were calculated between PRE-POST changes in relevant variables (muscle collagen content, ROM, passive torque at anatomical joint angle (Figure 5B), passive torque at standardized joint angle, maximal SOL muscle belly passive elongation), but no significant correlations were found.

(Insert Figure 4 approximately here)

(Insert Figure 5 approximately here)

DISCUSSION

The present study examined the effect of 24 weeks of habitual stretching in humans on intramuscular collagen content and passive MTU properties. The main finding is that SOL intramuscular collagen content does not seem to change after 24 weeks of stretching, despite a large increase in ROM and reduced passive resistance to stretch.

ROM

Previous studies where MTU responses to stretching have been examined *in vivo* include intervention periods of up to 10 weeks. In the present study, a duration of 24 weeks was chosen and thus the stretching volume was more than doubled. The 9° improvement in ROM is similar or greater than reported in former intervention studies (Guissard & Duchateau, 2004; Blazevich et al., 2014; Konrad & Tilp, 2014; Nakamura et al., 2017; Simpson, Kim, Bourcet, Jones, & Jakobi, 2017), and corresponds to an increase of more than 50 % (relative to anatomical joint angle). Joint ROM is governed by passive properties of relevant tissues

that cross the joint, such as muscle and tendon, but ligaments, joint capsule and other anatomical constraints also play a role. In addition, the tone of the in-series contractile muscle tissue is controlled by the nervous system, which means that factors related to neural control also contribute to joint ROM. It follows, that the physiological mechanisms for the observed changes in ROM and passive torque after habitual stretching are intricate.

Torque and MTU elongation during passive dorsiflexion

Passive torque was reduced in the stretching leg at fixed angles (anatomical and standardized joint angle), while at maximal joint angle, the passive torque was increased relative to PRE. Such a pattern fits well with what is previously reported (Nakamura et al., 2017), although several studies observed an increased ROM without changes in passive torque (Mahieu, Cools, De, Boon, & Witvrouw, 2009; Konrad & Tilp, 2014; Blazevich et al., 2014). Discrepancies between studies may relate to study protocols, stretching type, volume or individual responses related to e.g. training status or flexibility of participants. Overall changes in passive torque do not discriminate between the underlying physiological mechanisms, and reductions in torque may reflect neural factors affecting active muscle stiffness, but also potential structural changes such as reduced passive stiffness of the force-bearing tissues. Surprisingly, a reduction in passive torque was observed in the control leg following stretching. The bilateral reduction in passive torque may be attributable to a 'cross-education effect', which has previously been observed after unilateral strength training (Scripture, Smith, & Brown, 1894), but also reported after acute stretching exercise (Chaouachi et al., 2017; Killen, Zelizney, & Ye, 2018). Cross-education in passive tension supports the notion that neural adaptation is an important mechanism for gains in ROM following habitual stretching. The contributions of muscle and tendon to total MTU elongation during passive dorsiflexion were examined, and increased tendon elongation and a

tendency (P = 0.08) for increased muscle elongation was seen in the stretching leg at maximal joint angle. These results partly differ from the increased muscle strain and reduced tendon strain at maximal ROM reported after 3 weeks of stretching (Blazevich et al., 2014). An increase in muscle elongation during passive stretching to similar joint angles could suggest that stretching training induces intrinsic structural changes in the muscle, but this was not the case in the present study.

Muscular adaptation

SOL muscle thickness, fascicle pennation angle and fascicle length were unaffected by 24 weeks of stretching. Cross-sectional studies have observed differences in fascicle length between populations with different joint flexibility (Moltubakk et al., 2018), while most intervention studies have found no changes in fascicle length with 3–6 weeks of stretching (Blazevich et al., 2014; Konrad & Tilp, 2014; Nakamura, Ikezoe, Takeno, & Ichihashi, 2012). Stretching for 24 weeks did not induce changes at macroscopic level, which to some extent contrasts findings of increased gastrocnemius medialis fascicle length and thickness after 6 weeks of stretching (Simpson et al., 2017). Differences between studies may relate to different stretching protocols and methods.

The muscle consists of contractile muscle cells and the parallel elastic element, and although IMCT accounts for less than 10 % of the muscle cross sectional area, *in vitro* studies have shown that the collagen rich extracellular matrix is the main contributor to passive stiffness, leaving little role in passive force transmission for intracellular structural proteins such as titin (Lieber & Ward, 2013; Meyer & Lieber, 2011). The extracellular matrix is important for transfer of contractile forces, but limited information is available regarding function, mechanical properties and organization of the extracellular matrix (Kjaer, Jørgensen, Heinemeier, & Magnusson, 2015). Longitudinal collagen ‘cables’ have been observed in

close proximity to the muscle cells and are thought to contribute to active force transmission but also to passive stiffness of the muscle tissue (Gillies & Lieber, 2011; Borg & Caulfield, 1980). Opposite to the muscle fibre, which hypertrophies with loading and atrophies with unloading, the extracellular matrix is known to increase with altered use, both when load is increased, decreased (immobilization) and in excessive stretching in animal models (Lieber & Ward, 2013). Whether stretching exercise in humans, which is performed in much less volumes, has a similar effect is not known. Previous studies have demonstrated augmented intramuscular collagen synthesis in response to loading (strength training) (Miller et al., 2005; Holm et al., 2010; Crameri et al., 2004; Heinemeier et al., 2007), although the extent to which synthesis is translated into functional load bearing collagen content is not well elucidated. The muscle tissue load during stretching exercise is likely much less compared to that of strength training, and it is thus questionable if responses similar to those of strength training are at play after stretching exercise. Animal models where extreme stretching loads were applied have observed increases in intramuscular collagen content (fibrosis), but in addition to stretching, many such studies can also be seen as immobilisation studies (Williams & Goldspink, 1984), and fibrosis is a known response to unloading (Jarvinen, Jozsa, Kannus, Jarvinen, & Jarvinen, 2002). Therefore, studies that examine stretching exercise should likely apply more feasible stretching protocols. A recent animal study applied stretching somewhat similar to what is used in humans during sports and rehabilitation settings (15 days, 10 x 1 min / day) and found increases in markers for collagen synthesis, but at the same time, intramuscular collagen deposition was mitigated. It could be speculated that stretching exercise of limited volume contributes to a reorganisation of collagen, perhaps reducing density, which in turn plays a role for muscle elongation during passive loading. Despite agreement that collagen content and the extracellular matrix defines the passive resistance of muscle tissue (Meyer & Lieber, 2011; Lieber & Ward, 2013; Purslow & Trotter, 1994), the muscle collagen content has been

shown to be a poor predictor of passive muscle fibre bundle stiffness at least in human biopsy material (Smith et al., 2011; Lieber & Ward, 2013), but if such a relation exists in humans in vivo has not previously been examined. In the present study only weak correlations between pre-intervention collagen content and passive torque were observed (Figure 5A), and likewise weak relations between PRE-POST changes in collagen content and changes in passive torque were observed (Figure 5B). It should be noted that numerous factors contribute to *in vivo* joint stiffness, and it may be questioned if in fact a relation between intramuscular collagen content as measured by hydroxyproline and *in vivo* stiffness can be expected.

Limitations

One limitation of the present study is that, in order to minimize the risk of the participants, the PRE-biopsy was taken from the control leg while the POST-biopsy was taken from the stretching leg. Issues related to side-to-side differences could influence results, however, training legs were randomized and moreover, none of the participants participated in activities known to induce contralateral difference in muscle strength or joint flexibility. Also, muscle elongation during passive dorsiflexion may be slightly underestimated and tendon elongation overestimated due to stretching of the skin when approaching large joint angles. Finally, it should be remembered that many structures that all take up tension span the ankle joint, but the nature of *in vivo* studies includes the limitation of targeting specific structures.

Conclusion

Knowledge on the effects of human stretching training on intramuscular adaptations is scarce. Despite significant changes in passive torque, stiffness and ROM, we found no change in intramuscular collagen content in the SOL muscle after 24 weeks of stretching, and as such, our hypothesis of reduced collagen content could not be confirmed. Other mechanisms must

underlie the reduced passive resistance to stretch seen in the present study. Changes in the non-stretched leg support the notion of a cross-education effect involving neural adaptations, as is known for strength training. It remains clear that numerous factors contribute to ROM and to the response to stretching training. Future studies could shed light on the muscular response to stretching by combining detailed measurements of passive mechanical properties at the protein, cellular and tissue levels with analysis of mechanical properties of the *in vivo* MTU and limb.

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FIGURES

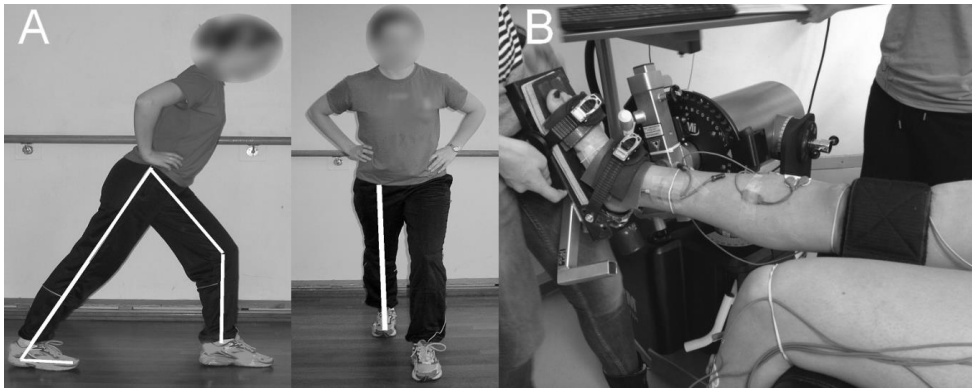


Figure 1. A) Stretching exercise, B) experimental setting in the isokinetic device. In A, participants were instructed to place the stretching leg as far posteriorly as possible, while pushing the heel down to the ground, the forefoot pointing forward. After 8 weeks, stretching was progressed such that two of four repetitions were performed with the knee joint flexed to approximately 45°, to ensure continuous stretching load on the soleus.

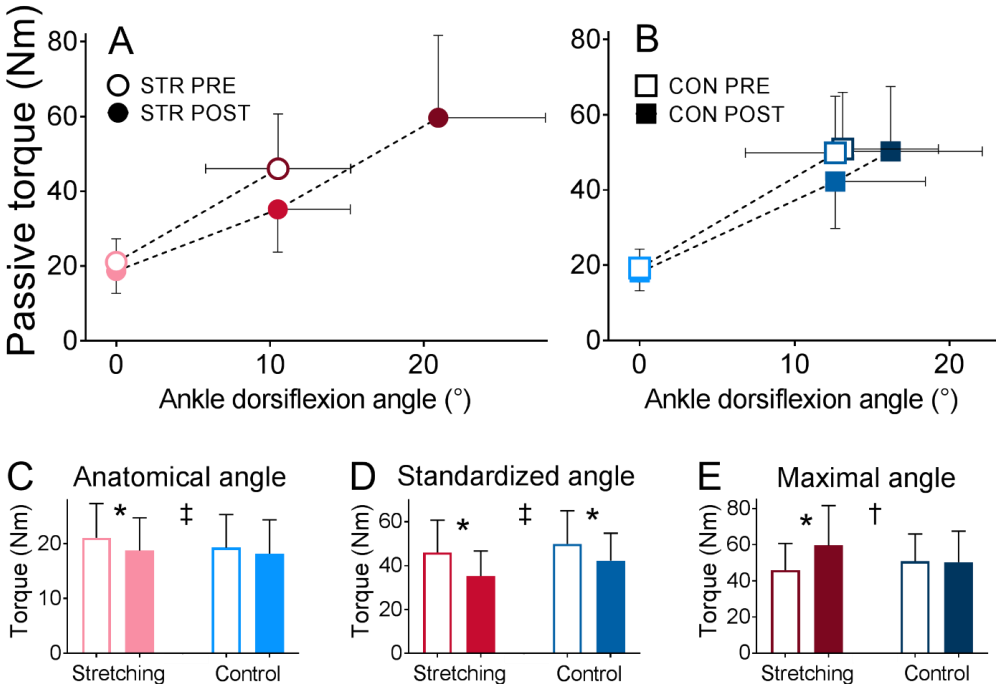


Figure 2. Passive torque during passive dorsiflexion. A) Stretching leg (STR) and B) control leg (CON) at anatomical, standardized and maximal joint angle, before (PRE) and after (POST) 24 weeks of stretching. C-E) Statistical analyses: † indicates an interaction effect ($P < 0.05$), ‡ indicates a time effect ($P < 0.0001$-0.05), * indicates significant post hoc tests ($P < 0.0005$-0.05). Note different scales on the Y axes of figures C-E.

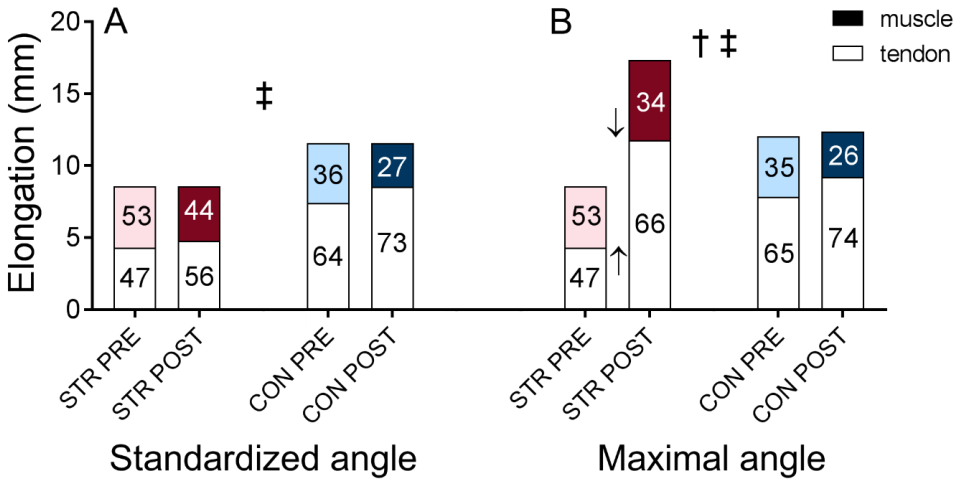


Figure 3. Soleus muscle belly and free Achilles tendon elongation during passive dorsiflexion. Stretching leg (STR) and control leg (CON) at standardized and maximal joint angle, before (PRE) and after (POST) 24 weeks of stretching. Numbers on the bars represent percent contribution to total muscle-tendon unit elongation. ‡ indicates a time effect for elongation of muscle and tendon at standardized joint angle ($P < 0.05$, post hoc tests indicate increased tendon elongation and decreased muscle elongation in CON), and for contribution at maximal joint angle ($P < 0.01$, ↓↑ indicate significant post hoc tests). † indicates an interaction effect for elongation of muscle and tendon at maximal joint angle ($P < 0.005$-0.05, post hoc tests indicate increased tendon elongation in STR).

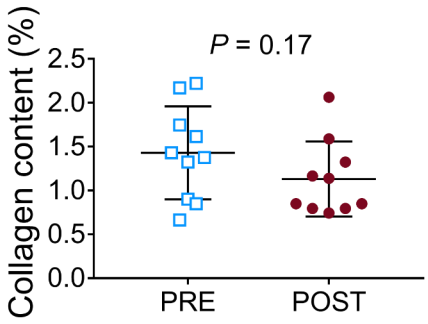


Figure 4. Soleus muscle collagen content (% of dry weight) in the control leg before (PRE) and in the stretching leg after (POST) 24 weeks of stretching.

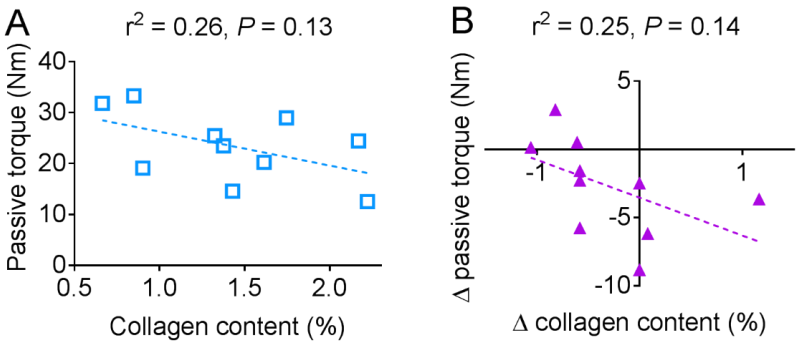


Figure 5. Correlation between collagen content and passive torque at anatomical joint angle. A) Prior to 24 weeks of stretching, B) change in passive torque and change in collagen content with 24 weeks of stretching.

TABLES

Table 1. Passive ankle joint stiffness and in the stretching and control legs before (PRE) and after (POST) 24 weeks of stretching.

		PRE	POST	*P* inter.	*P* time	Post hoc *P*
Passive joint stiffness ($Nm \cdot {}^{\circ -1}$)						
At standardized angle	Stretching	2.9 ± 1.5	1.9 ± 1.0	0.74	< 0.005	< 0.05
	Control	3.0 ± 1.5	2.1 ± 1.1			0.07
At maximal angle	Stretching	2.9 ± 1.5	3.3 ± 2.0	0.30	0.98	0.72
	Control	3.0 ± 1.5	2.5 ± 1.1			0.69

Table 2. Soleus muscle architecture of the stretching and control legs before (PRE) and after (POST) 24 weeks of stretching.

		PRE	POST	*P* inter.	*P* time	Post hoc *P*
Thickness (mm)	Stretching	18 ± 4	19 ± 4	0.35	0.10	0.14
	Control	18 ± 4	18 ± 5			0.83
Pennation angle (°)	Stretching	24 ± 5	22 ± 5	0.18	0.21	0.15
	Control	27 ± 9	27 ± 8			1.00
Fascicle length (mm)	Stretching	33 ± 8	35 ± 5	0.27	0.83	0.60
	Control	38 ± 4	36 ± 4			0.73

Appendices

Appendix I:

Studies I-III: Assessments from the Regional Committee for Medical and Health Research Ethics

Appendix II:

Study III: Stretching training program

Appendix III:

Studies I-III: Questionnaires

UNIVERSITETET I OSLO

DET MEDISINSKE FAKULTET

Jens Bojsen-Møller
Norges idrettshøgskole
Sognsveien 220
0863 Oslo

Regional komité for medisinsk og helsefaglig forskningsetikk sør-øst B (REK sør-øst B)
Postboks 1130 Blindern
NO-0318 Oslo

Telefon: 22 85 06 70
Telefaks: 22 85 05 90
E-post: juliannk@medisin.uio.no
Nettadresse: http://helseforskning.etikkom.no

Dato: 16.02.2010
Deres ref.:
Vår ref.: 2010/172

2010/172b Mekaniske egenskaper i muskel-sene-systemet hos utøvere som har drevet mangeårig systematisk bevegelighetstrening
Prosjektleder: Jens Bojsen-Møller
Forskningsansvarlig: Norges idrettshøgskole, Forskningssenter for trening og prestasjon

REK viser til søknad om godkjenning av forskningsprosjektet *Mekaniske egenskaper i muskel-sene-systemet hos utøvere som har drevet mangeårig systematisk bevegelighetstrening* som ble sendt inn til fristen 04.01.2010. Komiteen har vurdert søknaden i sitt møte 29. januar 2010 med hjemmel i helseforskningsloven § 10, jf. forskningsetikkloven § 4.

Saksfremstilling
Formålet med prosjektet er å få ny kunnskap om forskjellene mellom personer med stor bevegelighet/som har trent bevegelighet over lang tid ved å sammenligne mekaniske egenskaper ved muskel-sene-systemet hos utøvere som har drevet mangeårig systematisk bevegelighetstrening (landslagsutøvere i rytmisk gymnastikk) med utøvere i idretter/mosjonsaktiviteter uten spesielt fokus på bevegelighetstrening. Karakteristika som undersøkes er bevegelsesutslag (ROM), passiv motstand, generell leddmobilitet, lengdespenningsforhold i muskulatur, senestivhet og aktiv stivhet.

Vedtak
Etter søknaden fremstår dette prosjektet som en studie som har til hensikt å gi indikasjon om effekt av en bestemt treningsform for å bedre prestasjon i idrett eller dagliglivet. Det anses ikke som et medisinsk eller helsefaglig forskningsprosjekt, jf. helseforskningsloven § 4 a), og er ikke fremleggelsespliktig, jf. helseforskningsloven § 10, jf. § 2 og forskningsetikkloven § 4 annet ledd.

Komiteens vedtak kan påklages (jfr. forvaltningslovens § 28) til Den nasjonale forskningsetiske komité for medisin og helsefag. Klagen skal sendes til REK Sørøst B (jfr. forvaltingslovens § 32). Klagefristen er tre uker fra den dagen du mottar dette brevet (jfr. forvaltningslovens § 29).

Med vennlig hilsen

Stein Opjordsmoen Ilner (sign.)
leder

Julianne Krohn-Hansen
komitésekretær

Kopi: NIH, Avdeling for forskningsforvaltning og dokumentasjon, postboks 4014 Ullevål stadion, 0806 Oslo

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Tor Even Svanes	22845521	02.07.2012	2012/959
			Deres dato:	**Deres referanse:**
			22.05.2012	

Vår referanse må oppgis ved alle henvendelser

Jens Bojsen-Møller
Norges Idrettshøyskole
Sognsveien 220
0863 Oslo

2012/959 C Effekter av mangeårig bevegelighetstrening på struktur og funksjon i triceps surae muskel-sene komplekset .

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk i møtet 14.06.2012.

Forskningsansvarlig: Norges Idrettshøyskole
Prosjektleder: Jens Bojsen-Møller

Prosjektomtale:
Å drive bevegelighetstrening/å ha god leddbevegelighet antas generelt å ha en rekke positive innvirkninger innen helse, rehabilitering og prestasjon. Samtidig er effektene av mangeårig bevegelighetstrening ikke tilstrekkelig dokumentert gjennom forskning. Tidligere intervensjonsstudier har vart maksimalt 24 uker,mens det antas at endringer i mekaniske egenskaper i muskel og sene krever større stimulus. Formålet med denne studien er å avdekke eventuelle forskjeller i sener, muskulatur, bindevev eller nervesystem knyttet til langvarig bevegelighetstrening blant personer som har drevet med systematisk bevegelighets trening i >10 år. Ulike analyser av vev og funksjon vil bli foretatt på ett tidspunkt for å kartlegge de overnevnte egenskapene. Sett fra et overordnet perspektiv vil studien kunne øke kunnskapen om hvilke grupper som bør eller ikke bør drive bevegelighetstrening, og hvordan treningen evt. bør foregå.

Under søknadens punkt **1.d – Andre prosjektopplysninger**, anfører søker at det her omsøkte prosjektet er relatert til studien *Mekaniske egenskaper i muskel-sene-systemet hos utøvere som har drevet mangeårig systematisk bevegelighetstrening* (referanse 2010/172). Prosjektet har som formål å få ny kunnskap om forskjellene mellom personer med stor bevegelighet/som har trent bevegelighet over lang tid ved å sammenligne mekaniske egenskaper ved muskel-sene-systemet hos utøvere som har drevet mangeårig systematisk bevegelighetstrening (landslagsutøvere i rytmisk gymnastikk) med utøvere i idretter/mosjonsaktiviteter uten spesielt fokus på bevegelighetstrening.

Det gir begge studiene det samme utgangspunktet, fordi intervensjonsgruppen nå vil bestå av dansere fra henholdsvis Den Norske Opera og Ballett og Norges Dansehøyskole, samt en kontrollgruppe bestående av friske frivillige deltakere uten slik bakgrunn. Hensikten er igjen å sammenligne egenskaper ved muskel-sene-system.

Mekaniske egenskaper i muskel-sene-systemet hos utøvere som har drevet mangeårig systematisk bevegelighetstrening (referanse 2010/172) ble behandlet av REK sør-øst B 29.01.2010. Komiteen konkluderte den gang med at prosjektet falt utenfor helseforskningsloven, og som sådan ikke var fremleggelsespliktig for REK. Vedtaket ble begrunnet med at prosjektet hadde til hensikt å gi indikasjon om effekt av en bestemt treningsform, for å bedre prestasjon i idrett eller dagliglivet, jf. helseforskningslovens § 4 første ledd.

Komiteen mener at de samme vurderingene som ble gjort tidlig i 2010, er gjeldende også for dette prosjektet. Formålet med studien er å se på effekt av bevegelighetstrening for ulike grupper av friske og (etter prosjektleders egen vurdering) sunne mennesker. På et overordnet plan kunne man sagt at studien innehar en mulig overføringsverdi til for eksempel rehabiliteringsfeltet, men det er ikke det som er den reelle hensikten med prosjektet.

Prosjektet fremstår ikke som et medisinsk eller helsefaglig forskningsprosjekt, og faller derfor utenfor komiteens mandat, jf. helseforskningslovens § 2.

Vedtak
Prosjektet er ikke fremleggelsespliktig, jf. helseforskningslovens § 10, jf. helseforskningslovens §4 annet ledd.

REK antar for øvrig at prosjektet kommer inn under de interne regler som gjelder ved forskningsansvarlig virksomhet. Søker bør derfor ta kontakt med enten forskerstøtteavdeling eller personvernombud for å avklare hvilke retningslinjer som er gjeldende.

Komiteens avgjørelse var enstemmig.

Komiteens vedtak kan påklages til Den nasjonale forskningsetiske komité for medisin og helsefag, jfr. helseforskningsloven § 10, 3 ledd og forvaltningsloven § 28. En eventuell klage sendes til REK sør-øst. Klagefristen er tre uker fra mottak av dette brevet, jfr. forvaltningsloven § 29.

Vi ber om at alle henvendelser sendes inn via vår saksportal: http://helseforskning.etikkom.no eller på e-post til: post@helseforskning.etikkom.no.

Vennligst oppgi vårt referansenummer i korrespondansen.

Med vennlig hilsen

Arvid Heiberg
prof. dr.med
leder REK sør-øst C

Tor Even Svanes
seniorrådgiver

Kopi til: Norges Idrettshøyskole: olivier.seynnes@nih.no

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Jørgen Hardang	22845516	12.09.2011	2011/1394/REK sør-øst A
			Deres dato:	**Deres referanse:**
			15.06.2011	

Vår referanse må oppgis ved alle henvendelser

Jens Bojsen-Møller
Norges idrettshøgskole

2011/1394 A Effekt av 24 ukers bevegelighetstrening på mekaniske egenskaper i muskel-sene-systemet

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk i møtet 18.08.2011.

Forskningsansvarlig: Norges idrettshøgskole
Prosjektleder: Jens Bojsen-Møller

Formålet med studien er å avdekke eventuelle endringer i sener, muskulatur, bindevev eller nervesystem knyttet til langvarig bevegelighetstrening. Forsøket skal utføres på unge, friske studenter. Det skal gjøres undersøkelser, blant annet EMG, ultralyd og MR, tester av bevegelighet styrke og funksjon, og analyser av muskelvev og gjennom spørreskjema informasjon om tidligere skader. Ulike analyser av vev (biopsier fra muskelvev) og funksjon vil bli foretatt før, underveis i og i etterkant av 24 ukers bevegelighetstrening. I tillegg undersøkes om eventuelle endringer vedvarer etter at treningsperioden er avsluttet. Studien inkluderer 30 personer i intervensjonsgruppen og 15 i kontrollgruppen.

Det søkes om godkjenning for å opprette forskningsbiobanken "Muskelvev i forhold til langvarig bevegelighetstrening.". Ansvarhavende for biobanken er Jens Bojsen-Møller. Deltakerne skal samtykke til innsamling, bruk, oppbevaring og til overførsel av materialet til utlandet for analyse. Biobanken planlegges å vare til 2014.

Deltakerne skal rekrutteres blant studenter ved Idrettshøgskolen.

Ulemper ved deltagelse som forsøkspersoner i intervensjonsgruppen, vurderes som liten. Dette gjelder både selve treningen og gjennomføring av muskelbiopsier. Søkeren viser til at det ved Norges idrettshøgskole er tatt over 1600 biopsier de senere årene. Treningen vil kunne påføre forsøkspersonene noe smerte, men det angis at opplæring og overvåking vil minimere risiko for skader.

Komiteens vurdering

Studien er lagt opp som en form for pilotprosjekt. Det er ikke formulert klare forskningsspørsmål eller hypoteser. I informasjonsskrivet sies det at en håper "studien skal gi oss bedre dokumentasjon på hvilke endringer bevegelighetstrening medfører". Det sies lite om hva en antar at en vil kun finne. Dette er i søknaden heller ikke redegjort for den teoretiske bakgrunnen. Derfor blir det tilsynelatende litt tilfeldig

hvilke analyser og registreringer en vil gjøre. Det er trolig at det foreligger hypoteser som styrer datainnsamlingen, men at de ikke er kommet med i søknadens utforming. Det ville ha vært nyttig å gjøre hypotesene eksplisitte og dermed gi studien et mer presis design.

Komiteen antar at de registreringer som gjøres likevel kan ha en verdi som grunnlag for videre forskning. Ulempene for deltagerne synes små.

Vedtak

Komiteen godkjenner at prosjektet gjennomføres i samsvar med det som framgår av søknaden.

Godkjenningen gjelder til 31.12.2014.

Forskningsprosjektets data skal oppbevares forsvarlig, se personopplysningsforskriften kapittel 2, og Helsedirektoratets veileder for «Personvern og informasjonssikkerhet i forskningsprosjekter innenfor helse- og omsorgssektoren». Opplysningene skal ikke oppbevares lenger enn det som er nødvendig for å gjennomføre prosjektet, deretter skal opplysningene anonymiseres eller slettes.

Dersom det skal gjøres endringer i prosjektet i forhold til de opplysninger som er gitt i søknaden, må prosjektleder sende endringsmelding til REK.

Prosjektet skal sende sluttmelding på eget skjema, se helseforskningsloven § 12, senest et halvt år etter prosjektslutt.

Med vennlig hilsen,

Gunnar Nicolaysen (sign.)
Professor
Leder

Jørgen Hardang
Komitésekretær

Anne Schiøtz Kavli
Førstekonsulent

Kopi til: hans.andresen@nih.no,
Biobankregisteret ved nina.hovland@fhi.no
Norges idrettshøgskole: mailto:postmottak@nih.no

Effekt av 24 ukers bevegelighetstrening på mekaniske egenskaper i muskel-sene-systemet

Bevegelighetsprogram

Beskrivelse av treningen:

- Programmet består av statisk, passiv tøyning for triceps surae og hamstrings.
- Hver av stillingene skal holdes i 1 minutt, 4 repetisjoner. Gjør triceps surae og hamstrings vekselvis: TS – hamstrings – TS – hamstrings osv (totalt 8 tøyninger), så trenger du ikke å legge inn ekstra pausetid.
- Tøyningene skal gjøres med så høy intensitet som du tolererer. Du skal kjenne en solid tøyning, men ikke smerte.
- Unngå å «gynge» i stillingene, men gå langsomt lenger inn i tøyningen etter hvert som du kjenner at tøyningen slipper.
- Programmet skal KUN gjøres på det benet du har fått beskjed om. For det andre benet kan du ikke tøye systematisk. Uttøyning etter løping o.l., 1 repetisjon á 10-15 sek, er tillatt.
- Type oppvarming er frivalgt. Gjør gjerne bevegelighetsprogrammet etter dine ordinære treningsøkter, men så lenge du føler deg konfortabel med å tøye, trenger du ikke være spesielt varm. Temperaturen i vevet er ikke vist å påvirke effekten av tøyningen.

Gjennomføring:

- Treningsprogrammet skal gjøres HVER dag (7 dager i uken) i 24 uker. Dette MÅ du gå inn for!
- Har du glemt å tøye en dag, er du likevel ikke fortapt. Fyll ut treningsloggen korrekt. Det er et større problem om loggen ikke reflekterer sannheten, enn om du har gått glipp av noen få økter innimellom.
- Har du glemt en økt, er det OK å ta gårsdagens økt på morgenen, og dagens økt på kvelden. Men du kan ikke samle opp og gjennomføre flere økter samtidig (8-12 repetisjoner i samme økt): For å oppnå maksimal framgang er det viktig med jevnlig stimuli.
- Med jevne mellomrom vil vi i prosjektgruppen ta kontakt. Vi mener ikke å mase! ☺ Men innen forskning det stilles krav til at man følger litt med på at forsøkspersonene følger opp, gjør øvelsene riktig m.m.
- Hvis det oppstår noe som gjør at du er i tvil om du kan fortsette treningen (skade, alvorlig sykdom e.l.), ta kontakt med Marie så tidlig som mulig. Vi vil vurdere hvor langt treningsavbrekk du kan ha før du må tre ut av prosjektet. I tvilstilfeller kan vi sette deg i kontakt med ekspertise som kan vurdere situasjonen.

Tips for å hjelpe med gjennomføringen:

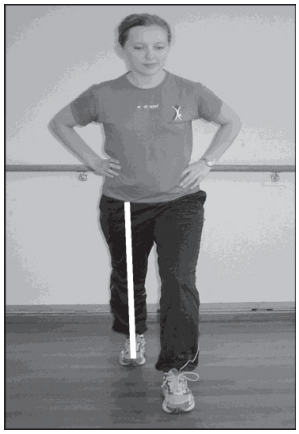
- Legg treningen inn i den daglige rutinen: Ser du på et TV-program hver kveld? Rett før tannpussen? Gå 10 minutter tidligere til skolen og gjør det i forkant av forelesning?
- Samarbeid med noen i klassen din: Det er trolig mer motiverende å tøye sammen, og/eller minne hverandre på treningen. Det gjør heller ingen skade å få noen som ikke er med i prosjektet (samboer e.l.) til å tøye sammen med deg. De trenger det sikkert. ☺
- Sett opp en alarm på telefonen, som repeteres hver dag. Evt. er Astrid, Producteev o.l. praktiske, gratis apper som kan holde orden på både tøyning og andre gjøremål for deg.
- Planlegg travle dager: Kvelden før eksamen, etter en kveld på byen, sent på julaften o.l. er det vanskelig å få prioritert tøying. Sørg for å få treningen unna på dagtid!

Progresjon:

- Etter noen uker vil du antagelig oppleve at det er vanskeligere å tøye slik at du føler en solid tøyning (kanskje spesielt i ankel). Dette betyr IKKE at treningsprogrammet er nytteløst!
- At du føler mindre tøyning kan skyldes at toleransen for tøyning har begynt å endre seg. Fortsett å tøye med god intensitet. Øvelsene kan gi god effekt selv om du kjenner litt mindre.
- Etter hvert som du blir mykere, bruk tipsene som er oppgitt for å utvikle øvelsene.
- Ta kontakt med Marie dersom du er bekymret for at øvelsene ikke fungerer som de skal.

Lykke til med treningen!

1. Triceps surae

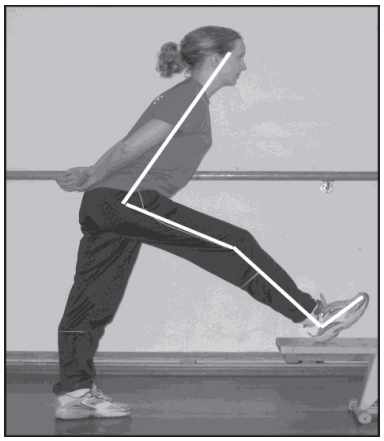


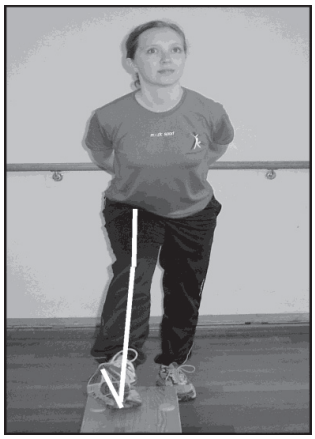
- Stå med treningsbenet en god meter bak det andre (utfall)
- Hælen på bakre ben skal være i kontakt med bakken
- Bakre fot må peke rett forover, i samme retning som hoftene
- Det bakerste kneet skal være tilnærmet strakt, men ikke overstrukket
- Skyv gjerne mot en vegg for å oppnå bedre tøyning

Progresjon når du blir mer bevegelig:

- Sett bakre ben lenger bak og senk hoftene, slik at vinkelen i ankelen blir større
- Bygg opp under forfoten på det bakerste benet, f.eks. med sammenbrettede sokker, noen aviser e.l.
- Se om du opplever mer tøyning ved å justere knevinkelen lett

2. Hamstrings





- Stå med treningsbenet på et trappetrinn, sofaen, en stol, e.l.
- Lett bøyd kne
- Nøytral posisjon i ankelleddet
- Pass på at bekkenet ikke roterer bort fra treningsbenet: Hold hoftekammene rett fremover, og med lik høyde på begge sider
- Len strak overkropp forover mot benet
- Støtt evt. på låret, men behold rett rygg

Progresjon når du blir mer bevegelig:

- Du vil kjenne mer tøyning ved å lene overkroppen litt lenger forover. MEN: Hvis du lener deg for langt, kan det bli tungt for ryggen å holde stillingen. Velg heller:
- Plasser treningsbenet litt høyere. Legg f.eks. anatomisk atlas under hælen! Etter hvert kan du bytte fra en lav stol til en høyere stol, e.l.

SPØRRESKJEMA FOR UTØVERE I RYTMISK GYMNASTIKK

1 Forsøksperson-nummer: _____ (fylles ut av forskerne)

2 Fødselsdato: _____

3 Hvilke idretter/treningsformer har du drevet med de siste 3 årene, utenom RG-treningen?

_____

4 Hvilke øvelser behersket du da du begynte å trene rytmisk gymnastikk?

4a	Spagat på gulvet, høyre ben	○ Ja	○ Nei	○ Vet ikke
4b	Spagat på gulvet, venstre ben	○ Ja	○ Nei	○ Vet ikke
4c	Sidespagat på gulvet	○ Ja	○ Nei	○ Vet ikke
4d	Bøye deg forover med strake knær og ta på gulvet	○ Ja	○ Nei	○ Vet ikke

5 Hvor gammel var du da du begynte å trene rytmisk gymnastikk? _____

6 Hvor gammel var du da du begynte å konkurrere? _____

7 Hvor gammel var du da du kom på landslaget (samling eller konkurranse)? _____

8 Hvor mange timer trener du totalt pr uke, konkurransesesongen 2010? _____

9 Hvor mange timer trener du bevegelighet pr uke, konk.sesongen 2010? _____

10 Hvilket ben er ditt beste i spagat? ○ Høyre ○ Venstre ○ Ingen forskjell

11 Hvilket ben foretrekker du å satse på i sprang? ○ Høyre ○ Venstre ○ Ingen forskjell

12 Beskriv eventuelle skader du har/har hatt i bena de siste 3 årene (type skade, hvilken periode, og om du har fått behandling for skaden):

_____

QUESTIONNAIRE, RHYTHMIC GYMNASTICS ATHLETE

1 Research subject no: [ ] (to be filled in by the researchers)

2 Date of birth: [ ]

3 Which sports or types of exercise have you practised the last 3 years, except RG training?

[ ]

4 Which exercises could you already do when you started practising rhythmic gymnastics?

4a	Splits on the floor, right leg	○ Yes	○ No	○ Don't know
4b	Splits on the floor, left leg	○ Yes	○ No	○ Don't know
4c	Side splits on the floor	○ Yes	○ No	○ Don't know
4d	Bend forward with straight knees, touch the floor	○ Yes	○ No	○ Don't know

5 How old were you when you started practising rhythmic gymnastics? [ ]

6 How old were you when you started competing? [ ]

7 How old were you when joined the national team (camps/competitions)? [ ]

8 How many hours do you practise each week (competitive season 2010)? [ ]

9 How many hours do you stretch each week (competitive season 2010)? [ ]

10 Which is your best leg for doing splits? ○ Right ○ Left ○ No difference

11 Which leg do you prefer to take off from in leaps? ○ Right ○ Left ○ No difference

12 If you have had any injuries to your legs during the last 3 years, please describe them (type of injury, when, and was the injury treated by health personell):

[ ]

SPØRRESKJEMA FOR PERSONER UTEN FOKUS PÅ BEVEGELIGHETSTRENING

1 Forsøksperson-nummer: _____ (fylles ut av forskerne)

2 Fødselsdato: _____

3 Hvilke idretter/treningsformer har du drevet med de siste 3 årene?

_____

4 Behersket du noen av disse øvelsene da du gikk på barneskolen?

4a	Spagat høyre ben	○ Ja	○ Nei	○ Vet ikke
4b	Spagat venstre ben	○ Ja	○ Nei	○ Vet ikke
4c	Sidespagat (guttespagat)	○ Ja	○ Nei	○ Vet ikke
4d	Bøye deg forover med strake knær og ta på gulvet	○ Ja	○ Nei	○ Vet ikke

5 Hvor gammel var du da du begynte med organisert idrett? _____

6 Hvor gammel var du da du eventuelt begynte å delta i konkurranser/stevner? _____

7 Hvor gammel var du da du evtentuelt kom med på landslaget (samlinger/konk.)? _____

8 Ca hvor mange timer trener du pr uke, gjennomsnittlig for 2010? _____

9 Ca hvor mange timer trener du bevegelighet pr uke, gjennomsnitt for 2010? _____

10 Hvilket ben er ditt beste i spagat? ○ Høyre ○ Venstre ○ Ingen forskjell

11 Hvilket ben foretrekker du å hinke på? ○ Høyre ○ Venstre ○ Ingen forskjell

12 Beskriv eventuelle skader du har/har hatt i bena de siste 3 årene (type skade, hvilken periode, og om du har fått behandling for skaden):

_____

SPØRRESKJEMA FOR PROFESJONELLE UTØVERE INNEN DANS

1 Forsøksperson-nummer: [ ] (fylles ut av forskerne)

2 Fødselsdato: [ ]

3 Hvilke idretter/treningsformer har du drevet med de siste 3 årene, utenom danse-treningen?

4 Hvilke øvelser behersket du da du begynte å trene dans?

4a Spagat på gulvet, høyre ben

4b Spagat på gulvet, venstre ben

4c Sidespagat på gulvet (guttespagat)

4d Bøye deg forover med strake knær og ta på gulvet

5 Hvor gammel var du da du begynte å trene dans? [ ]

6 Hvor gammel var du da du begynte å trene innenfor teknikken klassisk ballet? [ ]

7a Hvor gammel var du da du begynte med tåspiss sko? [ ]

7b Hvor mange timer per uke praktiserer du tåspiss, sesongen 2011/2012? [ ]

8 Hvor mange timer trener du totalt pr uke,i 2011/2012? [ ]

9 Hvor mange timer trener du bevegelighet pr uke, sesongen 2011/2012? [ ]

10 Hvilket ben er ditt beste i spagat?

11 Hvilket ben foretrekker du å satse på i sprang/hopp?

12 Eventuelle skader du har/har hatt i bena de siste 3 årene (type skade, hvilken periode, og om du behandling for skaden):

[ ]

SPØRRESKJEMA TILVENNINGSDAG

Forsøksperson-nummer: _____ Dato: _____

Trening de to siste dagene før test:

Måltider samme dag som testen (tidspunkt, innhold og omtrentlig mengde):

Biopsi vil bli gjennomført på samme dag for alle forsøkspersoner som skal være med på dette. Dato blir trolig 28. september. Du skal være på NIH 1 time ifm. dette. Det vil være mulig å ta biopsi på dagtid eller på kveldstid. **Har du mulighet denne dagen, og ønsker du å være med på dette?** Har fått bekreftet fra Pernille Botolfsen at de som har undervisning i forflytning 28. sept kan bytte til en annen dag. De som tar biopsi får også delta på MR-scanning, mest sannsynlig blir det 3-4 dager å velge mellom etter 28. sept.

For videre planlegging av testing, må vi vite hvilken **klasse** du går i. For de som skal ut i **praksis** i uke 41/42, må vi vite om du har praksis et sted som tilsier at du kan komme til testing på NIH på kveldstid/helg i forbindelse med praksisperioden, eller om testing må skje ukene før/etter praksis.

For **jenter** kan hormonsvigninger påvirke elastisiteten i vevene. Derfor må vi velge tidspunkt for de neste testene basert på menstruasjonssyklusen. Vi vet at dette kan variere litt, derfor vil vi avklare dette med deg når vi nærmer oss de neste testene. For å kunne planlegge omtrentlig, må du oppgi dato for første dag av forrige menstruasjon (evt. omtrentlig dato). For de som bruker p-piller vil hormonforløpet være noe annerledes, derfor ber vi om at du oppgir type p-pille hvis du bruker dette.

PRE

SPØRRESKJEMA PRE-TEST

Forsøksperson-nummer: _____ Dato: _____

Trening de to siste dagene før test:

Måltider samme dag som testen (tidspunkt, innhold og omtrentlig mengde):

Med hvilket ben foretrekker du å sparke en ball? ○ Høyre ○ Venstre

På hvilket ben vil du gjøre en ettbenssats? ○ Høyre ○ Venstre

Opplever du selv at det ene benet er stivere - hvilket? ○ Høyre ○ Venstre ○ Ca likt

For **jenter:** Første dato i forrige menstruasjon:

SPØRRESKJEMA 8-UKERS-TEST

Forsøksperson-nummer: _____ Dato: _____

Trening de to siste dagene før test:

_____

_____

_____

_____

_____

_____

Hvordan opplevde du intensiteten i tøyningen av legg den første uken du trente?

Hvordan opplever du intensiteten i tøyningen av legg DENNE uken?

Hvordan opplevde du intensiteten i tøyningen av hamstrings den første uken du trente?

Hvordan opplever du intensiteten i tøyningen av hamstrings DENNE uken?

For **jenter:** Første dato i forrige menstruasjon:

_____

SPØRRESKJEMA MID-TEST

Forsøksperson-nummer: _____ Dato: _____

Trening de to siste dagene før test:

_____

_____

_____

_____

_____

_____

Hvordan opplever du intensiteten i tøyningen av legg DENNE uken?

Hvordan opplever du intensiteten i tøyningen av hamstrings DENNE uken?

For **jenter:** Første dato i forrige menstruasjon:

_____

POST

SPØRRESKJEMA POST-TEST

Forsøksperson-nummer: _____ Dato: _____

Trening de to siste dagene før test:

Hvordan opplever du intensiteten i tøyningen av legg DENNE uken?

Hvordan opplever du intensiteten i tøyningen av hamstrings DENNE uken?

For **jenter:** Første dato i forrige menstruasjon:

Føler du selv at du har blitt mykere gjennom prosjektet? Hvordan merker du evt. dette?
(I tøyningsøvelsene? I dagliglivet? I trening? Annet?)

Når prosjektperioden er ferdig, har du planer om å fortsette bevegelighetstrening på egen hånd?
I større eller mindre grad enn i prosjektet? Samme eller andre muskelgrupper?